

**ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
SCHOOL OF VETERINARY MEDICINE**

**EPIDEMIOLOGICAL STUDY OF FOOT AND MOUTH DISEASE IN CATTLE
AND SMALL RUMINANTS IN SELECTED DISTRICTS OF GAMBELLA REGIONAL
STATE, SOUTH-WEST ETHIOPIA**

BY

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LIST OF ABBREVIATIONS

BHK	Baby Hamstere Kidney
cDNA	Complementary Deoxyribonucleic Acid
CPE	Cytopathic Effect
CSA	Central Statistic Agency of Ethiopia
DACA	Drug Administration and Control Authority of Ethiopia
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
EWCA	Ethiopia Wildlife Conservation Authority
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GBoARD	Gambella Bureau of Agriculture and Rural Development
GDP	Gross Domestic Product
GIS	Geographical Information System
GPNRS	Gambella Peoples National Regional State
GPS	Global Positioning System
GRBoFED	Gambella Regional Bureau of Finance and Economic Development
GNRP	Gambella National Regional Park office
HRP	Horseradish peroxidase
ICTV	International Committee on Taxonomy of Virus
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
km	Kilometer
km ²	Square kilometer
LPB-ELISA	Liquid Phase Blocking ELISA
M	Molar
Masl	Meter above sea level
min	Minute
ml	Milliliter
mm	Millimeter
μl	Microliter

MoARD	Ministry of Agriculture and Rural Development
mRNA	messenger Ribonucleic Acid
NAHDIC	National Animal Health Diagnostic and Investigation Centre
NCR	Non-coding Region
nm	Nanometer
NMAGBO	National Metrological Agency Gambella Branch Office
NSP	Non Structural Protein
nt	Nucleotide
NVI	National Veterinary Institute
°C	Degree Celsius
OD	Optical Density
OIE	Organization International des Epizooties
OPF	Oro-Pharyngeal Fluid
OR	Odds Ratio
PA	Peasant Association
PBS	Phosphate Buffer Saline
poly [A]	Poly- adenosine
Poly [C]	Poly-cytidyl
Poly [U]	Poly-uridine
RGD	Arginine-Glycine-Aspartic acid
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAT	South African Territories
SNNPRS	Sothorn Nation Nationalities Peoples Regional State
SPSS	Statistical Package for Social Science
UK	United Kingdom
USD	United States Dollar
UTM	Universal Transverse Mercator
VNT	Virus Neutralization Test
VP	Virus Protein
VPg	Virus Protein genome
WRL	World Reference Laboratory
χ^2	Chi-Square

ABSTRACT

A cross sectional study based on serological and questionnaire survey was conducted in 4 selected Districts of Gambella Regional State from September 2009 to April 2010 to determine the sero-prevalence of FMD, the involvement of small ruminants in the epidemiology of FMD, identify and characterize the serotypes of FMD virus circulating in the study area and assess epidemiological risk factors associated with FMD in cattle. A multistage random sampling was implemented to select the required sample size. Field observation, clinical examination, specimen collection and laboratory diagnosis, interviewing farmers, and reviewing secondary data source were methods followed to generate information. Sera were tested by 3ABC-ELISA at NAHDIC, Sebeta and FMD virus identification and characterization was carried out at Pirbright, UK. Results were analyzed using descriptive statistics, chi-square test and logistic regression. The overall seroprevalence of FMD was 12% (N = 1,152) for cattle and 1.43% (N = 768) for small ruminants. Cattle was 9.37 times more FMD seropositive than small ruminants. Seroprevalences of 17.74%, 11.86%, 9.42% and 8.97% were recorded in Itang, Gambella, Abobo and Lare Districts, respectively. The difference among which was statistically significant ($p < 0.05$). From the different risk factors analysed; herd size, age, contact with wildlife and cattle-small ruminant combination were significantly associated with FMD seroprevalence ($p < 0.05$). From the total of 338 cattle and 69 small ruminants clinically examined during an outbreak episode 124 (36.69%) of cattle showed signs and lesions suggestive of FMD but observable symptoms were not detected in small ruminants. FMD virus SAT-2 serotype was responsible for the occurrence of the investigated outbreak in the study area. The field isolates shared greater than 80% genetic similarity with the Benshangul-Gumuz and Sudan SAT-2 isolates. The present SAT-2 isolate had also showed antigenic relationship with the vaccine strains of Eritrea SAT2/ERI and Zimbabwe SAT2/ZIM (r_1 -values > 0.3 and 0.4 for VNT and LPB-ELISA), respectively. Questionnaire survey feedback generated from a total of 120 respondents indicated that FMD was prevalent and frequently affect the cattle population of the study area. Herd congregation sites, free animal movement, seasonal recurrence of flood and infected animal introduction into herd were complained as possible source of FMD infection and dissemination. This work generates preliminary baseline information on current status of FMD along with associated risk factors in the study area in particular and can also add information nationally on existing knowledge in general. Therefore, the identified FMD virus SAT-2 serotype should be considered in vaccine formulation, cattle and small ruminants must be surveyed regularly,

domestic animals should be protected from wildlife park entry and realizing regional approach of FMD control program with the neighboring regional states and the Sudan republic across the border is recommended.

Key words: Cattle, small ruminants, FMD, seroprevalence, serotype, risk factors, Gambella

1. INTRODUCTION

Agriculture is the main stay of life for the great majority of population of Ethiopia. The agricultural sector constitutes approximately 45% of the gross domestic product (GDP), more than 90% of foreign exchange earnings, 85% of employment opportunities and most of the domestic food supply (Anon, 2000). Livestock production which is one of the sub sectors of agriculture is an essential component of the overall farming systems. The contribution of livestock and livestock products to the agricultural economy is about 46% of the national foreign currency earnings (IBC, 2004; FAO, 2005). Ethiopia's ruminant livestock populations are the largest in Africa and constitutes about 47.6 and 47.8 million heads of cattle and small ruminants, respectively (CSA, 2008).

In spite of the great number and contributions to the national economy, productivity of ruminants in the country are very low due to poor management practices, poor nutrition, low genetic potential and high disease incidence (ILCA, 1991; GPNRS, 2003). Animal diseases are widespread in all agro-ecological zones of the country and reduce the production and productivity of livestock by 50-60% per year (Anon, 2000; Sahle *et al.*, 2004).

Foot and mouth disease (FMD) is one of the most important socio-economic livestock diseases of the globe (Perry *et al.*, 2006; Perry and Rich, 2007). Now a days as the result of globalization, FMD epidemics can change from local and regional spread to wide international spread (Knowles *et al.*, 2005; Cottam *et al.*, 2006). FMD is an extremely contagious, acute viral disease of all cloven hoofed animals and pigs, characterized by fever, loss of appetite, salivation, vesicular eruptions in the mouth, on the feet and teats and sudden death of young stock (Bronsvort *et al.*, 2004; Quinn *et al.*, 2005; FAO, 2007). The disease is caused by one of the smallest RNA viruses which belongs to the genus *Aphthovirus* of the family Picornaviridae (Sáiz *et al.*, 2002; OIE, 2004). Seven immunologically distinct but clinically indistinguishable serotypes of foot and mouth disease virus (FMDV), namely O, A, C, SAT-1, SAT-2, SAT-3 and Asia-1 are recognized worldwide. The disease is common in most developing nations of the world and it is highly endemic in most sub-Saharan African countries (FAO, 2006; Sumption *et al.*, 2008).

In Ethiopia, FMD is endemic, widely distributed and frequently noted in different farming systems and agro-ecological zones of the country (Asfaw and Sintaro, 2000; Sahle, *et al.*,

2004; Leforban, 2005). During the last ten years (1999-2008) on average over 77.7 numbers of FMD outbreaks were reported every year to Ministry of Agriculture and Rural Development. Five of the seven FMDV serotypes (O, A, C, SAT-1 and SAT-2) were identified in Ethiopia. Serotype O was more prevalent followed by types A, SAT-2, SAT-1 and C, respectively (Sahle, 2004; Gelaye *et al.*, 2005; Legesse, 2008; Ayelet *et al.*, 2009). Serotype C was not identified after 1983; however, a serotype C specific antibody in cattle was recently reported (Rufael *et al.*, 2008).

The impact posed by the disease is enormous. It causes high morbidity and affects animals' performance directly through reduction of milk yield, weight gain and meat production. Death of young animals and fertility impairment due to increased abortion rate are also the grave consequences of the disease (Woodbury, 1995; Doel, 2002). Pastoralists are severely affected by the direct impact of the disease since their livelihood is directly linked to livestock production (Rufael *et al.*, 2008; Bayissa, 2009). In Ethiopia over 95% of draught power for crop production originates from animals (Sahle *et al.*, 2004). FMD outbreak affects draft oxen working efficiency during the planting season and causes heavy losses in small scale mixed farming system and the burden may be severe impairing local food security (Sahle *et al.*, 2004; Bogale, 2005).

From a current global perspective, the indirect effect and the risk of the disease has a much greater impact than that of the disease itself. Much of the burden (cost) of containing outbreaks, as well as maintaining the ongoing capacity and measures intended to reduce the risk of FMD such as vaccination, surveillance, movement control and so on can incur an extremely high cost (Kitching *et al.*, 2006). The presence of FMD has also a negative impact on tourism and the integration of livestock and wildlife based activities. Currently, the single largest impact of FMD is undoubtedly its critical role as a restriction to international trade. FMD free countries are allowed to restrict or ban imports of livestock and livestock products and sometimes other products as well, as a potential risk of introducing FMD (Gulati *et al.*, 2005).

According to the information generated from MoARD (2009) agricultural marketing directorate on average Ethiopia has earned 102.2 million USD every year from exporting live animal and animal products. However, the impact of the disease in affecting the national export trade has been witnessed by import bans imposed by different countries at different

times. Following the 2001 outbreak of FMD in the United Kingdom, Saudi Arabia and Indonesia had imposed trade bans of our export meat and pickled sheep and goat skin, respectively. The 2006 FMD outbreak detected in Egypt also became the major cause of live cattle import ban to the Egyptian markets (MoARD, 2006). Thus, locally FMD reduces farmers' income and food availability for consumption. At national level, it slows economic growth by severely limiting international trade opportunities and hampering utilization of the available huge livestock resources for economic development and poverty alleviation (Bogale, 2005; MoARD, 2006; Megersa *et al.*, 2009).

Despite its wide socio economic impact, effective control and prevention of the disease is not achieved yet in most of the sub-Saharan African countries including Ethiopia (Sutmoller *et al.*, 2000; Vosloo and Thomson, 2004). The presence of complex epidemiological situations such as the involvement of wildlife in FMD maintenance and transmission, the existence of immune specific multiple serotypes and unpredictably emerging intratypic variants that can break immunity in many developing countries render them vulnerable to the spread and poor control of the disease (Hargreaves *et al.*, 2004; FAO, 2006).

Trans-boundary live animal movements are part of the main characteristics of husbandry systems in many pastoral areas of Africa; drought conditions, the use of communal grazing and watering points, refugee movements and frequent cross border trade have probably increased the risk of the entry and spread of FMD. Moreover, the use of vaccines is sub-optimal in relation to the size of livestock population and most of the FMD susceptible animal populations are found at risk (Sahle, 2004; Bogale, 2005; FAO, 2006; Rweyemamu *et al.*, 2008).

Similarly the role of small ruminants is not well established in the epidemiology of FMD. In sheep and goats the clinical signs can be very mild or absent, FMD can spread unnoticed and small ruminants may become carriers of FMDV for a long period of time. The involvement of small ruminants in FMD outbreaks has been widely reported in many European and Asian countries (Barnett and Cox, 1999; Leforban, 1999; Armstrong *et al.*, 2005). However, despite this cumulative evidence, research concerning FMDV transmission from sheep and goats has been limited in comparison to bovine species that require further investigations (Blanco *et al.*, 2002).

In Ethiopia regardless of the disease endemicity majority of outbreaks remain unrecorded and is not notified in a timely fashion. In pastoral areas disease surveillance is inadequate and sometimes absent; the transport of sampling material is difficult and expensive, the lack of well equipped regional animal laboratories, inaccessibility of certain areas and suboptimal routine surveillance and reporting rate are major problems (Sahle *et al.*, 2004; MoARD, 2006).

From the prevalence reported and serotypes identified at different areas of the country, there is a paucity of information on the status of the disease in the low land agro-pastoral areas of Gambella Regional State in western Ethiopia. Currently, in Ethiopia only a bivalent vaccine is manufactured from locally isolated FMD viruses of serotypes A and O (DACA, 2006). Since the different serotypes of the virus are antigenically different and require serotype specific vaccines, the serotypes of the present study area should be identified. With globalization of trade even areas where FMD is endemic can suffer from introduction of virus strains that are exotic to the region and hence continuous research is required (Brückner *et al.*, 2002).

For the development of adequate FMD control and prevention measures determining the involvement of small ruminants in FMD occurrence involving both serology and virus isolation is needed. Furthermore, Gambella is blessed with diverse flora and fauna and thus justifying the possible role of wildlife for the persistence of FMD virus in the area seems logical. The region is also exposed to trans boundary animal disease across the Sudan border and thus implementing sound epidemiological study is essential to generate important baseline information about the study disease.

Therefore, the main objectives of this study were;

- To determine the sero-prevalence of FMD in the study area.
- To determine the involvement of small ruminants in the epidemiology of FMD.
- To identify and characterize the serotypes of FMDV circulating in the study area and
- To assess potential epidemiological risk factors associated with FMD in cattle.

2. LITERATURE REVIEW

A general preliminary review of accessible background literature and previous studies related to the present study subject has been undertaken to get knowledge transfer and data collected were used as input for the current study. Additional relevant information was also assessed from various national and regional institutions as supplementary input in reference to study disease and livestock production system of the area.

2.1. Disease definition

Foot and mouth disease (FMD) is an extremely contagious, acute viral disease of all cloven-hoofed animals/ungulates and pigs, characterized by fever, loss of appetite, salivation, vesicular eruptions in the mouth, on the feet and teats and sudden death of young stock (Hedger, 1981; Thomson, 1994; Quinn *et al.*, 2005). It is one of the most important economical notifiable diseases of livestock owing to the infectious and transboundary nature of the disease (OIE, 2004). Significant economic losses are produced by its high morbidity and the international livestock and livestock product export trade restrictions imposed on affected countries (Geering and Lubroth, 2002; Bronsvoort *et al.*, 2004; FAO, 2007).

2.2. Etiology

Foot-and-Mouth Disease (FMD) is caused by one of the smallest known RNA virus which belongs to the genus *Aphthovirus* of family Picornaviridae (Sáiz *et al.*, 2002; OIE, 2004). Viruses within this family cause diseases of medical and agricultural importance such as poliovirus, common cold virus, human hepatitis A virus and FMDV (Pringle, 1999). The name Picornaviridae is derived from the Latin words 'pico', which means very small and 'rna' which refers to the RNA genome type. The genus name '*Aphthovirus*' comes from Greek word 'aphta' which refers to the vesicular lesions produced in cloven-hoofed animals (Matthews, 1979; OIE, 2004). The virus was discovered by Loeffler and Frosch in 1898 as the first viral causative agent of animal disease and its taxonomic classification was defined in 1963 by the International Committee of Taxonomy of Viruses (ICTV) (Melnick *et al.*, 1975).

Currently seven serotypes of the virus are recognized worldwide, but new subtypes with altered antigenic properties frequently emerge due to genetic instability of the virus. These

serotypes are clinically indistinguishable and immunity against one specific serotype does not confer against the other six (Verdaguer and Fita, 1996; Beard and Mason, 2000; Mason *et al.*, 2002; Zhao *et al.*, 2003; Bronsvoort *et al.*, 2004; Cottam *et al.*, 2006).

2.2.1. Virus morphology

The FMD viruses are characterized by having a naked nucleocapsids and icosahedral symmetry of protein shell with 22-30 nm in diameter (Melnick *et al.*, 1975; Cooper *et al.*, 1978; Robert and Bruce, 1981). The virion appears to be round particle with smooth surface (Clavijo and Kitching, 2003). The diameter of the virion capsid is ranging from 22-25 nm and the capsid is composed of the four major structural capsid proteins (VP1, VP2, VP3 and VP4) and contains 60 copies of each (Cooper *et al.*, 1978). One copy of each capsid protein assembles to produce a protomer; in turn, five protomers form a pentamer, and 12 pentamers make the complete capsid (Domingo *et al.*, 2002). Viral proteins (VP1-VP3) are exposed on the surface whilst the VP4 protein is located internally and interacts with the viral RNA (Strohmaier and Adam, 1982; Chow *et al.*, 1987; Acharya *et al.*, 1989).

2.2.2. Physicochemical properties of the virus

Foot and mouth disease virus is a small RNA virus that is enclosed with a non-enveloped protein shell (capsid). The capsid consists of polypeptides, which are devoid of lipo-protein, and hence is stable to lipid solvents like ether and chloroform (Cooper *et al.*, 1978). The virus is pH sensitive to both acidic and alkaline conditions. It is more stable between pH 7 and 9 at 4°C and -20°C but all strains are rapidly inactivated below pH 4 and above pH 11 (Domingo *et al.*, 2002; Greering and Lubroth, 2002). In milk and milk products, the virion is protected, and can survive at 70°C for 15 seconds and pH 4.6. In meat the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes. Two percent solution of caustic soda (NaOH) or KOH and 4% Na₂CO₃ in combination with detergent are effective disinfectants for FMDV contaminated objects, but the virus is resistant to alcohol and phenolic quaternary ammonium disinfectants. The FMDV is also sensitive to other chemicals like trypsin, which causes cleavage and denaturation of the vital capsid protein VP1 (Rowlands *et al.*, 1971; Russel and Edington, 1985; Rueckert, 1996).

At temperature below freezing point, the virus is also stable almost indefinitely. Exposure to 56°C for 30 minutes is sufficient to destroy most strains, although there is some variation

between strains in resistance to temperature and/or pH stress (Geering *et al.*, 1995). Sunlight has little or no direct effect on infectivity; any loss is due to secondary drying and temperature. The survival of airborne virus is mainly influenced by relative humidity with good survival above 60% relative humidity and rapid inactivation below 60% relative humidity (Geering and Lubroth, 2002). The size of droplet aerosol also plays a role in the survival or drying out of the virus, where a droplet aerosol size of 0.5-0.7 μm is optimal for longer survival of the virus in the air while smaller aerosols dry out. In dry conditions the virus also survives longer in proteins, e.g. in epithelial fragments (Donaldson, 1987).

2.2.3. Genomic organization of the virus

The genome of FMDV consists of a positive sense single stranded RNA molecule of about 8500 nucleotides in length, a molecular weight of 2.6×10^6 , and acts as a messenger RNA (mRNA) for the synthesis of virus proteins in the infected cell (Bittle *et al.*, 1982; Quinn *et al.*, 2005; Sangare, 2005).

The genome has a 5' non-coding region (NCR), a single open reading frame, and a short 3' NCR. The 5' non-coding region (NCR) is exceptionally long about 1300 nucleotides and consists of three segments: the highly structured S fragment capable of forming a large hairpin structure, an internal polyribocytidylate (poly[C]) tract of variable length followed by a non-translated segment which is predicted to form pseudo-knots. At the 3' end region the genome has a poly [A] tail of about 50-100 nt. and a short NCR of 89 bases of which the function is not known (Clarke *et al.*, 1987). Moreover, the polyadenylated 3' end region has 3B small virus encoded protein called virus protein genome (VPg) covalently attached to the 5' terminus. The protein 3B occurs in three different forms, 3B1, 3B2 and 3B3. The aphthoviruses are unique in having these three similar, but not identical, VPg encoding genes in tandem (Palmenberg, 1987; Belsham, 1993; Sangare *et al.*, 2003).

The main portion of the virus genome is a single very large open reading frame of 6996 nucleotides encoding a poly protein of 2332 amino acids (for serotype O) (Forss *et al.*, 1984). Based on initial cleavage products, the open reading frame is divided into four distinct regions for poly proteins namely the L, P1, P2 and P3 (Domingo *et al.*, 2002). The L protein represents the leader protein, where two initiation sites (AUG codons) have been identified in FMD virus, called Lab and Lb (Sangare *et al.*, 1988). The P1 gene product is the precursor of

the capsid proteins while P2 and P3 are precursors to non-structural proteins (Ryan *et al.*, 1989; Belsham, 1993).

2.2.4. FMD viral proteins

Structural proteins

The P1 gene product is the precursor of structural capsid proteins 1D, 1B, 1C and 1A and named viral proteins VP1, VP2, VP3, and VP4 (Ryan *et al.*, 1989; Belsham, 1993). The intermediate P1 precursor is initially processed with the help of viral protease 3C^{pro} to produce the four major structural capsid proteins (VP1-VP4) and the mature virion is produced after the encapsidation of the virion RNA (Cooper *et al.*, 1978). The VP1 is an important protein for epidemiological studies of FMD viruses because it is the most antigenic protein involved in cell attachment and carries an immunologically important G-H loop. VP3 is the most conserved surface exposed structural protein among different FMD viruses (Robert and Bruce, 1981; Acharya *et al.*, 1990; Barteling, 2002). FMD viruses have a high concentration of histidine residues lining the pentamer interfaces that also play a major role in virus instability at acidic pH (Stanway, 1990). Heat or acid treatment of the virus disrupts the interactions between VP2 and VP3 and the pentameric interfaces resulting in pentamer dissociation with release of the internal capsid protein VP4 and the RNA genome (Strohmaier and Adam, 1982; Chow *et al.*, 1987; Acharya *et al.*, 1989).

Importance of VP1 protein in the antigenicity of FMD virus

The antigenic diversity of FMD viruses has made the diagnosis and control of these viruses very difficult in countries where the disease is endemic. Several studies have shown that VP1 (encoded by 1D) is the most important protein in FMD virus both for its antigenic properties and as the virus-cell attachment site. Two regions of VP1 have been shown to induce antibodies involved in neutralization of viral infectivity (Bittle *et al.*, 1982; Pfaff *et al.*, 1982; Strohmaier and Adam, 1982). The main immunogenic site exposed on the surface of the virus particle is located at a highly disordered region (G-H loop) of the capsid while the C-terminus antigenic residues are highly ordered (Strohmaier and Adam 1982; Acharya *et al.*, 1989). Since VP1 is the only protein cleaved by trypsin it is believed to be responsible for both immunological and infectivity functions (Bittle *et al.*, 1982). Several studies have shown that mutations at the critical amino acid residues in 1D (VP1) can result in antigenic variations

in FMD viruses. Four non-overlapping neutralization sites and a fifth conformational site in FMD virus were identified (Crowther *et al.*, 1993; Aktas and Samuel, 2000). The main immunogenic site 1 of FMD viruses is located on the 1D region which makes the VP1 region the gene of choice to study variation and relationships between isolates (Kitson *et al.*, 1990; Crowther *et al.*, 1993; Lea *et al.*, 1994).

Importance of VP1 protein in virus-cell attachment

Several studies have reported that the Arginine-Glycine-Aspartic acid (RGD) sequence within the G-H-loop of the VP1 is involved in attachment of the virus to susceptible cell receptors (Lebermann *et al.*, 1991; Leippert *et al.*, 1997). The cleavage of VP1 by trypsin highly reduces or abolishes the ability of the FMD virus to bind and infect susceptible cell cultures (Baxt *et al.*, 1989). Similar work conducted by Liebermann and co-workers (1991) also reported that the highly conserved triplet RGD within the G-H-loop is responsible for binding of FMD virus to pig kidney cell receptors. FMD virus infection of susceptible cells is successfully blocked following the binding of antibodies directed against the RGD region as well as peptides representing part of its sequence. Although, the RGD has been reported to play the main role in cell-virus attachment, it is not the sole element in the binding process since removal of the C-terminus of VP1 in the absence of cleavage within the FMD virus loop also affects the attachment (Fox *et al.*, 1989).

Baranowski and co-workers (2001) also reported at least three different mechanisms for cell recognition by type C FMDV and suggest a potential for this virus to use multiple alternative receptors for entry even into the same cell type. The sequence variation around the virus binding site is responsible for the antigenic diversity of the virus (Fry *et al.*, 1990; Logan *et al.*, 1993). The FMD virus binding site to cell receptors is located within the most exposed site on the virus particle compared to other picornaviruses where it is found in a deep pocket on the virus surface, hidden from immune surveillance (Rossman *et al.*, 1985).

Non-structural proteins

The genome segments P2 and P3 precursors are processed into non-structural proteins which are involved in virus RNA replication and protein processing (Forss *et al.*, 1984; Acharya *et al.*, 1989; Belsham, 1993). The genome segment P2 encodes the non-structural proteins 2A, 2B, and 2C. While, P3 region is a precursor of 3A, 3B, 3C and 3D, respectively (Doel *et al.*,

1978; Ryan *et al.*, 1989). The protein 2A is a protease and cleaves itself liberating the precursor of the capsid proteins whilst 3C is the major protease and carry out the majority of the processing of the polyprotein (Strebel and Beck, 1986; Stanway, 1990). The protein 3D is the RNA-dependent RNA polymerase and is required for the replicative intermediate (RI) stage. The role of the proteins 2B, 2C, and 3A still remains unclear (Sangare, 2005).

2.2.5. FMD virus replication

In the initial event of the replication process, FMD virus uses among other picornaviruses its highly conserved triplet sequence (Arg-Oly-Asp) motif on the G-H loop to attach to specific receptors on the cell membrane (Fox *et al.*, 1989; Mateu *et al.*, 1996). These receptors mediate the release of the viral genome from the protein shell into the cytoplasm. The incoming RNA uses the host cell protein-synthesizing machinery causing shut down of host cell replication. Complementary negative (-) RNA strand synthesis of the positive (+) RNA strand is initiated by a virally-encoded RNA polymerase. Further synthesis of (+) RNA strands leads to the formation of multi-stranded replicative intermediates (RI) with a 3' poly [A] which are transcribed from the poly [U] tract in the RI. The RI generates a pool of (+) RNA for translation and some for synthesis of additional (-) RNA. As the protein level increases, some (+) RNA is packaged into virions (Rueckert, 1985). Proteolytic cleavages occur during shell assembly in a poly protein precursor of structural and non-structural proteins. Complete virus particles are released by cell lysis (Rueckert, 1985; Belsham, 1993). The cycle is completed in 5 to 10 hours (Sangare, 2005).

2.2.6. Antigenic variation

The concept of antigenic variation came from the observation of Vallée and Carré in 1922 that an animal that has recovered from FMD virus infection can be re-infected and develops clinical signs. Subsequently, cross challenge experiments in cattle have allowed the classification of FMD virus into seven immunologically distinguishable serotypes: O, A which exist in Asia, South America and Africa, and Asia-1 and the SAT types (South African Territories SAT-1, SAT-2 and SAT-3) which occur in Asia and sub-Saharan Africa, respectively (Brooksby, 1982). Type C currently occurs only in East Africa (Bastos, 2001).

Serological studies and the observation in the degree of virulence of the virus in recovered animals have shown that there are significant differences between strains within each serotype

(subtypes) (Brooksby, 1982). The study on field isolates of type C using a panel of monoclonal antibodies identified several antigenic groups showing variation in FMD virus epitopes (Mateu *et al.*, 1996). Progress made in the understanding of the genetic differences underlying observed antigenic variation, has played a major role in the epidemiology of the disease. Today, nucleotide sequencing is routinely used to identify the genetic relationships between different isolates and historical strains. In this way the origin of a virus can be traced in outbreak conditions (Sangare, 2005).

Mutation

Antigenic variation can be caused by nucleotide mutations or recombination in the RNA viral genome. Studies revealed that the rates of mutations of the European serotype FMDV RNA genome can reach 10^{-2} substitutions per nucleotide site per year (Gebauer *et al.*, 1988). Similar studies conducted on SAT-1 and SAT-2 FMD viruses have estimated nucleotide changes of 1.64 % and 1.54%, respectively per year for the VP1 gene (Vosloo *et al.*, 1996). This rapid mutation rate is a million times greater than the rates in their natural hosts (10^{-8} to 10^{-9} nucleotide substitution per year) (Domingo *et al.*, 1990). These mutations may give rise to variant viruses that can be a source of new outbreaks (De La Torre *et al.*, 1985 and 1988; Domingo *et al.*, 1990; Vosloo *et al.*, 1992 and 1996). Mutations that lead to conformational changes produce a population of neutralizing escape variants (Stave *et al.*, 1988; Domingo *et al.*, 1992; Sanyal *et al.*, 1997).

Continual modification of the genome of FMD viruses isolated from persistently infected cattle and buffalo have been reported (Malirat *et al.*, 1994; Dawe *et al.*, 1994; Vosloo *et al.*, 1996). Antigenic changes occur following replication of a virulent FMD virus in partially immune populations of cattle and a large number of genetic and phenotypic variants have also been generated after limited replication in cell cultures (Sobrino *et al.*, 1986).

Recombination

The mechanism of recombination in FMD virus has been described as the modification of the surface proteins due to segment crossing over during co-infection of the animal cells by more than one FMD virus serotype (King *et al.*, 1985; Krebs and Marquardt, 1992). Crossing over or reciprocal recombination involves the even exchange of homologous sequences. Non-reciprocal recombination, on the other hand, involves the uneven replacement of a sequence

which results in the loss of one of the variant sequences involved in the recombination event. This procedure has been reported to be responsible for severe types of mutational change that may affect the susceptibility of the natural hosts (King *et al.*, 1982).

Quasi-species concept

RNA viruses have genomes that replicate in the absence of repair mechanisms; they evolve very rapidly with a mutation frequency per nucleotide site of 10^{-3} to 10^{-5} substitutions per year. The high rate of error during RNA replication in the picorna viruses gives rise to a range of multiple co-circulating viral genomes within a host (Van Regenmortel *et al.*, 1997). A dominant virus genome sequence called a master or consensus sequence in regard to the specific environment emerges and competes with the others in the virus population. This is defined as the quasi-species principle of RNA viruses (Holland *et al.*, 1982; Sobrino *et al.*, 1986; Domingo *et al.*, 1992; Van Regenmortel *et al.*, 1997). At a certain time a shift or change in the equilibrium will alter the consensus sequence and cause one of the variants to dominate. FMDV populations exist as mixtures of related but non-identical genomes that can have a competitive potential, and generate a dominant variant in a viral population (Domingo *et al.*, 1992).

2.3. Epidemiology

2.3.1. Global distribution of the disease

FMD has occurred in most parts of the world except Greenland, Iceland, New Zealand and the smaller islands of Oceania. Outbreak was not reported in Australia, USA, Canada and Mexico since 1970, 1929, 1952, and 1954, respectively. Europe has experienced a number of sporadic outbreaks since the cessation of vaccination on the continent during 1990 - 1991 (Samuel and Knowles, 2001; Rweyemamu and Astudillo, 2002). Currently almost all European countries are recognized by the World Animal Health Organization (OIE) as free of FMD without vaccination. The disease is common in most developing nations of South America, Asia, Middle East and it is highly endemic in most sub-Saharan African countries (FAO, 2006; Sumption *et al.*, 2008).

2.3.2. Distribution of the disease by serotypes

Foot and mouth disease serotypes are not uniformly distributed in the regions of the world where the disease still occurs. Africa has the greatest diversity of FMD serotypes. Six of the seven serotypes of FMD virus (i.e. all but Asia-1) are prevalent in Africa. Three of the South African Territories (SAT) serotypes are unique to Africa. Asia contends with four serotypes (O, A, C, Asia-1), and South America with only three (O, A, C). Serotype Asia1 is restricted only to Asia subcontinent and the capacity to invade free areas is common to all serotypes (Donaldson, 1999; Valarcher *et al.*, 2004; FMD Homepage-Maps, 2006; Rweyemamu *et al.*, 2008).

Type O is the most widely prevalent serotype in the world followed by serotype A. South America has had a genetically stable type O virus for nearly the past 50 years under study. Serotype C appears extremely rare and disappearing from the world as a whole with the exception of Kenya and some foci of Brazil in South America probably because of the circulation of this particular serotype extinct in wildlife (FAO, 2006). The last confirmed case was in Kenya in 2005 and the Amazon region of Brazil in 2004. Historically it is the rarest of the FMD types to have occurred in Africa, having been recorded only in three countries, namely Ethiopia, Kenya and Angola (Rweyemamu *et al.*, 2008).

The SAT serotypes have an intimate and probably ancient association with African buffalo (*Syncaerus caffer*) that is instrumental in their maintenance (Vosloo *et al.*, 2002; FAO, 2006). Of the three SAT types, SAT-3 has the most restricted distribution to South African countries, while serotypes SAT-1 and SAT-2 are widespread and occur throughout sub-Saharan Africa and have also made recent incursions into the Middle East (Thomson, 1994; Bastos and Sangare, 2001).

Now a days as the result of globalization, FMD epidemics can change from local and regional spread to wide international spread (Knowles *et al.*, 2005; Cottam *et al.*, 2006). It should be noted that with globalization of trade even areas where FMD is endemic can suffer from introduction of virus strains that are exotic to the region (Brückner *et al.*, 2002). The risk of FMD entry into free areas is low through legal trade of animal and animal products from zones or countries officially recognized as FMD free by the OIE. However, smuggling of animal products is a significant issue and the probable main route of virus introduction into FMD free areas (WRL, 2007).

2.3.3. The disease situation in sub-Saharan Africa countries

The epidemiology of foot and mouth disease (FMD) in sub-Saharan Africa is probably more complicated than in any other regions of the world. Not only six of the seven serotypes prevalent in Africa (only Asia1 has never been recorded), but marked regional differences in the distribution and prevalence of serotypes and intratypic variants occur. Furthermore, wildlife plays a unique and important role in the epidemiology of the disease in Africa although this aspect has been adequately investigated only in Southern Africa (Hedger *et al.*, 1973; Ferris and Donaldson, 1992; Bastos, 1998; Hargreaves *et al.*, 2004; FAO, 2006).

Regardless of the disease endemicity in nearly all countries of sub-Saharan Africa, the majority of outbreaks remains unrecorded and is not notified in a timely fashion due to trade restrictions and pastoral systems where disease surveillance is inadequate and sometimes absent; the transport of sampling material is difficult and expensive, few African laboratories are able to confirm the diagnosis of FMD (FAO, 2006; Rweyemamu *et al.*, 2008). Transboundary live animal movements are part of the main characteristics of husbandry systems in many African regions; drought conditions, refugee movements and frequent cross border trade have probably increased the risk of the entry and spread of FMD. In most pastoral areas of the continent livestock trade is active across the border, dynamic and predominantly driven by price differential and hence, livestock movement (both formal and informal) plays an important role in the spread and epidemiology of FMD (Vosloo *et al.*, 2002; Rweyemamu and Astudillo, 2002; Leforban and Gerbier, 2002; Sahle, 2004).

East African countries are not only having large livestock populations but also the highest concentration of wildlife in the world. Farming is dominated by agro-pastoral and pastoral communities and it is characterized by communal grazing and migrations. This cluster probably contains several major FMD endemic foci and the most complicated FMD situation in the world. There are also wide genetic variations in the virus strains and the role of African buffalo in the maintenance and transmission of FMD serotypes and the role of other potential wildlife reservoirs of the disease that occur in this epidemiological unit have not been systematically studied (Sahle, 2004; Bogale, 2005; FAO, 2006; Rweyemamu *et al.*, 2008).

The situation of FMD in infected areas indicates that FMD types continually spread within endemic regions, and periodically and unpredictably give rise to virus types that break immunity and cause regional epidemics. In this cluster as in other parts of Africa, the use of

vaccines is sub-optimal in relation to the size of population and most of the FMD susceptible animal populations are found at risk. Countries in southern Africa, contrary to the general trend in Africa, have been largely successful in controlling FMD to ensure access to international markets (Sutmoller *et al.*, 2000; Vosloo and Thomson, 2004; Hargreaves *et al.*, 2004).

2.3.4. Susceptible hosts

FMD is highly contagious and affects over 70 domestic and wild life species of animals; however, not all FMD viruses have the same host range (Hedger, 1981; Sáiz *et al.*, 2002). As a whole the sensitive species belong to the mammalian order of *Artiodactyls*. Of the domesticated species, cattle, pigs, sheep, goats and buffalo are susceptible to FMD. Similarly, many species of wild life, such as deer, antelope, wild pigs, Warthogs, elephants, giraffes, camels and llamas, hedgehogs and tapirs may become infected with FMD viruses. Except African buffalo, the importance of other wild life involvement in the epidemiology of FMD is not well studied (Moutou, 2002; OIE, 2004). Human infections have been reported but are extremely rare and mild (Geering and Lubroth, 2002).

The susceptibility of cloven-hoofed species varies with breed of animal and strain of the virus. The disease is considerably less obvious or sub-clinical in breeds of cattle, sheep and goats indigenous to Africa and Asia, where FMD is endemic; and these animals are believed to have been the source of infection for countries previously considered disease free (Kitching, 2002a; Kitching and Hughes, 2002; Kitching and Alexanderson, 2002).

2.3.5. The role of carrier animals

The notion that FMDV could cause persistent infection (i.e., carrier animals) was provoked by observations from FMD outbreaks in the field and the question was fully resolved in 1959 by Van Bekkum and others after the confirmation of the presence of infectious FMDV in oro-pharyngeal (OP) fluid samples of cattle convalescent from infection for many weeks. Persistent infection is defined as animals being virus positive for at least 28 days after infection. It could be induced in cattle, sheep and goats and that live virus could be isolated from the pharyngeal area of such animals. Pigs, however, clear the infection in 3-4 weeks and so do not become carriers (Woodbury, 1995; Salt, 1998).

Regarding wildlife, it is well established that the African buffalo can carry the virus for up to 5 years (Hedger, 1972; Thomson *et al.*, 1984; Condy *et al.*, 1985). Other cloven-hoofed wildlife species including deer and impala, which may become acutely infected, are unlikely to be involved in the carrier problem. The maximum duration of the carrier state in cattle, sheep, goats, African buffalo and water buffalo is 3.5 years, 9 months, 4 months, 5 years and 2-24 months, respectively (Thomson *et al.*, 1984; Bastos *et al.*, 2000).

The target region involved in persistent infection in cattle is the pharynx, more specifically, the dorsal soft palate as well as the dorsal part of the pharyngeal ceiling located above the soft palate. Detection of the viral genome or culturing of the virus from OP fluid samples by using the probang cup from the indicated sites is the most consistent way of identifying carrier animals.

However, no significant histo-pathological changes have been reported to occur in this area even in acute infection (Thomson *et al.*, 1984; Bastos *et al.*, 2000). The mammary gland and testicles as well as the pituitary, pancreas and thyroid have been suggested as potential locations of FMDV persistence sites other than the pharynx (Gurhan *et al.*, 1993; Dawe *et al.*, 1994).

The factors determining the development of persistent FMDV infection are poorly understood; however, the carrier state can develop either after the acute stage or without any clinical disease, for instance, in vaccinated animals exposed to live virus. Nevertheless, in both situations, the main part of the virus replication is controlled, either immediately by the vaccine-induced immunity or after a few days by antibody being produced (Alexanderson *et al.*, 2002). However, the host response is not efficient in clearing the infection in the pharynx and may harbour virus even in animals with antibodies. This may provide a mechanism for the maintenance of the virus in nature and the cause of acute episodes of disease and may contribute to the emergence of new antigenically variant viruses (Doming *et al.*, 1992; Sahle, 2004).

Transmission evidence from carrier animals

Natural as well as experimental transmission of the three SAT serotypes of FMDV from carrier African buffalo to cattle were the best direct evidence so far demonstrated (Dawe *et al.*, 1994; Bastos *et al.*, 1999; Bastos *et al.*, 2000). Transmission from carrier cattle to

uninfected cattle was also indicated in SAT-2 serotype outbreaks (Alexanderson *et al.*, 2002). The risk of transmission from carrier African buffalo to cattle is significant and that transmission from carrier cattle to uninfected cattle cannot be excluded. The virus normally disappears from carrier sheep and goats much sooner than from carrier cattle and hence carrier sheep and goats may be considered as of lower risk than carrier cattle. Trigger conditions of the virus in carriers are yet undefined. Therefore, persistent infections or carrier animals could play an important role in the epidemiology of FMD and has major consequences for the international trade in livestock (Salt, 1993; 1998; Bastos *et al.*, 2000; Alexanderson *et al.*, 2002; Kitching *et al.*, 2007).

Carriers under field conditions

After clinical recovery, up to 80% of ruminant animals may become persistently infected. The prevalence of carriers in a population depends on the species, the incidence of disease (or infection) and the immune status of the population. The prevalence of carrier African buffalo has been described to be very high (50-70%) under free-living conditions (Hedger, 1972; Condey *et al.*, 1985). The detection of carrier domesticated animals can be a relatively frequent finding in endemic areas. For example, a survey in Asiatic Turkey found 15-20% of cattle and sheep to be carriers and a survey for carriers in Brazil after a vaccine breakdown detected around 50% after 6 months (Sutmoller and Gaggero, 1965; Gurhan *et al.*, 1993).

2.3.6. Molecular epidemiology

Phylogenetic analysis of the VP1 region of FMD viruses has been used extensively to investigate the molecular epidemiology of the disease worldwide. The techniques have assisted in studies of the genetic relationships between different FMDV isolates, geographical distribution of lineages and genotypes, and the establishment of genetically and geographically linked topotypes and tracing the source of virus during outbreaks (Vosloo *et al.*, 1992; Bastos, 2001; Bastos *et al.*, 2003; Knowles and Samuel, 2003; Sangare *et al.*, 2003; Sangare, 2005).

Currently, DNA sequencing and phylogenetic trees are widely used to illustrate the genetic relationships between viruses. Sequence differences of 30% to 55% of the VP1 gene were obtained between the seven serotypes of FMD virus while 15% to 20% sequencing difference within each subgroups (genotypes, topotypes) were observed (Knowles and Samuel, 2003).

Since 1987, the analysis of the genetic distance and phylogenetic resolution of the sequence of VP1 encoding gene have provided crucial epidemiological information covering different degree of genetic relationships between field isolates (Samuel *et al.*, 1999). Virus isolates from the same epizootic differ by $\leq 1\%$, viruses belonging to the same epizootics (common origin) differ by $< 7\%$, viruses of the same genotype differ up to 15% and viruses from different genetic lineage, differ by $\geq 20\%$ (Sahle, 2004). Sequence data has also been instrumental in identifying outbreak resulting from improperly inactivated vaccine and for refuting vaccine involvement in outbreaks (Murphy *et al.*, 1999).

2.3.7. Serotypes and subtypes/topotypes

There are seven recognized serotypes of FMDV, namely O, A, C, South African Territories SAT-1, SAT-2, SAT-3 and Asia-1. Recovery from one serotype does not confer immunity against the other six. Hence, viruses were assigned to different serotypes on the basis of lack of cross protection following infection (convalescent animals) or vaccination. Viruses showing partial cross protection were categorized to the same serotype but to different subtype (Mateu, 1995). Within the seven serotypes over 65 subtypes have also been described using biochemical and immunological tests; and new subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. Based on knowledge generated to date, SAT-2 viruses appear to be particularly diverse, with the largest number of topotypes, whilst serotype C, has the fewest on the African continent. The term topotype is used to reflect the presence of genetically and geographically distinct evolutionary lineages (Samuel and Knowles, 2001).

Topotype richness in Africa is thus summarized as SAT-2 > SAT-1 = A > O > SAT-3 > C (Bastos *et al.*, 2001; Reid *et al.*, 2001; Sangare *et al.*, 2001; Sangare., 2002; Vosloo *et al.*, 1992). The importance of subtype/topotype is that a vaccine may have to be mass-produced to the subtype present in the area in which the vaccine is being used (OIE, 2004). At present, a sequence of FMDV is increasingly being used to establish intratypic variations of FMDV and classifying viruses into genotypes and lineages (Sahle, 2004).

2.3.8. Source of infection and mode of transmission

FMD is very contagious because a small dose of the virus is infectious and several routes of FMD virus infection and excretion have been reported. The primary mode of transmission is

via respiratory aerosols since the virus can replicate mainly in the respiratory tract of animals and a large amount of the virus particles are excreted from this area although the virus may occur in all the secretions and excretions of infected animals during the acute phase of infection (Geering *et al.*, 1995; Kitching, 1998).

Other important means of spread is by direct contact between infected and susceptible animals. In densely populated areas the disease may spread extremely rapidly because of the high level of challenges from infected animals. FMD virus also readily spreads indirectly by a variety of inanimate objects including animal food stuff, beddings, farm equipment, livestock holding areas, transport vehicles and so on that have been contaminated with acutely infected animals excretions and secretions such as saliva, milk, faeces and urine. Outbreaks of FMD can occur because of viruses escaping from research and vaccine production centers and the semen of infected bull can be a source of infection by artificial insemination (Fraser, 1991; Radostits *et al.*, 2000).

Personnel handling infected animals can be contaminated on hands, clothes or in nasal passages with live FMD virus and mechanically carry virus to susceptible animals by direct contact (Kitching *et al.*, 2007). A person in contact with infected animals can serve as a source of infection for 24 hours post infection (Bogale, 2005). Dogs, cats, horses and birds can transmit the disease mechanically (Bavor, 1997; Quinn *et al.*, 2005).

The spread of FMD virus by wind over 250 km across the sea and 60 km across the land has been reported in temperate climates during an outbreak of the disease (Kitching, 1992; Geering and Lubroth, 2002). Early recognition of disease, followed by slaughter of infected livestock and the introduction of movement controls reduces the risk of mechanical spread. However airborne spread of the virus cannot be controlled by these means (Kao, 2001; Gloster and Alexanderson, 2004).

Airborne spread of the virus is determined by the numbers and species of animals affected, the virus strain, the environmental conditions, and the species and number of animals located downwind. Infected pigs emit high levels of airborne virus which can be as high as 4×10^6 infectious units per day, but are relatively resistant to infection by the airborne route (Mann and Sellers, 1990; Kitching, 1992). In contrast cattle, infected with FMD virus emit or excrete less airborne virus between 10^3 to 10^8 infectious virus units per day as an aerosol, but are highly susceptible to airborne infection of respiratory route and a dose of 20 TCID₅₀ (Tissue

culture infective dose) of virus is sufficient to establish infection (Donaldson *et al.*, 1987). Sheep and goats excrete airborne virus at levels similar to cattle but are thought to be less susceptible to airborne infection than cattle due to their smaller respiration volume (Gloster and Alexanderson, 2004). The virus is stable in aerosols at a relative humidity above 55-60% and at temperatures below 33°C. Sunlight and the pollution complex termed “the outside air factor” have minimal direct effects on virus survival (Gloster and Alexanderson, 2004).

2.3.9. Clinical signs

The clinical signs of the disease can range from a mild or inapparent in sheep and goats to a severe disease in cattle and pigs. The severity of clinical signs of the disease varies with the strain of the virus, the exposure dose, the age and breed of the animal, the host species and their status of immunity (OIE, 2004). The incubation period of the virus in natural infections is usually between 2 and 3 days and could be as long as 14 days in cattle and lasts for 4 to 8 days in sows. The incubation time may be even shorter in experimental infections. Viremia in cattle lasts for 3-5 days and the lesions develop 2-14 days post infection (Burrows *et al.*, 1981; Donaldson *et al.*, 1987; Kitching, 2002a).

In acutely infected cattle, the initial signs are fever or a rise in temperature (40-40.6°C), dullness, anorexia and marked drops of milk production. Within 24 hours, there appears excessive salivation, the saliva hanging in long, ropy strings and leads to nasal discharge (Quinn *et al.*, 2005). These signs are accompanied by vesicle formation on the dorsum of the tongue, soft palate, dental pads, lips and gums, which result in opening and closing of their mouth with characteristic smacking sound. Vesicles may also be found in areas where there is high friction, such as interdigital space and coronary band of feet with consequent lameness, on nostrils, muzzle and teats (Woodbury, 1995; Sahle, 2004). Pregnant cows may abort, presumably as a consequence of fever (Murphy *et al.*, 1999) and young calves up to 6 months of age may die suddenly before the appearance of any clinical signs of the disease due to virus infection of the developing heart muscle (myocarditis) (Radostits *et al.*, 1994; 2000).

Morbidity can approach 100%, mortality in adult animals is rare and usually less than 1% but it can exceed 50% in young animals (Woodbury, 1995). Most infected animals recover within 2 to 3 weeks although infection may delay recovery of mouth, feet and teat lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight, and breeding problems. A lactating animal may not recover to pre infection production status because of

damage to the secretory tissue. A chronic panting syndrome characterized by dyspnoea, anorexia, and hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (Burrows *et al.*, 1981).

In sheep and goats the clinical signs of FMD are very mild or inapparent (Donaldson and Sellers, 2000). The disease can easily be overlooked until and unless individual animals are carefully examined for disease lesions. In sheep and goats if clinical signs occur, it may include dullness, fever, and small vesicles (0.5-2 mm) or erosions on the dental pad, lips, gums, and tongue (Uppal, 2004). Mild lameness may be the only sign. In lame animals, there may be vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort and nursing lambs may die without showing any clinical sign (Hughes *et al.*, 2002).

In swine, lameness is often the first sign. Other signs include fever about 40-40.6°C, anorexia, reluctance to move, and squeal when forced to move. These signs are followed by formation of vesicles on the coronary bands, heels, snout and in the interdigital space (foot involvement is usually severe). Mouth lesions are not too common and when they occur they are smaller and of shorter duration than in cattle and tend to be a dry-type lesion; there is no drooling; sows may abort; and piglets may die without showing any clinical sign (Radostits *et al.*, 1994; 2000).

2.3.10. Immune response

The FMD virus infection elicits a rapid and broad spectrum humoral and cellular response that will protect against infection with homologous and antigenically related viruses. Even though immunity to FMD virus is thought to be primarily of humoral, there is an evidence of a cellular immune involvement mediated by T-helper lymphocytes and T-cytotoxic cells that plays a significant role in the development of the immune response to FMDV infection (McCullough *et al.*, 1992; Sanz-Parra *et al.*, 1998; Rigden *et al.*, 2002). Infection with one serotype produces complete protection against the same virus serotypes, but there is little or no protection against heterologous viruses (Fraser, 1991).

Infection of susceptible cattle with FMDV results in a rapid rise of serum-neutralizing antibody that can be detected from 4-5 days post-infection and reaching peak at around 28 days. The development of this serum response coincides with resolution of lesions, termination of viremia and the reduction of virus excretion. For successful clearance of virus,

phagocytosis is assisted by antibody-dependent opsonisation of virus (McCullough *et al.*, 1992; Rigden *et al.*, 2002).

Serotype specific immunity is based on the presence of neutralizing antibodies to VP1, which develops 7 to 21 days after infection. Immunoglobulin M (IgM) is most prevalent in the early convalescent serum and is less specific to the different serotypes than immunoglobulin G (IgG), which is produced in the later stage of infection and manifests highly specific reaction. Healing of lesions and a clinical recovery in infected animals would not occur until a few days after IgG1 antibodies have developed. The localized antibodies (IgM and IgA) response occurred in FMD virus infection in the mucosal secretions of the oropharyngeal area of the respiratory tract, the primary site of virus replication (Burrows *et al.*, 1981). These localized specific antibodies in the pharyngeal fluid of cattle develops 7 days after exposure to the virus, while IgG activity reaches peak in serum only 14-21 days after infection (Mulcahy *et al.*, 1990).

Recovered cattle become immune to re-infection caused by the same type of virus, for about one year (Murphy *et al.*, 1994). Host factors such as species, age, breed, health conditions, physiological and immune status of an animal and the viral factors including dose, volume, viral strains can affect the level of immune response to FMD virus infection (Sanz-Parra *et al.*, 1998; Rigden *et al.*, 2002).

Nicholls and his co-workers (1985) reported that calves deprived of maternal antibodies from one week to six months responded as well as, or better than 18 months old cattle to initial vaccination against FMD. In young pigs, the immune system is less mature and can only respond effectively to FMD vaccination 2 weeks after birth and less antibody response to FMD virus infection and vaccination for African Zebu cattle breeds than those in Europe was reported (Francis and Black, 1986).

2.3.11. Pathology

Foot and mouth disease virus may be detected in esophago-pharyngeal fluids and lymph nodes which constitute the site of primary replication after infection by the respiratory tract through inhalation (Burrows *et al.*, 1981).

In cattle, at all sites of predilection a single or multiple vesicles ranging from 2 mm to 10 centimeters can be observed. Initially a small-blanchd whitish area develops in the epithelium; fluid fills the area and a vesicle (blister) is formed; vesicle enlarges and may coalesce with adjacent ones. The vesicles are easily ruptured within 24 hours leaving a raw eroded surface and healing occurs within one to two weeks of rupture. Gray fibrinous coating forms over the eroded area that becomes yellow, brown or green till the epithelium is restored (Woodbury, 1995).

Coronary band inflammation (coronitis) and separation of the skin is seen on the wall of the hoof. The vesicle in the interdigital space is usually large because of the stress on the epithelium caused by movement and weight and animals can lose their hooves in severe cases (Donaldson, 1987). Animals that die may have grayish or yellowish streaking in the myocardium indicating degeneration and necrosis. These findings are known as 'tiger heart'. Skeletal muscle lesions can occur but are rare (Woodbury, 1995). There has also been supportive evidence that FMD virus replicates in the bovine mammary gland and mastitis may occur due to secondary bacterial infection. Moreover, histological studies have revealed the presence of clumps of necrotic secretory epithelial cells in the mammary gland alveolar tissue. A week after the onset of the disease in cattle, an increase in the number of alveoli containing necrotic cells, and luminal exocytosis of all alveoli occurs with concomitant increase in non-secretory areas (Blackwell *et al.*, 1983).

2.4. Diagnosis

The diagnosis of FMD is based on clinical, epidemiological and laboratory observations (OIE, 2004). Any time delay in laboratory tests may hinder the implementation of control measures. Therefore, rapid, sensitive and reliable diagnostic procedures are required. The immediate determination of the specific serotype of field virus and its molecular characterization are also essential in order to administer emergency vaccination with appropriate antigen and to trace the source of outbreak (Re´mond *et al.*, 2002; Sangare, 2005).

2.4.1. Clinical diagnosis

A presumptive diagnosis is justified when a rapidly spreading disease characterized by vesicular lesions on the feet and mouth is observed affecting cattle, pigs, sheep and goats. In

cattle, FMD could be considered whenever salivation and lameness occur simultaneously and when a vesicular lesion is seen or suspected (Radostits *et al.*, 2000). However, clinical diagnosis in sheep and goats is more difficult because FMD is often mild or subclinical. Moreover, diagnosis from clinical signs alone can be complicated by other vesicle forming viral disease of livestock and hence laboratory confirmation must always be sought (Quinn *et al.*, 2005).

2.4.2. Laboratory specimens

Specimens that can be collected for laboratory diagnosis of FMD may vary depending on the diagnostic method applied. Vesicular fluid usually contains the highest quantity of virus and epithelium coverings or flaps each about 0.5 gram from oral, foot or mammary lesions of affected animals, esophageal-pharyngeal fluid from convalescent animals and about 5 ml of blood with anticoagulant from viremic animals in the early stage of the disease onset are appropriate samples of choice for viral isolation (OIE, 2004). Whole blood of about 10 ml for serum antibody detection and from dead animals; about 10 grams of tissue samples from lymph nodes, thyroid, adrenal gland, kidney, spleen and heart are also recommended for viral isolation (Geering *et al.*, 1995). Samples should be collected into a transport medium consisting of equal amount of phosphate buffer and glycerol at pH 7.2-7.6 with antibiotics (Geering and Lubroth, 2002).

2.4.3. Antigen diagnostic tests

There are a number of diagnostic tests recommended for use in support of clinical diagnosis either to detect live virus, virus antigen or viral genome directly for the presence of infection (OIE, 2004).

Virus isolation

The isolation and characterization of the virus is the golden rule for the diagnosis of a viral disease. The isolation and serotype identification of the virus should be done in a virus-secure laboratory to prevent the escape of the agent since the virus is highly contagious and economically devastating (OIE, 2004).

Primary cells like bovine thyroid cells or lamb kidney cells are very sensitive, but laborious to maintain. Established cell lines such as BHK-21 may also be used, but are less sensitive (OIE, 2001). A suspension of epithelial sample is prepared by grinding the sample with sterile sand, using a sterile pestle and mortar with a small volume of tissue culture media and antibiotics. Media is added so that the final volume is ten times that of the epithelial sample, producing a 10% suspension (OIE, 2000). The suspension is centrifuged at 13000 rpm for 10 minutes and the supernatant obtained from the specimens are inoculated onto susceptible cells. The specimen is then incubated at 37°C and examined for cytopathic effect (CPE) 24 to 48 hours post infection. The virus is cytocidal, and its cytopathic effects include cell rounding, cell detachment and destruction and redistribution of internal cellular membranes. If no CPE is observed, the cells are frozen and thawed followed by 2 blind passages on fresh cell culture before they are declared negative for the presence of the virus (Kitching, 1998; Geering and Lubroth, 2002; Clavijo and Kitching, 2003). The virus may also be isolated by inoculation of tissue suspension in unweaned mice (Geering and Lubroth, 2002).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Due to the rapid spread of FMD virus and the tremendous economic consequences of the disease, it is essential to have a diagnostic test that is sensitive, accurate, rapid and easy to use. Recent advances in molecular biology have resulted in the development of the nucleic acid recognition method called Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). The test can be used to amplify the RNA genome fragment of FMD virus in tissue culture fluid or in clinical samples. Specific primers have been designed to distinguish between each of the seven serotypes and *in-situ* hybridization techniques have been developed for investigating the presence of FMD virus RNA in tissue samples (Woodbury *et al.*, 1995).

Unlike many living organisms where the hereditary information is enclosed within a DNA genome, FMD virus has a RNA genome that can be sequenced directly, but RNA is unstable and is usually first transcribed into cDNA prior to performing the nucleotide sequencing (Sangare, 2005). Reverse transcriptase when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes. Pairs of oligonucleotide primers are chosen to flank the DNA region of interest that is amplified by a Taq DNA polymerase enzyme. Following cycles of DNA denaturation by heat, primer annealing by cooling, and strand extension with a thermo stable enzyme such as Taq polymerase, DNA is synthesized

from a very small amount of template. In epidemiological studies of FMD virus, nucleotide sequencing of the VP1 gene has been used extensively to determine the relationship between the field isolates. The technique is also routinely used to investigate genetic variation, molecular evolution in carrier animals, and to identify the source of infection in outbreak conditions (Vosloo *et al.*, 2002).

Subsequent improvements in PCR technique have reduced the time required for viral detection and for the accurate characterization of FMD viruses from diagnostic samples (Locher *et al.*, 1995; Bastos, 1998). The test is extremely sensitive due to the requirements for only small quantities of template and the possibility of making multiple cycles of PCR. Specificity can also be high as only targeted viral RNA regions are amplified. In the end, the test supplies genetic material suitable for sequencing thus providing the most detailed epidemiological information for tracing the origin of an outbreak (Reid *et al.*, 20001).

Enzyme-linked immunosorbent assay (ELISA)

The test enzyme-linked immunosorbent assay (ELISA) was developed with a number of applications which include the detection of antigen, and antibody (Hamblin *et al.*, 1984). Later, the technique was improved by making of high-titer serotype-specific antisera, by the use of inactivated 146S virus antigens which give less cross-reaction and a higher sensitivity compared to the infective virus, and the establishment of a positive/negative threshold (Roeder and Le Blanc Smith, 1987; Hamblin *et al.*, 1987). Currently, two procedures are used, the sandwich ELISA for antigen detection (Roeder and Le Blanc Smith, 1987) and the liquid phase blocking ELISA for antibody detection (Hamblin *et al.*, 1986); both tests are routinely used in the diagnosis of FMD.

Sandwich ELISA

In the sandwich ELISA, the plates are coated with rabbit antisera (capture sera) to each of the seven serotypes of FMD virus (OIE, 2000). Test samples as well as controls are added to the plates. Guinea-pig-antisera to each of the serotypes of FMD virus are added to the corresponding wells followed by rabbit anti-guinea-pig serum conjugated to an enzyme. Plates are washed between each step to remove unbound reagents. A color development after the addition of a substrate indicates a positive reaction (OIE, 2000). The sandwich ELISA is used to study antigenic relationships of field isolates and the selection of vaccine strains.

Cross-reactions between serotypes occur in ELISA and can be overcome by the optimization of all reagents. Unlike the monoclonal antibodies, the use of polyclonal antibodies can detect smaller antigen concentrations and makes the technique more effective for detecting and typing of FMD virus (Alonso *et al.*, 1992).

2.4.4. Antibody detection tests

FMD virus infection can be diagnosed by the detection of a specific antibody response. The tests generally used are virus neutralization test (VNT) and ELISA (Hamblin *et al.*, 1987). Serological tests are particularly useful for the surveillance following an outbreak to identify silent infections in species like sheep and goat, which show little or no signs of disease. In recent years, emphasis has also been put on the differentiation of vaccinated animals from those infected, including animals which may have had contact with infectious virus after vaccination and may become persistently infected. The non-structural proteins (mainly proteases and RNA polymerases) expressed during the viral replicative cycle elicit an antibody response in infected animals which theoretically is not present in animals vaccinated with purified viral particles (Rémond *et al.*, 2002; Bruderer *et al.*, 2004).

Virus neutralization test (VNT)

For years, the conventional means of serological surveillance has been through virus neutralization test (VNT). The test relies on the detection of antibodies to structural, capsid proteins (VP1-VP4) of FMD virus (Rémond *et al.*, 2002). The virus neutralization test is based on the principle that serum specific neutralizing antibodies bind to the virus and then block the virus from entering cells in cell cultures. The test is serotype specific but it requires cell culture facilities and takes 2-3 days to provide results. The virus suspension (100 TCID₅₀) is mixed with a serum sample incubated and inoculated onto the susceptible cell cultures. The growth of virus in cell cultures is manifested by cell destruction or cytopathic effect (CPE). No CPE confirms the presence of FMDV antibodies in serum samples (Bruderer *et al.*, 2004; Robiolo *et al.*, 2006).

Liquid phase blocking ELISA (LPB-ELISA)

The blocking ELISA detects and quantifies specific FMD antibodies across all the serotypes in both infected and vaccinated animals (Hamblin *et al.*, 1987; McCullough *et al.*, 1992). The LPB-ELISA had been adopted by a large number of laboratories worldwide (Mackay *et al.*, 1998) to replace the VNT for routine screening because it is quicker, more reproducible, correlates well with the VNT and does not suffer from the biological variability that is inherent in VNT (Mackay *et al.*, 1998). However, the test is not without its problems. The percentage of animals giving false positive results varies from up to 4% in normal unvaccinated cattle to 18% in those which are stressed. For this reason, it is usually recommended that the low positive LPB-ELISA results are confirmed by VNT, particularly for the purpose of international trade (Esterhuysen *et al.*, 1995; OIE, 2000; OIE, 2001).

3ABC- ELISA

Non structural proteins (NSPs) (2C, 3AB and 3ABC) are exclusively synthesized during the FMDV replication cycle. Thus, detection of antibodies against the non structural proteins helps to discriminate infected from vaccinated or naive animals. Of the NSPs the polyprotein 3ABC is the most immunogenic and has been extensively studied (Bruderer *et al.*, 2004). Therefore, antibody to the 3ABC is only found in virus-infected animals but not in vaccinated animals and it is considered as a useful indicator of FMD virus infection regardless of the serotype involved (De Diego *et al.*, 1997; Mackay *et al.*, 1998). The immune response to 3ABC appeared early after infection and antibody to 3ABC could be detected for longer than antibody to any other NSP (Mackay *et al.*, 1998). It was also reported that the presence of antibody to one or more of the other NS proteins (2C, 2A, 3D) is an indication of previous infection (De Diego *et al.*, 1997; Mackay *et al.*, 1998).

2.4.5. Differential diagnosis

Diagnosis based on clinical signs alone is complicated by the fact that at least two other viral disease of livestock, vesicular stomatitis (VS) and swine vesicular disease (SVD) produce lesions which are identical to those of FMD. Horses are affected by vesicular stomatitis but not by FMD or SVD. Swine vesicular disease affects only pigs. Lesions induced by bovine papular stomatitis, bovine herpes mammillitis, infectious bovine rhinotracheitis, bovine mucosal disease, malignant catarrhal fever, rinderpest in cattle, bluetongue, parapox-virus,

peste des petits ruminants and footrot in sheep may also be mistaken for FMD and all these listed diseases should be considered during the diagnosis (Re´mond *et al.*, 2002).

2.5. Economic impact of FMD

Foot and mouth disease (FMD) remains one of the most important livestock diseases of the world, given its highly infectious nature, its broad economic impacts on animal wellbeing and productivity, and its implications for successful access to domestic and export markets for livestock and products (Perry *et al.*, 2006; Perry and Rich, 2007). The impacts of the disease vary markedly between developed and developing countries, and also within many developing countries depending on the species involved, the genotype of animal, the level of productivity, the significance of livestock to livelihoods, and the effectiveness of indigenous coping mechanisms for controlling the effects of FMD (Perry *et al.*, 2002b; Catley *et al.*, 2004).

At farm or individual level, the direct effects of an outbreak of FMD in terms of morbidity, mortality, expenditure to treat the disease, and measures taken to avoid further spread can substantially lower farm incomes, particularly in susceptible highly productive animals (James and Rushton, 2002). Overt clinical disease affects animals' performance directly and causes reduction of milk yield in dairy cattle by 20%, growth rate of beef animal by 10-20%, pig meat production up to 20% per annum and inflict fertility impairment due to increased abortion rate up to 10% (Doel, 2002). Moreover, death of very young animals, culling of unproductive and chronically infected animals, and loss of valuable breeding stock and disruption of livestock improvement programs are also attributed to the direct effect of the disease (Perry *et al.*, 1999; Catley *et al.*, 2004).

In less commercialized production systems in Africa and Asia where indigenous breeds predominate, these direct effects may be minor (Randolph *et al.*, 2002). However, livestock often serve multiple purposes in these systems, such as providing draught power, so that the temporary lameness reported up to 60-70% of working buffalo and cattle caused by FMD can also lower farm income by reducing crop production or transport services. Furthermore, if the disease affects livestock kept by the poor who have few such productive assets, the impact on their livelihoods can be especially devastating and similarly pastoralists severely suffer by the direct impact of the disease since their livelihood directly depend on livestock production (Perry *et al.*, 2002a; Doel, 2002; Rufael *et al.*, 2008; Bayissa, 2009).

From a current global perspective, the indirect effect and the risk of the disease has a much greater impact than that of the disease itself. Much of the burden (cost) of containing outbreaks, as well as maintaining the ongoing capacity and measures intended to reduce the risk of FMD such as vaccination, surveillance, movement control and so on can incur an extremely high additional economic cost (Kitching *et al.*, 2006). In developed nations containment measures especially stamping out policies are seen to be associated with animal welfare and environmental impacts that increasingly draw attention. Measures taken to contain an outbreak can also affect other sectors, such as the impact on tourism in the recent UK outbreak (Thompson *et al.*, 2002). The presence of FMD has also a negative impact on the integration of livestock and wildlife based activities, which is believed by many to be ecologically and financially desirable. Thus the disease can exert considerable influence on the development of integrated land use policies in South African countries (Sutmoller *et al.*, 2000).

Currently, the single largest impact of FMD is undoubtedly its critical role as a restriction to international trade. The highest value markets for livestock products are in FMD free countries, and these countries are allowed to restrict or ban imports of livestock products and sometimes other products as well as a potential risk of introducing FMD (Gulati *et al.*, 2005). The presence of FMD in a country is considered as binding constraint to developing an export sub-sector within the livestock industry, and, therefore, contributes to unavoidable loss. Unfortunately, FMD is widely distributed in the developing world, in particular Africa, South America, south East Asia, regions of the world that support 75 per cent of the world's poor and thus hampering utilization of their huge livestock resources for economic development and poverty alleviation (Rweyemamu and Astudillo, 2002; Rich and Winter, 2007).

2.6. FMD control and prevention

2.6.1. Control rationale

Achieving the first goal of the Millennium Development Goals to reduce poverty by half by the year 2015 will require sustained growth by developing countries, and much emphasis has been placed on the importance of economic growth in the processes of poverty reduction (Bourguignon, 2004). At the same time, poverty reduction is a complex process, with no single 'silver bullet' solution. The complexities include the need for action at many levels,

ranging from national-level policies that promote economic growth and equity, down to infrastructure development and technological innovations targeted at the priorities of the rural farmer. In this view control and eradication of FMD can prevent huge amount of economic loss and hence it contributes indispensable values for poverty reduction particularly in developing countries through effective livestock resource utilization (DFID, 2004).

Therefore, FMD control can be an important component of poverty reduction strategies for livestock enterprises of many but not all developing countries, depending on the competitive advantage held by the country in livestock resources, on the potential for engagement in export markets for livestock products, on the role of livestock in livelihoods, and on the importance of FMD relative to other diseases (Ravallion, 2004; Fuentes 2005; Scoones and Woolmer, 2006).

Moreover, effective control and implementations of appropriate strategies are a precondition for linkages to international markets for the reduction of risk of the disease spread. On the other hand, for pastoralists who are not integrated with markets or have the potential to be so, FMD control may open up new opportunities that diversify income sources and improve market access. A second case is where farmers were relied on livestock as a source of traction and nutrition (in the form of meat and milk), FMD control has a number of positive market and non-market benefits for producers: healthier animals provide a stable source of draught power, and also possible additional income through the renting of animals to other farmers; increased household milk production allows producers to spend scarce resources on other necessities; and reduced vulnerability (Perry *et al.*, 2002a; Randolph *et al.*, 2002).

2.6.2. Global control scenario

The control of FMD varies markedly between endemic and disease free classes of the world, and also within many developing countries due to the impact differences posed by the disease. These discrepancies in impact shape some markedly heterogeneous incentives for FMD control and eradication, which become of particular importance when setting priorities for poverty reduction in developing countries (Perry and Rich, 2007). In most developing countries on top of other epidemiological factors, the demands for its control or eradication are complicated by the presence of other competing animal disease constraints with a higher direct impact on livestock enterprises, and by competition for the financial resources and infrastructure necessary to control the disease. Furthermore, the lack of infrastructure, human

resources and movement controls in many developing countries render them particularly vulnerable to the spread and poor control of the disease (Perry *et al.*, 2002b; Sangare, 2004b).

2.6.3. Control strategies in endemic regions

Vaccine and vaccination

All FMD vaccines are produced by growing live virus in BHK-21 cells in roller bottles or suspension under bio-secure conditions in large volumes. The virus is harvested, concentrated and inactivated, and mixed for use with a buffer adjuvant, either oil or aluminum hydroxide or saponin (Cox *et al.*, 2006).

The first aim of disease control is local reduction of the incidence of infection and thus in endemic areas, the disease can be controlled by combinations of vaccination, movement restriction and regular inspection of susceptible animals (Woodbury, 1995; Radostits *et al.*, 2000; Quinn *et al.*, 2005). The choice of vaccine should be based on sound epidemiological and virological information pertinent to the areas where disease is to be controlled. Vaccines must be formulated taking into account the virus types and subtypes prevalent in the area; therefore, frequent checking of the serotypes and subtypes during an outbreak is also needed because FMDV frequently changes during natural passage through various species (Gonzalez *et al.*, 1992).

Cattle are normally vaccinated twice, starting at age when any maternally derived immunity will no longer interfere with the development of active immunity, and then every 4 or 6 months, depending on the likelihood that they will be exposed to the infection. For proper protection, calves originating from vaccinated and unvaccinated dams should be vaccinated at about 6 months and 2 weeks of age, respectively (Gerland, 1999). Vaccination programmes must ensure that no less than 85% of the cattle population is vaccinated at intervals sufficiently short to maintain constant immunity (Bogale, 2005). Following the first dose of vaccine, immunity will develop only after 4-5 days and in ruminants primary vaccination induces immunity that lasts for about 3-6 months. Therefore, frequency of vaccination varies from one to three times a year in different countries (Radostits *et al.*, 2000; Cox *et al.*, 2006).

FMD vaccines will not prevent infection in cattle exposed to live virus, and may not prevent disease if the vaccine strain is antigenically different from the field virus or the exposure to

2.6.4. Control strategies in disease free regions

Stamping out

In disease free regions the main objective is to prevent the introduction of infection, through strict implementations of all important preventive measures. But when the disease occurs in FMD free country, stamping out is the first option to eradicate the disease (Fraser, 1991; Suttmoller *et al.*, 2003).

Stamping out consists of the killing and disposal by burning or burial of all susceptible livestock on infected farms and their immediate contact farms that are most likely infected. Here, quarantine of the premises (including movement of personnel and vehicles), rapid diagnosis and decontamination of the premises are essential. This measure is accompanied by compensation payment to livestock owners (Radostits *et al.*, 2000). The use of this strategy to combat the outbreak of FMD that occurred in UK in 2001 has stimulated a larger debate. This proved the reassessment of the policy and re-consideration of vaccination as part of alternative and more flexible FMD control strategy, that the Netherlands adopted emergency vaccination to control the outbreak in 2001 (Rossides, 2002). The objective of this emergency FMD vaccination is to provide protective immunity, as rapidly as possible to susceptible stock and to reduce the amount of virus released, and thereby limit the spread of disease (Bogale, 2005).

The eradication of FMD in sub-Saharan Africa by means of test and slaughter is impractical because of the vastness of the region and limitation of infrastructures. Slaughtering out would necessitate testing, identification and destruction of large number of animals, requiring the mobilization of large numbers of field staff, well established diagnostic laboratory facilities and compensation funds (Hunter, 1998).

2.7. Foot and mouth disease situation in Ethiopia

2.7.1. Disease status

In Ethiopia, foot and mouth disease is endemic and a notifiable disease; the national animal and plant health regulatory directorate sends monthly and annually official reports to OIE (Leforban, 2005; MoARD, 2009). The disease is widely prevalent and previously used to

occur frequently in the pastoral herds of the marginal lowland areas of the country. However, this trend has been changed and currently the disease is also frequently noted in the highlands of the country (Sahle, 2004). The national picture of FMD status by summarizing the outbreak data reported to MoARD in the years 1999-2008 is presented in figure 1.

2.7.2. Spatial distribution

FMD is widely distributed in all areas of Ethiopia, although the level of the disease prevalence may show significant variations across the different farming systems and agro-ecological zones of the country.

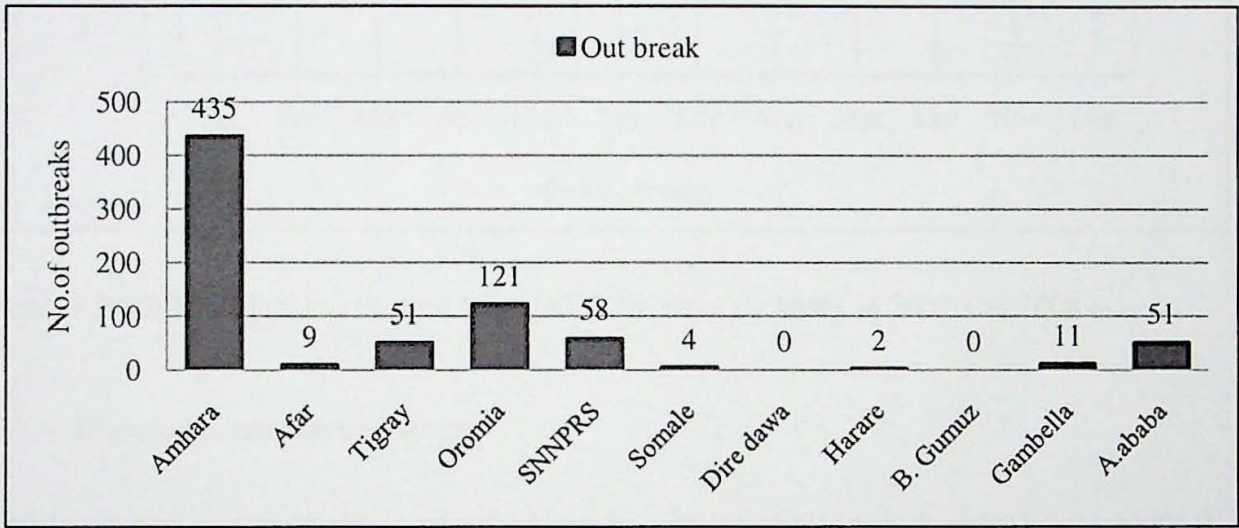


Figure 1: FMD outbreaks reported to MoARD during 1999-2008 by Regional States

2.7.3. Temporal distribution

According to MoARD, division of animal and plant health regulatory directorate disease outbreaks report summary, FMD occurs at any time of the year however, the highest outbreaks of the disease are observed during the heavy rainy and extreme dry seasons of the years, respectively (Figure 2). Various researchers reported that this might be associated with factors such as drought. During dry seasons especially pastoralists are obliged to move their herds long distances in search of pasture and water and thereby transmission of highly contagious diseases like FMD exacerbated at herd gathering sites or communal points (Rufael, 2006; Legesse, 2008; Molla, 2009; Bayissa, 2009). On the other hand in most rural parts of the country during rainy seasons of the year wide areas of farm land is planted with

crops. During this time huge numbers of domestic animals are kept on confined small plots of communal grazing lands that could favor occurrence and transmission of the disease (Personal observation).

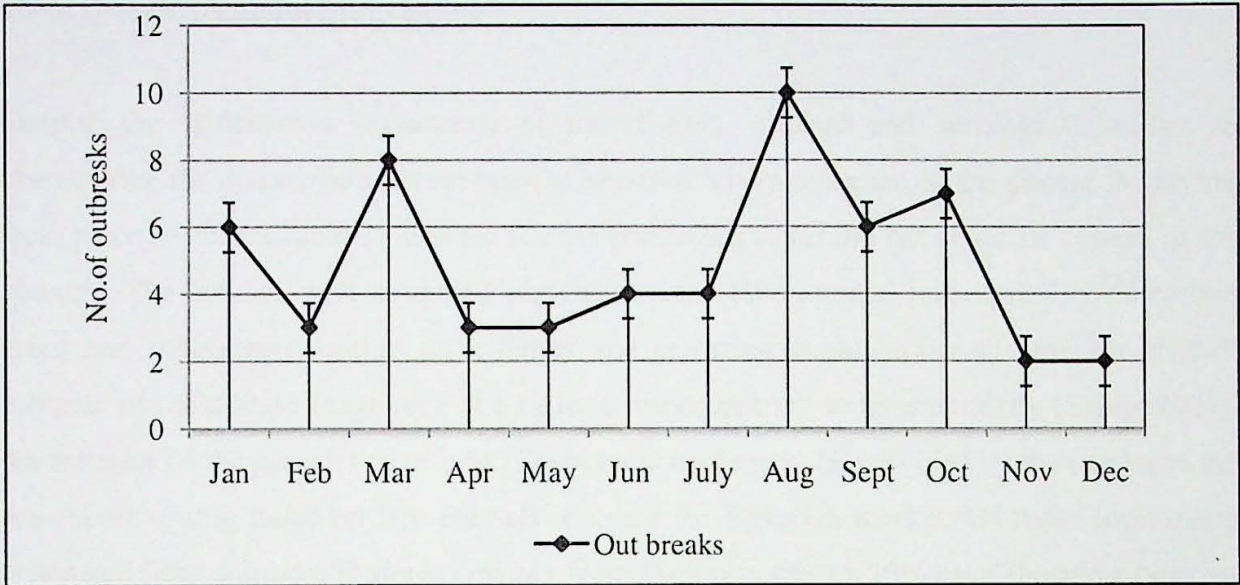


Figure 2: FMD outbreaks reported to MoARD by monthly bases in 2007 and 2008

2.7.4. Disease occurrence frequency

FMD outbreaks are reported frequently throughout the country (Asfaw and Sintaro, 2000). In the past ten years (1999-2008) on average over 77.7 numbers of FMD outbreaks were reported to MoARD annually. The outbreaks occurred every year, but most were reported in 1999 and 2001 each 176 outbreaks and in 2004, 134 outbreaks, respectively (Table 1). However, considering the reporting rate (35%), the figures provided are definitely underestimated and do not reflect the reality of the epidemiological situation in the country due to endemic nature of the disease and the unreported cases by farmers (Sahle, 2004; MoARD, 2009).

Table 1: Numbers of FMD outbreaks reported to MoARD during 1999-2008

Year	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Average
Total outbreak	176	84	176	60	27	134	26	36	35	23	77.7

2.7.5. Disease magnitude/prevalence

Despite the widespread occurrence of the disease, clinical and serological studies to characterize the disease have never been exhaustive. The prevalence of the disease is varying from place to place (Table 2), and the studies conducted so far did not cover all corners of the country. The lack of well equipped regional animal laboratories, inaccessibility of certain areas and suboptimal routine surveillance and reporting could hinder to have the overall estimate of the disease magnitude at a national view contrary to its endemicity (Sahle, 2004). An estimate of the prevalence of infection in local cattle may be provided by the results of the pre-export testing made on live animals destined for Egyptian market. Of those local cattle originated from southern Ethiopia (mainly from Borana zone) 15-20% have showed a positive result to ELISA NSPs test indicating that the animals have been infected by FMD virus and for this reason rejected from export (MoARD, 2006).

Table 2: Overall sero prevalence reports of FMD in different parts of the country

Location	No. of cattle tested	Sero-positive	Prevalence (%)	References
Borana	920	193	21.0	Rufael, 2006
Afar	765	43	5.60	Jembere, 2008
Borana	768	177	23.0	Bayissa, 2009
South Omo	770	63	8.20	Molla, 2009

The prevalence estimates of FMD during outbreak investigation were found to be high. The overall sero-positivity of 66.7% in cattle, 9.3% in sheep and 7.2% in goats (Legesse, 2008) and 44.20% in cattle (Nigussie, 2009) were reported after laboratory diagnosis from blood samples collected during the early outbreak investigations of the disease.

2.7.6. FMD virus serotypes identified

Research findings and records from National Animal Health Diagnostic and Investigation Centre (NAHDIC) and National Veterinary Institute (NVI) of Ethiopia indicate that five of

the seven FMDV serotypes (O, A, C, Southern African Territories SAT-1, and SAT-2) were identified in Ethiopia and the isolated serotypes were responsible for FMD outbreaks during 1974-2007. In terms of species, these serotypes were identified from bovine, swine, ovine, and caprine samples collected from the outbreak areas. Cattle were found to be infected with all circulating serotypes of FMDV, where as swine had only serotype O (Sahle, 2004; Gelaye *et al.*, 2005; Legesse, 2008; Nigussie, 2009; Ayelet *et al.*, 2009).

During the period of 1957-73, 62 outbreaks of serotype O, 24 of serotype C and 12 of serotype A were recorded (Berson *et al.*, 1972). Following this period, until 1992, FMD outbreaks due to serotypes O and A were common (Haileyesus, 1988; Roeder *et al.*, 1994). Serotypes O and SAT-2 viruses were identified as the major causative agents of FMD during 1988-1991 (Roeder *et al.*, 1994). FMDV serotypes O and C were first recorded in Ethiopia in 1957 while serotype A was identified in 1969 (Martel, 1974; 1975). Serotype C was not identified after 1983. It seems to have disappeared from Ethiopia. However, a recent report of serotype C specific antibodies in cattle in Ethiopia indicates that circulation of serotype C viruses in the country may have gone unnoticed (Rufael *et al.*, 2008).

The first isolation of SAT-2 was in 1989 in a sample collected from cattle raised on Leben Ranch, Borena Zone, in southern Ethiopia (Roeder *et al.*, 1994); the virus was detected for the next 2 years in 1990 and 1991. After an apparent gap of 16 years, serotype SAT-2 was recorded in 2007 from a bovine sample collected from Bambas, Benshangul-Gumuz, western Ethiopia bordering Sudan (Ayelet *et al.*, 2009). Recently the presence of serotype SAT-2 specific antibodies in cattle in Ethiopia has been reported (Rufael *et al.*, 2008). SAT-2 may have been introduced by animal movement across the border with Sudan because SAT-2 is endemic in Sudan (OIE, 2001; Vosloo *et al.*, 2002).

The presence of FMDV serotype SAT-1 in Ethiopia was isolated and reported for the first time by Legesse, in 2008, from three species of animals; cattle, sheep and goats. Although SAT-1 has not been previously reported in Ethiopia it might be circulating within wildlife and infrequently transmitted to domestic animals (Martel, 1974; 1975; OIE, 2001). FMDV recovered from the outbreaks in Ethiopia during 1981-2007 witnessed that serotype O (73.30%) was most prevalent followed by types A (19.50%), SAT-2 (4.10%), SAT-1 (1.80%) and C (1.40%), respectively (Gelaye *et al.*, 2005; Gelaye *et al.*, 2007; Ayelet *et al.*, 2009).

2.7.7. Impact of FMD under Ethiopian condition

The most direct economic impact of FMD is the loss or reduced efficiency of production, which reduces farmers' incomes. In Ethiopia, where the local economy is heavily dependent on livestock, the burden may be severe and local food security impaired (MoARD, 2006). The impact of reduced productivity of animals can be long lasting and diseases can have lasting effects on livestock output in a number of "hidden" ways (such as delays in reproduction leading to fewer offspring and the consequences of a reduced population) which often exceed the losses associated with clearly visible illness. At the local level, FMD reduces farmers' income and food availability for consumption. At the national level, FMD slows economic growth by severely limiting trade opportunities (Bogale, 2005).

Morbidity rate associated with the disease was found to be high although mortality of less than 1% was reported. Exotic and cross breed cattle were found more affected than indigenous ones and high death rate was recorded in young stock (Legesse, 2008; Nigussie, 2009). In Ethiopia, over 95% of draught power for crop production originates from animals (Sahle *et al.*, 2004). However, FMD outbreak affects draft oxen working efficiency during the planting season and causes heavy losses in small scale mixed farming system. It causes considerable losses of milk yield and weight gain among dairy and fattening stock (Sahle, 2004; Bogale, 2005).

Data summary of the last 7 years (2002-2008) obtained from MoARD division of agricultural marketing directorate indicates that Ethiopia earns on average 102.2 millions USD every year from exporting live animal and animal products (Table 3). However, the impact of the disease in affecting the national export trade has been witnessed by import bans imposed by different countries at different times. Following the 2001 outbreak of FMD in the United Kingdom, Saudi Arabia and Indonesia had imposed trade bans of our export meat and pickled sheep and goat skin, respectively. Therefore, FMD causes substantial economic loss to farmers and to the nation from embargoes of livestock and livestock product trade (Megersa *et al.*, 2009). The 2006 FMD outbreak detected in Egypt also became the major cause of live cattle import ban to the Egyptian markets (MoARD, 2006).

Table 3: Animal and animal product exported and foreign currency earned (2002-2008)

Items	Mean volume exported per year	Mean values earned in millions of USD per year
Meat and meat products	5,575.6 tons	15.1
Skin	7,745.7 tons	55.4
Hide	4, 381.9 tons	6.6
Animal product	461.3 tons	0.3
Live animals	152,179.9 heads	24.8
Total		102.2

Source: MoARD, agricultural marketing directorate (2009)

2.7.8. FMD control program in Ethiopia

At present, FMD is considered as one of the most important livestock diseases demanding urgent control intervention that should result in minimizing the impact of FMD to the level that won't be a major cause of international trade barrier. Conversely, the complex nature of the disease, its wider distribution across the country and absence of proper kind and amount of FMD vaccine within Ethiopia demanded that control strategies be implemented progressively on a short and medium to long-term basis. Measures such as disease free zone establishment and mass vaccination of the national cattle herds may have important contributions to minimize the impact of the disease. However, these measures will require huge financial and logistic resources that their consideration should be viewed from long term perspective (MoARD, 2006).

Therefore, the short term FMD vaccination program give emphasis to the control of all outbreaks occurring in the country through ring vaccination and vaccination of all export cattle before entering the quarantine stations. To protect export animals from contracting the disease while being kept in quarantine sites cattle found within 10 km radius of these sites could be vaccinated. All dairy animals should also be vaccinated (MoARD, 2006). Currently, a bivalent vaccine manufactured from locally isolated FMDV serotypes A and O is produced by the NVI. The virus is propagated from cell culture and absorbed into aluminium hydroxide gel and inactivated with 0.3% formaldehyde and adjuved with saponin. The recommended dosage and route of administration of the vaccine is 4 ml per head of cattle subcutaneously, preferably in the dewlap region. The first vaccination requires two injections at 6 months of

interval. Immunity develops 2-3 weeks after vaccination and may last for one year (DACA, 2006).

3. MATERIALS AND METHODS

3.1. General overview of the study region

The Gambella People's National Regional State (GPNRS) is located in the extreme end of southwestern part of the country, about 777 kilometers west of Addis Ababa, between the geographical coordinates of 6°28'38" to 8°34' North Latitude and 33° to 35°11'11" East Longitude. The total land area of the region covers about 34,063 km² which is equivalent to 2.78% of the national land areas. Gambella borders the regions of Benshangul-Gumuz in the north, Oromia in the northeast, SNNPRS in southeast and the remaining western and southwestern portion is bordered with Sudan republic (GRBoFED, 2007).

Administratively, Gambella Regional State is sub-divided into three zones and a city council, which is further divided into 12 Districts and 220 *Kebeles*/peasant associations which form the basic administrative or social units of the region. The major indigenous nationalities in Gambella are of Nilotic origin and include: Mejengir, Anyuwa, Nuer and minority populations of Komo and Oppo. Based on the estimation of CSA (2008), the human population of the region is 306,916, constituting only 0.4% of the countries' total population. The population density of the region is around 7.4 people per km².

As elsewhere in the country more than 85% of the population of Gambella depends on agriculture and animal husbandry in the rural settings. According to the national agricultural sample survey of CSA (2008), the livestock population of the region enrolls 212,59 cattle, 48,114 sheep, 54,638 goats, 336 equines, 173,844 poultry and 35,122 beehives.

The region is categorized into three agro-ecological zones, the mid-highland, lowland and semi-arid agro ecological zones. The elevation ranges from 350-500 masl in the western and from 500-2000 masl in the eastern and southeastern part of the region. The precipitation fluctuates from 900-1500 mm to 1900-2100 mm with increase of elevation under normal conditions. A quarter of the region is estimated to be covered with forest and known for its diverse fauna and flora (NMAGBO, 2009).

3.2. Description of the study area

3.2.1. Location

The present study was conducted in four selected Districts of Gambella People’s Regional State. Two of the Districts, namely Gambella and Abobo, are found in the lowland agro-ecological zone of the region, while Itang and Lare Districts are categorized in semi-arid zone. The District towns’ are located 777, 822, 827 and 867 kilometers away from the national capital city and measures an altitude of 452, 470, 426 and 413 masl, respectively. Geographical location and map of the study Districts and point locations of sampled *Kebeles*/PAs are indicated on Figure 3 and 4, respectively.

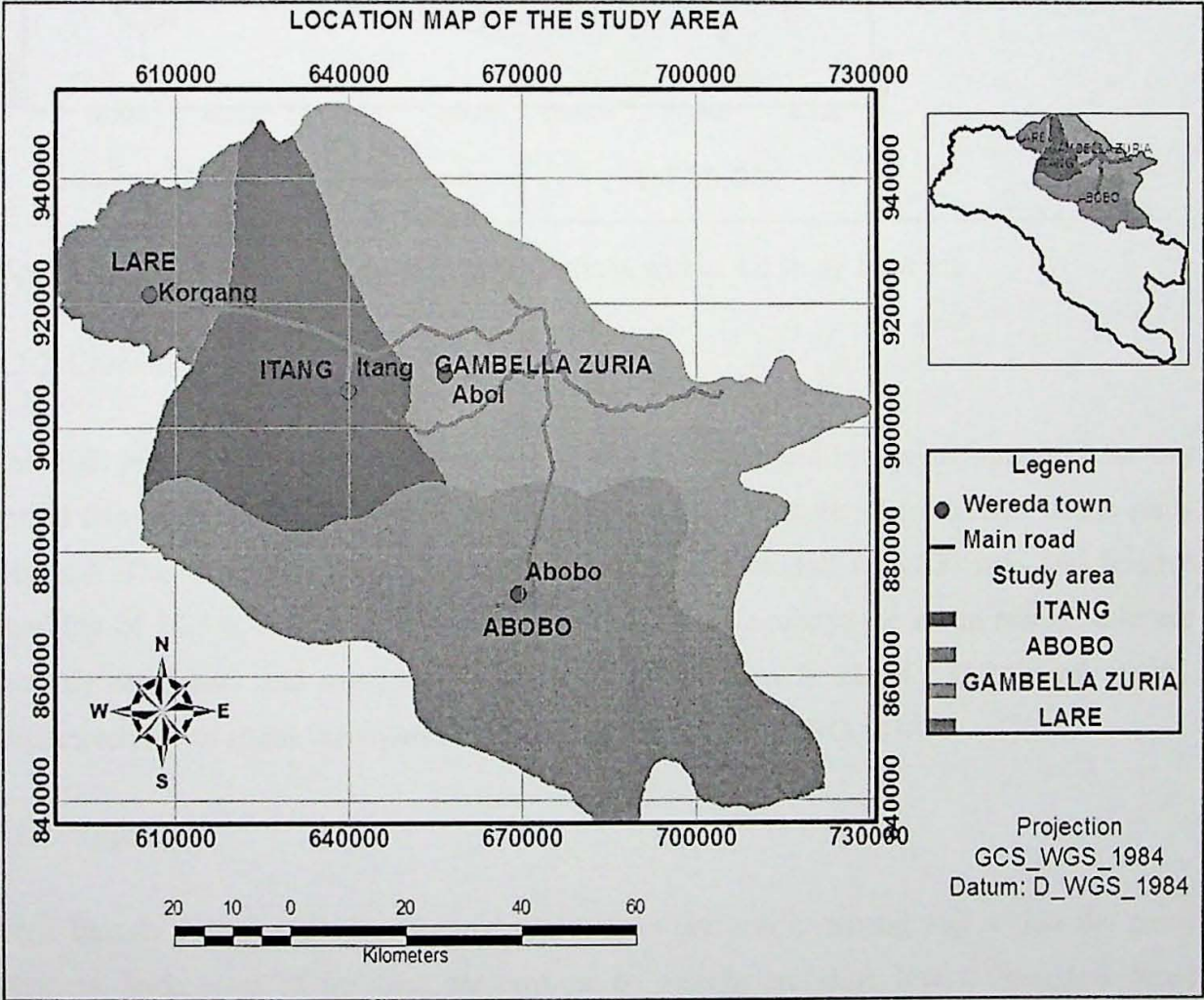


Figure 3: Geographical location and map of the study Districts

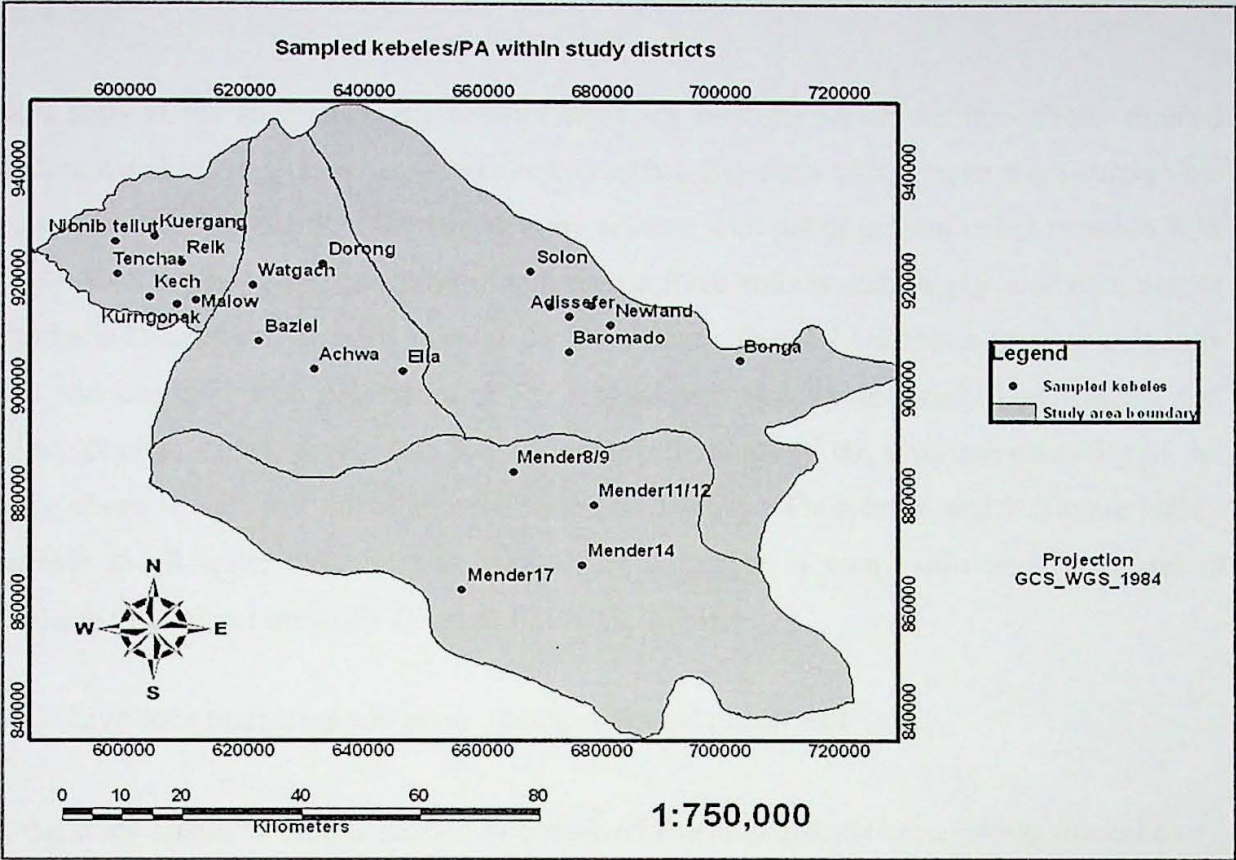


Figure 4: Point locations of sampled *Kebeles*/PAs within the study Districts

3.2.2. Climate

The high precipitation of the wet period (May to October) and low precipitation of the dry period (November to April) with mono modal pattern are climatic characteristic of the study Districts. The area receives an overall average annual rainfall of 1200 mm and relative humidity of 52.8%. It is one of the hottest places in the country; the mean annual, average monthly maximum and minimum temperature of the area is 28.4°C, 35.7°C and 19.9°C, respectively, with slight variations among the Districts (NMAGBO, 2009).

3.2.3. Vegetation

Even though the vegetation cover and types lacks uniformity among and within the study Districts, wide areas of the land are covered by vegetation. High forest, open and dense woodlands with high diversity of species, savanna grassland, grassland, bushes and shrubs are the major vegetation types known to the area. However, the topography being flat plain and the soil is water logged the vegetation cover in Lare District is low and the land cover is dominated by vast grasslands (GBoARD, 2009).

3.2.4. Soil

Most soils of the Itang and Lare District areas are vertisols which are imperfectly drained predominated by very deep heavy textured, cracking clay soils which make them susceptible for surface logging and flooding during rainy seasons with pH of the soil rating between 7.38 - 7.69. Well drained clay, dark reddish to brown colored soils is dominating in Abobo, part of Gambella District and on both sides of the Baro River. Parts of Gambella District are sandy clay and clay soil with pH values of 7.97. Moreover, soils of the study Districts are non saline, organic carbon levels and the total nitrogen values of the soils are generally in the range of low to very low throughout the Districts. Available Phosphorus and Sulfur are highly variable in all soils; Calcium and Magnesium dominate in many soils and Potassium is adequate throughout the study Districts (GPNRS, 2003).

3.2.5. Livestock marketing and major routes of animal movement

In the study Districts there is neither an organized livestock market nor a known market day except for Gambella (Table 4). Marketing of animals is done at the nearby towns on any day provided that there is a buyer and a seller.

Table 4: Market characteristics of the study Districts

District	Market		Presence of allotted area for market
	Place	Day	
Abobo	Abobo	Not fixed	No
Gambella	Gambella	Monday	Yes
Itang	Itang	Not fixed	No
Lare	Korgeng	Not fixed	No

Free livestock movement usually takes place in any direction across the Sudan border, between the neighboring regional states, within and among the Districts of the same region (Figure 5) for the access of better market opportunities.

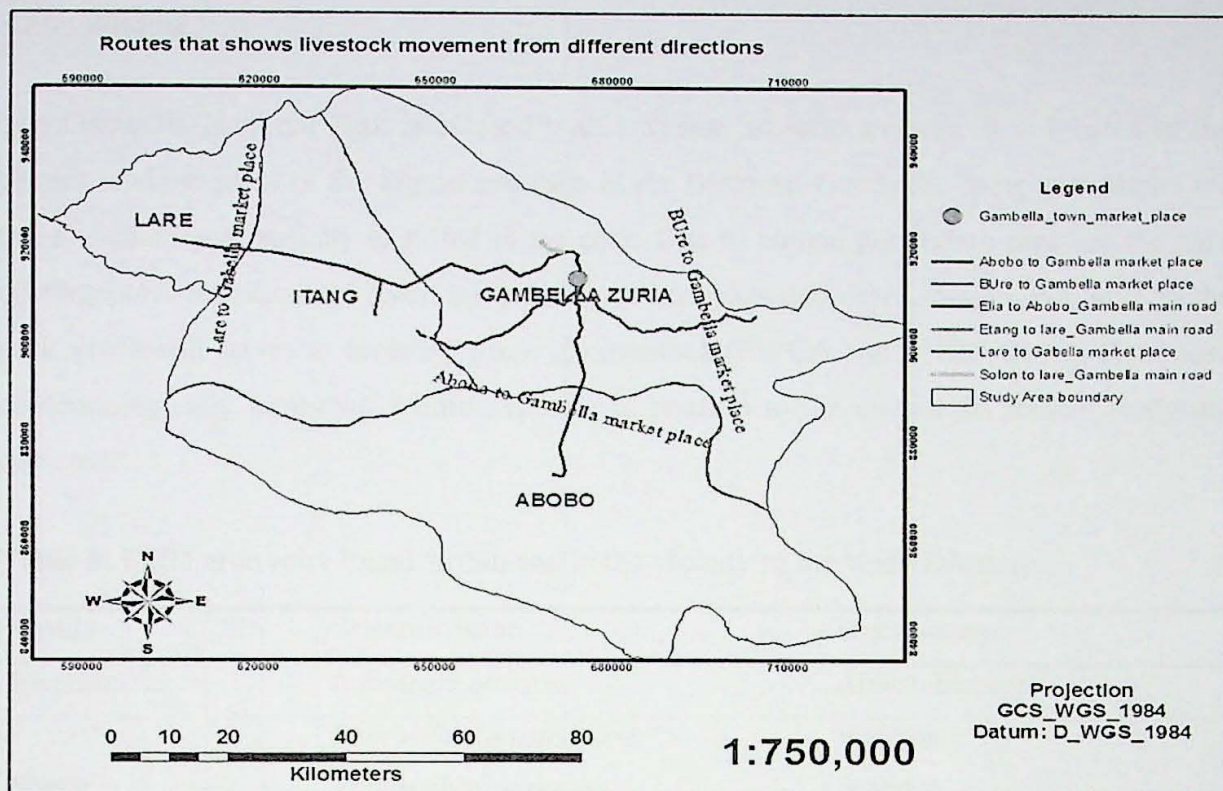


Figure 5: Livestock market routes within the study Districts

3.2.6. Socio-economic activities

The economic activities of the study areas are predominantly agrarian. Farming by oxen is not practiced by indigenous people and agricultural production is minimal. Small-scale farmers use hand tools and fire to clear the land and hence agricultural production is just for subsistence. Maize, sorghum, ground nuts, oilseed, tubers and fruits are major subsistence crops. The Anyuwa peoples are sedentary agriculturists and depend more on crop production mainly along the river bank while the Nuer nationalities are semi-sedentary pastorals. Moreover, both indigenous ethnic groups are engaged in fishing as well as edible wild fruit gathering.

Livestock production is the dominant activity by Nuer agro-pastoral community inhabiting most of the wide treeless portions of Itang and Lare Districts. Livestock rearing is not practiced to a noticeable degree by Anyuwa ethnic group living in the rural areas of Gambella and Abobo Districts except in some of its localities. However, in and around Gambella town and Bonga, previously called refugee camp areas of the District and around Abobo State farm areas livestock production is highly exercised by settlers who came from north and south parts of the country due to drought and famine during the Derg regime.

3.2.7. Wildlife

The Gambella National Park is blessed with a variety of wild animals. It is located in the central lowland plain of the region and falls in six Districts. Gambella, Itang and Abobo are three of them and partially engulfed in the park. Due to human population pressure the park also supports thousands of livestock grazing on the grass and other forages available in the park which still serves as breeding place for livestock (EWCA and GNRP, 2009). Here, also epidemiologically important wildlife species in relation to the disease in present study are depicted.

Table 5: FMD reservoirs found within and in the vicinity of the study Districts

Family	S/N	Scientific name	English name
Elephantidae	1	<i>Loxodonta africana</i>	African Elephant
Suidae	2	<i>Phacochoerus aethopicus</i>	Warthog
	3	<i>Potamochoerus porcus</i>	Bushpig
	4	<i>Hylochoerus meinertzhageni</i>	Giant Forest Hog
Giraffidae	5	<i>Giraffa camelopardalis reticulata</i>	Giraffe
Bovidae	6	<i>Sylvicapra grimmia</i>	Grmm's Duiker
	7	<i>Ourebia ourebi</i>	Oribi
	8	<i>Oreotragus oreotragus</i>	Kilipspringer
	9	<i>Tragelaphus scriptus</i>	Common Bushbuck
	10	<i>Alcelaphus bucelaphus</i>	Lelwel Hartebeest
	11	<i>Damaliscus korrigum</i>	Topi
	12	<i>damaliscus lunatus</i>	Tiang
	13	<i>Hippotragus equinus</i>	Roan Antelope
	14	<i>Kobus ellipsiprymnus</i>	Waterbuck
	15	<i>Kobud defassa</i>	Defassa Waterbuck
	16	<i>Kobus kob leucotis</i>	White-eared Kob
	17	<i>Kobus megaceros</i>	Nile lechwe
	18	<i>Redunca redunca</i>	Bohor Reedbuck
	19	<i>Syncerus caffer</i>	African Buffalo

Source: EWCA and GNRP (2009)

3.2.8. The Nuer agro-pastoral livestock management system

The Nuer agro-pastoralist practice an oscillation form of movement of their herds related to the raise and fall of the main rivers. In the heavy rainy season from June to November, the Baro river over flow the banks and flood wide areas of grazing plains. During this time the Nuer moves up from river banks and lives in higher or uplifted limited dried areas of Itang District and some parts of Lare along the main road side. During the main dry season temporary camps are established near by the rivers as the herds are moved around to take advantage of water and green pasture and stay there from December to May. During heavy wet season the small area of dried pastureland left out of the flood forces huge number of livestock to rely on scarce reserve of feed. The scenario is reversed during extreme dry season; many animals are concentrated along the river bank because of bush and non palatable lignified grass burning up. Natural pasture comprises the major source of feed.

3.2.9. Study animal population

Indigenous breeds of cattle, sheep and goats that are kept under traditional management system were studied. The major cattle breed is the Nuer (zebu) which is known for its good milk yield performance and trypanotolerant qualities. The study population consisted of 85,523 cattle and 54,480 shoats (Table 6).

Table 6: Study animal populations by Districts

Animal	District				Total
	Lare	Itang	Gambella	Abobo	
Cattle	34,726	23,000	17,563	10,234	85,523
Sheep and goats	22,233	16,055	7,500	8,692	54,480

Source: Districts' socio economic survey (2006)

3.3. Study design

A cross-sectional study design was implemented to determine the sero-prevalence of FMD in cattle and small ruminants. Sheep and goats, which have had contact or kept in close proximity to cattle herd, were included in the study to see whether small ruminants are risk

factors or not for the epidemiology of FMD. Serum was sample of interest to determine the prevalence of the disease by using 3ABC-ELISA test.

Species, age, sex, animal composition, herd and flock size, and wildlife contact history of sampled animals were considered as major risk factors to the occurrence of the disease and properly recorded on prepared format during the survey (Annex 5 and 6). Sampled animals were grouped into three age categories based on their dental eruption status (Annex 4). Moreover, purposive study design was used for outbreak investigation so as to isolate and characterize the serotypes and subtypes of FMD virus circulating in that particular ecology. For this specific task, tissue and oro-pharyngeal fluid specimens were collected from infected and apparently healthy in contact animals for diagnostic testing. Additional relevant epidemiological information was generated by interviewing appropriate individuals. This was further strengthened by careful field observations and secondary data sources.

3.4. Sample size determination

Win Episcopy 2.0, an improved epidemiological software, was used to determine the sample size with the under mentioned assumptions; 5% absolute precision, 95% confidence interval, 50% expected prevalence, which gives 384 cattle and 384 small ruminants. To increase the representativeness or precision, the sample size was increased by three folds for cattle and two folds for small ruminants. Accordingly, a total of 1,920 animals (1,152 cattle and 768 sheep and goats) were included in the study to determine sero-prevalence of FMD. The number of animals included in the study were distributed proportionally over the study Districts (Annex 1 and 2).

During an outbreak investigation accessible animals of 338 cattle and 69 small ruminants were clinically examined for the presence of lesions. Tissue samples from active lesions of tongue, mouth and foot, one from each organ (n=3) from cattle were collected. Similarly oro-pharyngeal fluid probang from cattle (n=24), sheep (n=7) and from goats (n=8) were collected for virus isolation and characterization.

3.5. Sampling technique

Multistage sampling technique was applied for the determination of seroprevalence of the disease. Four Districts were purposely selected from the lowland and semi-arid ecological zones of the region, based on their accessibility, geographical location, proximity to livestock market, huge ruminant population density and composition, and possibility of contact with wild animals in nearby parks and sanctuary. From each District 30% of *Kebeles*/Peasant Associations (PAs) were selected randomly from District's PA list (Annex 3). From each *Kebele*/PA 20% of privately owned herds were selected randomly to obtain primary sampling unit. An effort was made to include different herd sizes and composition in the PA. Finally, 10% of individual animal from each herd was selected randomly as secondary sampling unit to attain the required number of sample size (Thrusfield, 2005). During FMD outbreak a field investigation was conducted purposively at the specific site of the outbreak within the study Districts.

3.6. Questionnaire survey

Participatory approach of the study encompasses interviewing of appropriate stakeholders using pre tested semi-structured questionnaires. To this effect, in each of the study District, herd and/or flock owners from which sample were drawn, voluntary village leaders, animal health experts and development agents were interviewed. The questionnaire was designed with following main objectives of assessing: the perception and understanding of the communities about FMD, major diseases and livestock production constraints in the study area with particular reference to FMD, and collection of the most important epidemiological risk factors that could be associated with FMD so as to avoid bias which would most likely misguide the study.

For the present study a total of 120 households were selected randomly and interviewed during blood sample collection and outbreak investigation side by side. The proportion was allowed to be flexible depending on the number of herd and/or flock owners included in the sample within each District and PAs to attain appropriate sample size required for the study. Local language translators were used for interviewing informants in different areas of the study Districts.

Before the commencement of outbreak investigation, linkage was established with appropriate personnel of the study Districts to arrive timely at specific outbreak site. Key informants selected from the PAs were interviewed to obtain the history of any outbreak in association to small ruminants and cattle, to determine the characteristics of specific outbreaks and assessment of potential risk factors. This was the first step of the investigation process where tentative diagnosis of the disease can be made (Putt *et al.*, 1988). The questionnaire used for outbreak investigation and important risk factor assessment was indicated in Annex 10 and 11, respectively.

3.7. Study methodology

3.7.1. Field observation

In order to arrive at sound and unbiased conclusions with respect to this study, undertaking visual assessment of the target areas was of paramount importance. Therefore, field observations and exploration have been considered in all the representative sample areas.

3.7.2. Clinical examination

Soon after arrival at the specific outbreak sites during disease outbreak investigation, the animals were examined from a distance for evidence of salivation and lameness in each individual animal owner's homestead since there was no crush in the area. Salivating and/or limping animals were restrained for thorough examination and sampling. The mouth cavities of salivating animals were widely opened and examined for evidence of intact and/or ruptured vesicles, erosions and ulcers on the tongue, dental pad and mucosa of the oral cavity. The hooves of lame animals were thoroughly washed with water and then carefully examined for similar lesions particularly on the coronary bands and interdigital spaces of the hooves. Small ruminants in contact to the outbreak-affected cattle were similarly examined. This was performed to identify the clinical picture of FMD in cattle and small ruminants. Sampling was done only when active lesions suggestive of FMD were encountered.

3.7.3. Sample collection

Serum

Whole blood was collected from the jugular veins of randomly selected cattle and small ruminants into 10 ml sterile vacutainer tubes and stored overnight at room temperature for serum collection. The serum was then transferred into a sterile cryovial bearing the identification number, species, village, age and sex and transported in an icebox to Gambella Regional State Bureau of Agriculture and Rural Development for refrigeration and then stored at -20°C. Finally, at the end of each sampling sera were transported in cold chain to National Animal Health Diagnostic and Investigation Centre (NAHDIC), Sebeta, for laboratory analysis. The samples were stored there at the recommended temperature of -20°C until ready for laboratory investigation (OIE, 2004).

Tissue and oro-pharyngeal (OP) fluid samples

Active tissue samples were collected from the gum, tongue and interdigital space of cattle in one outbreak. Epithelial tissue was collected from un- ruptured or freshly ruptured vesicles and placed in a bottle with transport medium composed of equal amount of glycerol and 0.04 M phosphate buffer with antibiotics (OIE, 2004). Samples of OP fluid were collected from apparently healthy cattle and small ruminants that were found in close proximity to the affected herd or flock. The OP fluid was collected by a probang cup (Annex 13) and poured into a 20 ml bottle. The fluid was then examined and about 2 ml of it containing cellular material was added to a container of 5 ml containing about 2ml of transport medium (OIE, 2004). Then, the samples were labeled and transported to NAHDIC, Sebeta, in similar fashion as for serum. Both tissue and OP fluid samples were kept at -70°C until submitted to the WRL, England.

Specimen shipment process to WRL

Preserved tissue and OP fluid sample were further processed before shipment to WRL, England. A total of five pooled samples (one tissue and four OP fluids) were prepared in 2 ml cryotubes and sealed with Parafilm (Table 7). The pooled samples were sent in dry ice to the WRL. Sample submission to the WRL was facilitated by National Animal Health Diagnostic and Investigation Centre (NAHDIC), Sebeta.

Table 7: Tissue and OP fluid samples submitted to WRL for virus characterization

Outbreak locations		Species	Number of sample		
District	PAs/Kebele		Tissue	OP fluid	Pool
Gambella	Newland	Bovine	3	-	1
Gambella	Baromedo	Caprine	-	8	1
Gambella	Addis sefar	Bovine	-	12	1
Lare	Riek	Bovine	-	12	1
Lare	Riek	Ovine	-	7	1
Total			3	39	5

3.7.4. Serological diagnostic tests

FMDV 3ABC-Ab ELISA (SVANOVIR®)

Sera collected from bovine species were tested by FMDV 3ABC-Ab ELISA for the detection of antibody to poly protein called 3ABC which is a useful indicator of FMD virus infection regardless of the serotype involved (Haas, 1997; Mackay *et al.*, 1998). Antibody to 3ABC is found only in virus infected animals but not in vaccinated animals (De Diego *et al.*, 1997). Therefore, in this study the test was undertaken for screening and determination of positive and negative proportions of the test sera.

Briefly, the test was carried out stepwise as per the manufacturer's manual. First all reagents were kept at room temperature and then test sera and controls were diluted to 1:40 concentrations using sample dilution buffer. The test was carried out in 96 well microplate (Annex 8). Duplicates of 50 µl diluted controls and test sera were dispensed into selected test plate's wells. The test plates' odd column wells were coated with 3ABC FMD viral antigens while the even column wells were coated with control antigens. The plates were sealed with lids and incubated for 30 minutes at 37°C with continuous shaking. A lyophilized horseradish peroxidase (HRP) conjugate was reconstituted with 6 ml PBS-Tween buffer and prepared immediately before use. The solution was left one minute and thoroughly mixed. Then 50 µl of the reconstituted HRP conjugated anti-species IgG was added into each well of the microtiter plates. The plates were kept for another 30 minutes at 37°C incubation. To remove unbound reagents, the plates were washed six times between each step by filling the wells with about 300 µl pre-diluted phosphate buffered saline (PBS) Tween buffer using distilled

water at 1:20 concentrations. After washing the plates, 50 µl of substrate solution was added into test wells and kept for 30 minutes at room temperature in a dark place. The reaction was terminated by adding 50 µl of stop solution to each well of the plate and within 15 minutes after addition of stop solution (sulphuric acid). ELISA reader was used to measure the optical densities (OD) of the controls and test samples. Optical densities were measured at 405 nm in a microplate photometer and calculations of the results were estimated in two steps as follows:

i. Corrected OD Values (OD_{Corr})

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

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

ii. Percent Positivity Values (PP)

All corrected OD values for the test sera as well as the Negative Control (Neg C) are related to the corrected OD values of the positive control as follows:

$$PP\ (\%) = \frac{\text{Test sample or Neg C (OD}_{Corr})}{\text{Positive Control (OD}_{Corr})} \times 100$$

Result interpretation

Percent positivity value	< 48	≥ 48
Interpretation	Negative	Positive

CHEKIT FMD-3ABC Bo- Ov ELISA

Unlike 3ABC-Ab-ELISA (SVANOVIR®) which is used for testing only bovine sera, the CHEKIT FMD 3ABC Bo-Ov test is more general and used for detecting antibodies against the pathogen responsible for FMD in serum samples of bovine, ovine and caprine origin. The two tests general principles are the same but the test procedures and the result interpretation

method of the CHEKIT FMD 3ABC Bo-Ov test is different from the previous one. The test was preformed as follows:

All reagents were allowed to room temperature before using them and each sample and control was prediluted at 1:100 concentrations in a tube using CHEKIT-FMD-3ABC Sample Diluent. Then 100 µl prediluted sample and control was dispensed into the appropriate wells of the microtiter plate. The microplate was covered with lid and incubated for 60 minutes at 37°C in a humid chamber. After incubation each well was washed three times with 300 µl CHEKIT- Wash Solution which was diluted at 1:10 using distilled water and the liquid contents of all wells were aspirated following washing. CHEKIT-FMD-3ABC-Anti-Ruminant-IgG-PO conjugate was added into each well. The pervious step was repeated for second cycle plate incubation and washing. Then 100 µl CHEKIT-TMB Substrate was added into each well of the test plate. The Substrate was incubated at room temperature for 15 minutes. To stop the color reaction 100 µl CHEKIT-Stop Solution was dispensed into each well and finally the result reading was recorded using a photometer at wavelength of 450 nm within 2 hours after the addition of the stop solution. Plate lay out used for the test is indicted on Annex 9.

Result calculation:

The OD of the positive control (OD_{pos}) and the OD of the samples (OD_{sample}) are corrected by subtracting the OD of the negative control (OD_{neg}):

Positive control:

$$OD_{pos} - OD_{neg}$$

Analyzing the samples in relation to the negative and the positive controls with the formula:

$$Value (\%) = \frac{OD_{sample} - OD_{neg}}{OD_{pos} - OD_{neg}} \times 100$$

Sample :

$$OD_{sample} - OD_{neg}$$

Interpretations of results:

Value	< 20 %	≥ 20 % to < 30 %	≥ 30 %
Interpretation	Negative	Suspect	Positive

The test was repeated for suspected samples.

3.7.5. Diagnostic tests employed at WRL, Pirbright

Virus isolation

Tissue and OP fluid samples were initially inoculated onto primary bovine thyroid cell culture and the cytopathic effect (CPE) of the virus was investigated. Destruction and rounding of the infected monolayer cell culture within 48 hours of virus inoculation was considered as the presence of cytopathic effect due to the existence of live FMD virus. The supernatant of positively reacted cell culture was tested by antigen detection ELISA and the serotype of FMD virus was determined (Roeder and Le Black Smith, 1987).

Reverse transcriptase polymerase chain reaction (RT-PCR)

Polymerase chain reaction was used to amplify the genome fragments of FMD virus from the specimen. RNA extraction and RT-PCR were conducted according to the protocol described previously (Knowles *et al.*, 2005). The thermal profiles used for amplification of the VP1 sequence of SAT-2 serotype was as follows: 42°C for 30 min, 94°C for 5 min, 35 cycles of 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 90 seconds, followed by a final extension of 50°C for 5 min. After PCR, deoxyribonucleotide triphosphates and primers were removed by using GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions.

DNA Sequencing

PCR amplicons were sequenced by using the DTS Quick Start Kit (Beckman Coulter, Fullerton, CA, USA). The sequencing reactions were run on a CEQ8000 Automated Sequencer according to the manufacturer's instructions.

Phylogenetic analysis

Each VP1 coding region was amplified by RT-PCR and the products were directly sequenced to obtain the complete VP1 sequence, which were compared with other relevant FMDV VP1 sequences within the same serotype. VP1 nucleotide sequences were aligned by using Bio Edit 7.0.5.3 and Clustal W. The alignments were used to construct distance matrices by using the Kimura 2- parameter nucleotide substitution model in the program MEGA 4.0. Mid point-rooted neighbor-joining tree was then constructed with MEGA 4.0 software. The robustness

of the tree topology was assessed with 1,000 bootstrap replicates by using the model in MEGA 4.0.

The result was interpreted according to Samuel *et al.* (1999), where $\leq 1\%$ nt. sequence difference indicated that the virus isolated from the same epizootic, $< 7\%$ nt. sequence difference indicated that virus belonging to the same epizootics (common origin), virus of the same genotype differ up to 15% and viruses from different genetic lineages differ by $\geq 15\%$ nt. sequence.

Vaccine strain selection (r1-values determination)

The antigenic relationship between a field isolate and a vaccinal viral strain (r1-values) was determined by VNT and LPB-ELISA test at WRL. This test used an antiserum raised against a reference vaccine strains found in the WRL for FMD. The blocking ELISA titers of this reference serum against antigen prepared from the homologous vaccine strain were compared with the corresponding titres of the serum against a field isolate to determine how antigenically 'similar' the field virus is to the vaccine virus. The r1-values were computed as:

$$r1 = \frac{\text{resporical titer of reference serum against field virus}}{\text{resporical titer of reference serum against vaccine virus}}$$

Interpretation of r1-values:

In case of VNT:

$r1 = \geq 0.3$. Suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

$r1 = < 0.3$. Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect.

In case of LPB-ELIZA:

$r1 = 0.4 - 1.0$. Suggests that there is a close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

$r_1 = 0.2 - 0.39$. Suggests the field isolate is antigenically related to the vaccine strain. The vaccine strain might be suitable for use if no closer match can be found provided that a potent vaccine is used and animals are preferably immunized more than once.

$r_1 = < 0.2$. Suggests that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect.

3.8. Study area mapping

Study District mapping, displaying of point locations of sampled kebles/PA and the tracking of the main livestock marketing movement routes within the study Districts were done with the collaboration of GIS experts of Gambella Regional State Bureau of Finance and Economic Development Department of Cartography and Statistics. Germinetrex Global Positioning System (GPS) was used for geographical point locations and market routes tracking reading records. The recorded data of GPS was transferred into the computer loaded with GIS program called Germin driven program and then the interface of GPS data was displayed on the soft ware. Finally an Arc Geographical Information Systems (GIS) program was chosen and the required features of the map were displayed on previously existing map of the study districts. Projection of the map with its major map elements such as (directions, legend, title and scale) was done by converting latitudinal and longitudinal measurement values into meters using Universal Transverse Mercator (UTM) program from the GIS computer software.

3.10. Data management and analyses

Microsoft Excel-2007 and SPSS-15 statistical software programmes were used for data entry and analyses, respectively. The data were coded before the entry of information from questionnaire and laboratory result recording format to convert the information obtained from each subject into a format that SPSS can understand. Then quantification of the disease occurrence and the questionnaire survey results were analyzed using descriptive statistics. The chi-square (χ^2) test was performed to determine the association between putative risk factors and the disease outcome. Logistic regression was employed to explain the disease outcome variable in terms of explanatory or independent variables. In all the analyses, 95% confidence level and $p \leq 0.05$ was set for significance.

4. RESULTS

4.1. Sero-prevalence

From the total of 1,152 bovine and 768 small ruminants' sera examined 12.00% (138) and 1.43% (11) were positive, respectively (Table 8). The prevalence was higher in cattle than small ruminants and the difference was statistically significant ($\chi^2 = 71.609$; $p = 0.000$).

Table 8: Prevalence of FMD in cattle and small ruminant by District

District	Cattle				Small ruminant			
	Sera tested	Test (+)	Prevalence (%)	95% CI	Sera tested	Test (+)	Prevaence (%)	95% CI
Lare	468	42	8.97	6.38-11.56	313	6	1.92	0.40-3.44
Itang	310	55	17.74	13.49-21.99	226	1	0.44	0.42-1.30
Abobo	138	13	9.42	4.55-14.29	106	2	2.50	0.47-5.47
Gambella	236	28	11.86	7.73-15.99	123	1	0.94	0.77-2.65
Overall	1,152	138	12.00	10.12 -13.88	768	11	1.43	0.59-2.27

(+) = Positive, CI = Confidence Interval

The highest bovine FMD prevalence of 17.74% was recorded in Itang followed by prevalence of 11.86% in Gambella and 9.42% in Abobo Districts while the lowest prevalence of 8.97% was observed in Lare District. The difference among the Districts was statistically significant ($\chi^2 = 14.631$; $p = 0.002$).

In small ruminants, species specific prevalence of 2.45% for sheep and 1.16% for goats was observed (Table 9). The difference between small ruminant species was statistically not significant ($p > 0.05$).

Table 9: FMD prevalence in small ruminants by species

Species	Number of sera tested	Number of sera (+)	Prevalence (%)	95% CI
Sheep	163	4	2.45	0.08-4.82
Goats	605	7	1.16	0.30-2.01
Total	768	11	1.43	0.59-2.27

4.1.1. Prevalence in relation to host intrinsic risk factors

FMD seroprevalence comparison was done in different age and sex groups of cattle and small ruminants. An increasing seroprevalence trend was observed in both cattle and small ruminants with increasing of age from 10.25% (23/293) in groups less than 2 years, 13.19% (22/257) from 2-3 years and 15.45% (93/602) in greater than 3 years in cattle and from 0.41% (1/243), 1.03% (3/292) and 3.00 (7/233) in similar age groups in small ruminants (Figure 6). The difference was statistically significant among cattle age groups ($\chi^2 = 14.459$; $p = 0.001$); however, there was no significant statistical ($p>0.05$) associations in case of small ruminants.

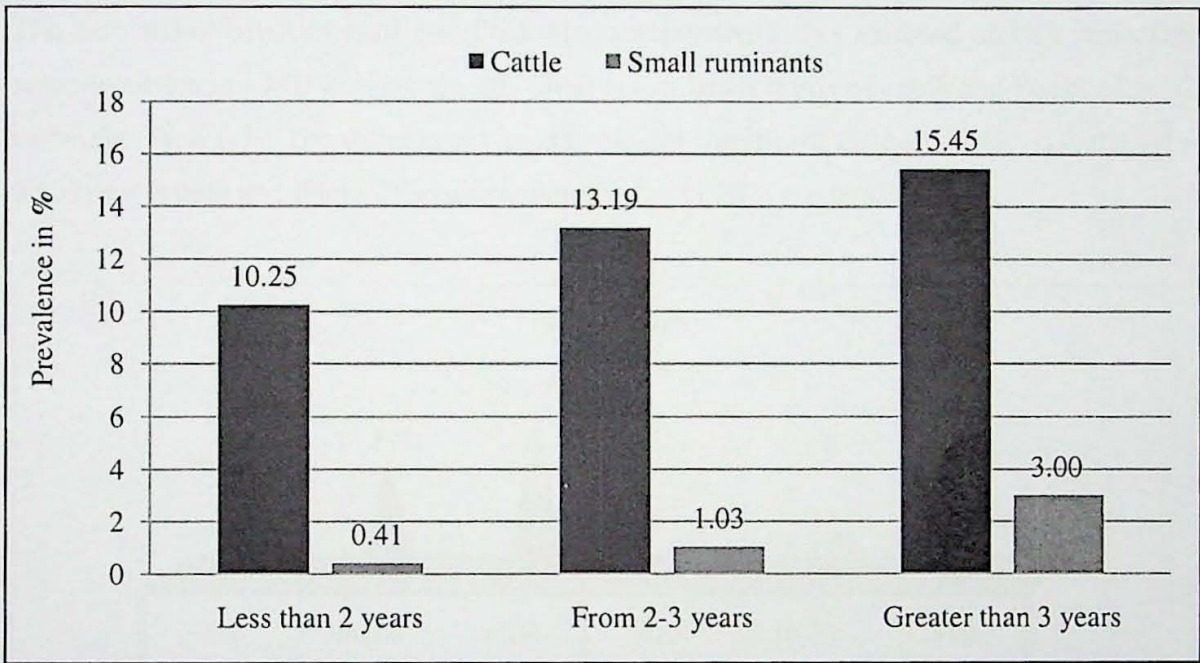


Figure 6: FMD prevalence of cattle and small ruminants by age groups

The prevalence estimate for female and male cattle was 14.19% and 8.01%, respectively (Table 10). The difference in seroprevalence was significant ($\chi^2 = 9.576$; $p = 0.002$). In small ruminants seroprevalence of 1.41% in females and 1.55% in males was recorded but the difference was statistically not significant ($p>0.05$).

Table 10: Sero-prevalence of cattle and small ruminants by sex

Sex	Cattle				Small ruminants			
	Sera tested	Test (+)	Prevalence (%)	95% CI	Sera tested	Test (+)	Prevalence (%)	95% CI
Female	740	105	14.19	11.68-16.70	639	9	1.41	0.50-2.32
Male	412	33	8.01	5.39-10.63	129	2	1.55	0.58-3.68

4.1.2. FMD prevalence in relation to host extrinsic risk factors

The association between herd and flock size seropositivity was assessed and an increasing seroprevalence of FMD occurrence was observed in larger herds of cattle and flocks of small ruminant (Figure 7). The difference was statistically significant for both herds of cattle ($\chi^2 = 9.699$; $p = 0.008$) and flocks of small ruminant ($\chi^2 = 11.343$; $p = 0.003$).

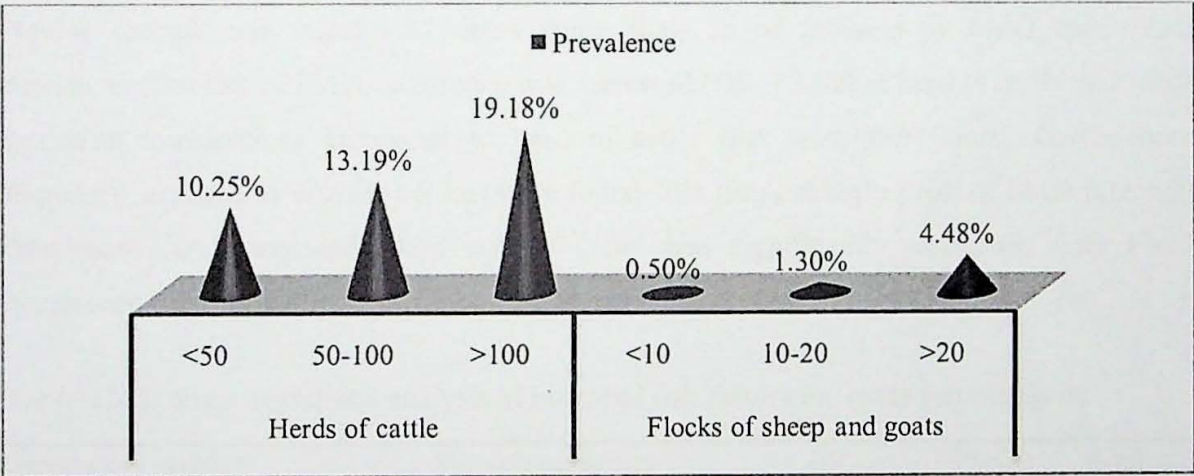


Figure 7: FMD prevalence in relation to herd and flock size

Furthermore, other risk factors such as herd composition and contact history with wildlife were considered. High seroprevalence was recorded in cattle that were kept together with small ruminants than herds of cattle without and the prevalence was varied significantly ($p < 0.05$). Animals with frequent history of wildlife contact have manifested greater FMD prevalence than those with less contact (Table 11).

Table 11: FMD prevalence in relation to animal composition and wildlife contact history

Risk factors	Sera of cattle			95% CI
	Tested	Test (+)	Prevalence (%)	
Animal composition				
only herds of cattle	1010	113	11.19	9.25-13.13
cattle and shoat	142	25	17.61	11.34-23.61
Wildlife contact history				
rare	468	42	8.97	6.38-11.56
frequent	684	96	14.04	11.44-16.64

4.1.3. Logistic regression analysis

The multivariable logistic regression analysis included effects of species, history of herd contact with small ruminants and wildlife, herd size, sex and age of animals (Table 12). Bovine species was found 9.37 times more likely to be infected by FMD than small ruminants. The risk of FMD occurrence was increased (OR = 1.82) in herd of cattle and small ruminant combinations compared to herd of cattle that were kept alone. Cattle those frequently exposed to wildlife contact were found 2.68 times at higher risk of FMD infection than those rarely exposed. Herd size of cattle was significantly associated with FMD occurrence.

Table 12: Logistic regression analysis of potential risk factors for cattle seropositivity

Explanatory variable	Variable categories	OR	95% CI	p-value
species	Bovine vs. small ruminant	9.37	5.03 -17.43	0.000
Herd contact with small ruminant	Yes/no	1.82	1.11-2.99	0.017
Herd size	Large vs. small herd size	2.97	1.76- 5.03	0.000
	Medium vs. small size	1.75	1.08- 2.83	0.023
Age	Old vs. young	2.08	1.26- 3.42	0.004
	Adult vs. young	1.15	0.62-2.14	0.665
Wildlife contact	Present/absent	2.68	1.73-4.15	0.000
Sex	Female/male	1.55	1.00-2.40	0.049

OR, odds ratio; vs. versus; CI, confidence interval; large herd > 100 animals, medium herd 50-100 animals, small herd < 50 animals; old > 3 years, adult 2-3years, young < 2 years old

Animals kept in large herd size were found 2.97 times more FMD sero-positive than animals kept in small herd size groups. Animals greater than 3 years old were found 2.08 times more FMD sero-positive than young animals (those found below 2 years old). Similarly the odds of FMD in females were 1.55 higher than the odds of males.

Breeding of cattle in isolation from not getting contact with small ruminants and wildlife, keeping of small herd size and young animals may be found more protective which could mitigate the risk of acquiring FMD infection and dissemination.

4.2. Clinical examination of FMD outbreak

Out of 338 cattle clinically examined 124 (36.69%) of animals showed lesions and signs typical of FMD infection. However, characteristic signs and lesions suggestive of FMD infection were not observed in all 69 clinically examined small ruminants. The difference between cattle and small ruminants was statistically significant ($\chi^2 = 36.405$; $p = 0.000$).

The major important clinical signs observed during the outbreak investigation were salivation and lameness. Mouth lesions consisted of erosions and ulcers on tongue and dental pad whereas foot lesions comprised erosions on the interdigital space and the coronary bands. In severe cases the hooves of affected animals tended to separate from the coronary bands. Reluctant to move and lagging behind herds and refusal of grazing were also seen as common features of clinically affected animals. Some observed FMD lesions in cattle during the outbreak investigation are shown on annex 12.

4.3. FMD virus isolation and characterization

From tissue and or-pharyngeal fluids samples collected during an outbreak from the study area SAT-2 serotype FMD virus which belongs to topotype XIII was identified at World Referral Laboratory(WRL), Pribright, England (Table 13). The two pooled samples positively reacted to all the tests employed at the WRL. From the other pooled OP fluid sample collected from goats in Gambella, only the presence of the genetic material (RNA) of FMD virus was confirmed by RT-PCR. On the other hand, neither the virus nor their genetic material (RNA) was identified from sheep OP fluid sample examined at the FMD World Reference Laboratory. The summarized test result is shown in table 13.

Table 13: FMD virus serotype and toptype identified at WRL, Pirbright

Outbreak locations (District)	Species	Sample			Test employed and virus identified			
		Type	Number	Pool	Culture (CPE)	RT-PCR	LPB-ELISA	
							Serotype	Topotype
Gambella	Bovine	Tissue	3	1	+	+	SAT-2	XIII
Gambella	Caprine	OPF	8	1	-	+	-	-
Gambella	Bovine	OPF	12	1	-	-	-	-
Lare	Bovine	OPF	12	1	+	+	SAT-2	XIII
Lare	Ovine	OPF	7	1	-	-	-	-
Total			42	5				

(+), Positive test result; (-), Negative test result; CPE, Cytopathic effect; RT-PCR, Reverse transcriptase polymerase chain reaction; VNT, Virus neutralization test; LPB-ELISA, Liquid phase blocking ELISA; OPF, oro-pharyngeal fluid sample

4.3.1. Sequence based phylogenetic analysis

The recovered SAT2/ETH/42/2009 and SAT2/ETH/48/2009) viruses were compared based on the complete sequencing of 648 nucleotide of VP1. The viruses were shared 99.54% genetic similarity with each other. The WRL, nucleotide sequence difference analysis confirmed that the nucleotide sequence difference of the two identified viruses was $\leq 1\%$ and hence these viruses were isolated from the same outbreak. The two viruses were also showed $>80\%$ genetic similarity with virus isolates of SAT2/ETH/2/2007 Ethiopia and SAT2/SUD/1/2008, SAT2/SUD/2/2008, SAT2/SUD/6/77 and SAT2/SUD/9/77 Sudan isolates. These viruses are homologous, geographically clustered and formed a single genetic lineage called toptype XIII. The genetic relationship of the isolates with the other SAT-2 serotypes is displayed on phylogenetic tree (Figure 8).

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Gambella	Caprine	OPF	8	1	-	+	-	-
Gambella	Bovine	OPF	12	1	-	-	-	-
Lare	Bovine	OPF	12	1	+	+	SAT-2	XIII
Lare	Ovine	OPF	7	1	-	-	-	-
Total			42	5				

(+), Positive test result; (-), Negative test result; CPE, Cytopathic effect; RT-PCR, Reverse transcriptase polymerase chain reaction; VNT, Virus neutralization test; LPB-ELISA, Liquid phase blocking ELISA; OPF, oro-pharyngeal fluid sample

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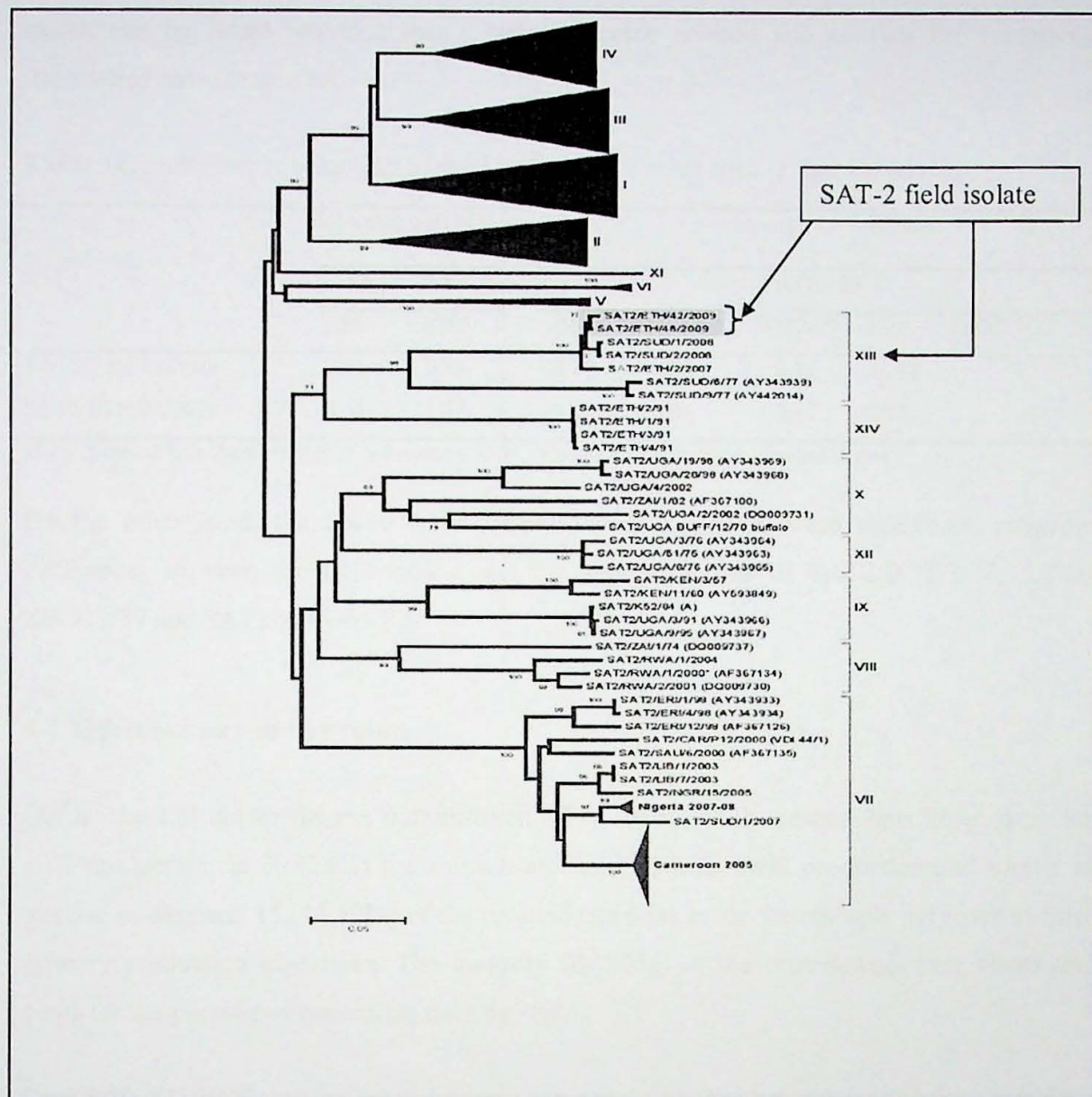


Figure 8: Phylogenetic tree; relationship between the field isolate with other SAT-2

4.3.2. Selection of vaccine strain match of the SAT-2 field isolate

The SAT-2 field isolate of the study area (Gambella) was examined both by virus neutralization test (VNT) and LPB-ELISA to select antigenically related vaccinal strains. The result shows that the field isolates were antigenically related to SAT-2 Eritrea (SAT2/ERI) and SAT-2 Zimbabwe (SAT2/ZIM) isolates. SAT2/ERI vaccine strain is more protective than SAT2/ZIM vaccine strain, because the r1-value of the former is higher than the r1-value of the latter (table 14). The vaccine strain SAT2/ZIM might be recommended for use if no closer

match can be found provided that a potent vaccine is used and animals are preferably immunized more than once.

Table 14: Antigenic relationship of field isolates with other SAT-2 vaccine strains

Field isolate	r1-value by VNT		r1- value by LPB-ELISA				
	SAT2 - RVS		SAT2 - RVS				
	ERI	ZIM	ZIM	ZIM11/89	BOT3/77	ERI	KEN65/82
SAT2/ETH/42/2009	0.61	0.35	0.21	0.08	0.11	≥0.88	-
SAT2/ETH/48/2009	0.53	0.32	0.21	0.31	0.13	0.88	-

ERI = Eritrea; ZIM = Zimbabwe; BOT = Botswana; KEN = Kenya; RVS = Reference vaccine strains

On the other hand, the above table clearly shows that there were significant antigenic differences between the field isolate and the vaccine strains of SAT2/BOT/3/77, SAT2/ZIM/11/89 and SAT2/KEN/65/82.

4.4. Questionnaire survey result

Out of the 120 questionnaires administered 75 (62.50%) of the respondents keep cattle for milk production; in 30 (25%) the animals are used both for milk production and source of traction power; and 15 (12.50%) of the respondents raise cattle for multiple purposes as their primary production objectives. The majority (80.83%) of the interviewees rear sheep and goats for the purpose of generating cash by sale.

Over 90% (110/120) of the interviewees responded that disease and inadequate veterinary services were major problems of livestock production in the area. A noticeable proportion of 30% (36/120) animal keepers complained looting or animal raiding as the main production problem next to disease and veterinary services. Animal diseases such as trypanosomoses, internal and external parasites, contagious bovine pleuropneumonia (CBPP), foot and mouth disease and hemorrhagic septicemia (HS) were prioritized from one to five in order of importance by the respondents, respectively (Figure 9).

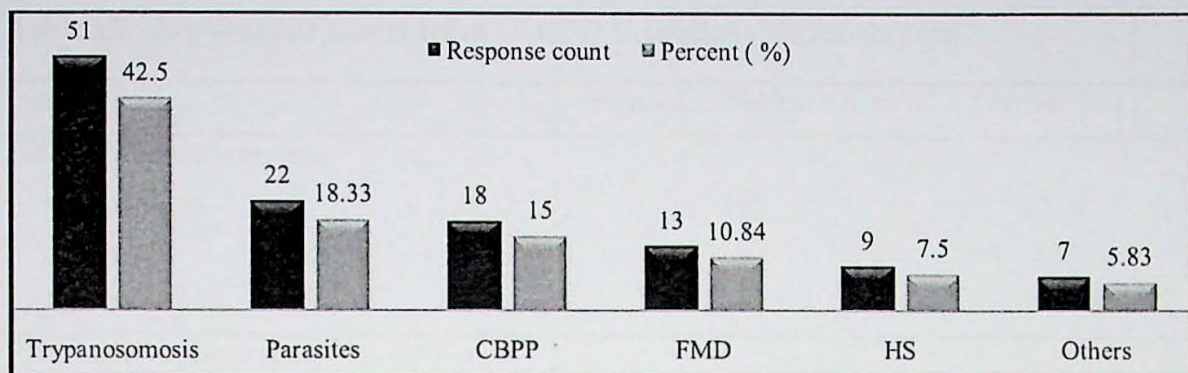


Figure 9: Major livestock diseases of the study area

Of the total interviewees 105 (87.50%) reported that FMD occurrence was common to the area and frequently affect their herds. Around 87 (72.50%) of herd owners agreed that FMD was temporally associated with rainy (wet) season but 18 (15%) and 15 (12.50%) of them indicated possibilities of occurrence in the dry season and any time of the year, respectively. Out of 120 individuals consulted 78 (65%) said FMD has no effect on sheep and goats while 3 (2.5%) said the disease has effect; 39 (32.5%) said the effect is unknown (Table 15).

The other important information generated from farmers verified the frequent contact of their herds and flocks with many species of wildlife existing in Gambella national park and its surrounding. Data obtained from Gambella regional national park office and Ethiopia Wildlife Conservation Authority (EWCA) strengthened the suggestions of the herd owners but contact frequency of wildlife and domestic animals vary from District to District on the bases of vegetation coverage. The role of wildlife in the transmission of FMD in the present study animal species was totally not known by all respondents. All individual key informants underlined prophylactic measures like vaccination against FMD was not undertaken so far in the area to mitigate the effect and occurrence of the disease (Table 15). Discussions held with animal health personnel in the respective study areas and recorded data at District level also supplemented the views of the farmers.

Seventy nine or 65.83% of informants associated the occurrence of FMD outbreak with incidence of flood, contact of animals at market place and communal points as the possible source of infection. Descriptive response summary of the same question showed 41 (34.17%) of farmers were relating the risk of outbreak occurrence with movement and introduction of infected animal(s) to their herds in addition to the aforementioned risk factors (Table 15).

Table 15: Questionnaire survey result on FMD in relation to the study area

Inquiry	Respondent feedback count	Percent (%)
FMD occurrence in the area		
common	105	87.50
rare	9	7.50
not known	6	5.00
FMD season of occurrence		
wet season	87	72.50
dry season	18	15.00
any time	15	12.50
FMD effect on sheep and goats		
present	3	2.50
absent	78	65.00
not known	39	32.50
Domestic animals contact with wildlife		
common	84	70.00
rare	36	30.00
Wildlife disease that can affect animals		
known	0	0.00
not known	120	100.00
FMD vaccination in the area		
present	0	0.00
absent	120	100.00
Likely source of FMD occurrence		
seasonal occurrence of flood, contact at communal points and marketing places	79	65.83
infected animal free movement and new animal introduction into herd	41	34.17

Respondents from Lare and Itang Districts reported that milk yield reduction was the major impact of FMD followed by animal sale price fall. Farmers from Abobo and Gambella Districts had said FMD seriously affect both milk production in cows and traction power of male animals in addition to its significant effect on market value. More than 98% of the informants witnessed that morbidity rate was high but death due to FMD was not common.

Communal grazing and watering point resource utilization was dominant in all the study Districts. Grazing land and watering point tribal ownership did not exist. The presence of cross national border free movement of animals was reported by herd owners of Lare and some inhabitants of Itang Districts due to common border with Sudan. Furthermore, movement of animals within the Region or Districts and between neighboring regional states was commonly observed. Animal composition assessed during the survey showed that some farmers owned only herds of cattle, some had herds of cattle and flocks of sheep and goats and few of them possessed only either flock of sheep or goats or in combinations. During the night time segregation of animals by age and species was not exercised by most farmers of Lare and Itang Districts. All species and age groups of animals had been kept in the same homestead (Annex 13).

The farmer's ability to identify FMD infection was cross checked by enquiring the clinical signs and local vernacular name for FMD. Respondents described the disease by mentioning major clinical signs of excessive salivation and appearance of lesions on the mouth and feet of affected animals. Different vernacular names of FMD were recorded from different ethnic groups in sampling locations. In the study area it was named as *Dat* by Nuer, *Anjicho* by Kembata, *Shilimat* or *Maze* by Tigire, *Okolchisa* by Oromo and *Maze* by Amhara; which indicate in general lesions on the foot and mouth of animals. Farmers in Abobo and Gambella Districts described their farming system as crop-livestock mixed production while those in Lare and Itang prioritized animal rearing as their major activity.

5. DISCUSSION

The overall sero-prevalence of 12% observed in bovine species in this study was in close agreement with prevalence of 15-20% reported in local cattle originated from southern Ethiopia which were pre tested for Egyptian market (MoARD, 2006). Compared to the present findings lower prevalences of 5.6% and 8.18% were reported from Afar Regional State and South Omo Zone of SNNPRS (Jembere, 2008; Molla, 2009). On the other hand relative to the current serological survey higher bovine sero-prevalences of 26.5%, 21% and 23% had been reported (Sahle, 2004; Rufael, 2006; Bayissa, 2009) at national level and from Borana pastoral areas of Oromia Regional State at different times. Abu-Elzein *et al.* (1987) in Sudan and Hafez *et al.* (1994) in Saudi Arabia also reported sero-prevalences of 16% and 53%, respectively, from FMD virus infected cattle. The observed prevalence variation may be resulted from differences in individual animals breed, immune status, production system and the type of diagnostic tests employed.

In small ruminants an overall prevalence of 1.43% was analyzed and species specific prevalence of 2.45% for sheep and 1.16% for goats were estimated. This was in close harmony with the respective 2% and 4% FMD prevalence records of sheep and goats in the Sudan (Abu-Elzein *et al.*, 1987). Serological survey conducted in Saudi Arabia reported that 20% of sheep and 9% goats had sero-converted to FMD virus infection (Hafez *et al.*, 1994). Although the proportion of positive sera of small ruminants obtained in this study is small, the result clearly demonstrates the involvement of small ruminants in FMD occurrence in the study area. Similar serological evidence was reported in Morocco by Blanco *et al.* (2002) and in Ethiopia by Legesse (2008). Furthermore, FMD epidemics have been confirmed in several countries as the consequence of sheep and goats involvement such as in Turkey (Samuel *et al.*, 1999), Balkan countries (Leforban, 1996), Morocco (Donaldson, 1999), Greece (Tsaglas, 1995), United Kingdom and Ireland (Uppal, 2004).

The magnitude of FMD prevalence significantly varied across the Districts during the study period in cattle. The highest prevalence of 17.74% obtained in Itang District might be attributed to the seasonal occurrence of flooding. In the heavy rainy season the Baro River overflow the banks and floods wide areas of grazing plains. During this time the Nuer agro pastoralist moves up their animals from the river banks and lives in higher or uplifted limited dried areas of Itang District especially along the main road side. Moreover, by this time even

from neighboring Districts of the region herd keepers frequently move their animals to Itang District and stay there until the flood recedes and, therefore, highly contagious diseases like FMD can be exacerbated. This finding was in agreement with the works of Valarcher *et al.* (2005) and Renyemamu *et al.* (2008). The authors reported that flooding of the Mekong River in Vietnam, Cambodia and Laos displaces farming families and their livestock every 2-3 years to seek temporary refuge on higher land where animals are aggregated at higher density and rapid transmission of FMD at such times is common. When animals return to their villages as the flood recedes, FMD viruses are seeded out into the communities and generate slowly moving epidemics spreading from place to place.

The second highest prevalence of 11.86% recorded in Gambella District may be associated with contact of animals at marketing place. In selected Districts for the present study there was neither an organized livestock market nor a known market day except for Gambella. For the purpose of access of market; cattle, sheep and goats have been frequently transported from various corners of the region sometimes from the nearby regional states to Gambella and, hence, possibility of occurrence of infectious diseases like FMD could be high. This finding agrees with a number of previous studies that have described contact of animal at marketing places as one of an important contributing factors for the occurrence of FMD virus dissemination (Vosloo *et al.*, 2002; Leforban and Gerbier, 2002; Sahle, 2004; FAO, 2006).

Significant sero-prevalence association was observed between cattle frequently having contact with wildlife versus those rarely communicated with wildlife. The risk analysis result showed those frequently having contact with wildlife was more likely to be at risk than those with rare contact. Except Lare all study Districts are partially engulfed in Gambella national park. Domestic and wild animal interaction was common because the park was not protected from animal entry. FMD potential reservoirs such as African buffalo, warthog, bush pig, antelopes live in the park and may be acting as source of infection for FMD. Several studies in southern African countries have shown the involvement of wild animals in the maintenance and transmission of SAT types of FMD virus (Sutmoller *et al.*, 2000; Hargreaves *et al.*, 2004; Sangare, 2005).

Breeding of cattle and small ruminants together is associated with high risk of FMD sero-conversion as the result of infection. Similar studies in Nigeria (Obi and Newman, 1988) and South America (Barnett and Cox, 1999), where communal farming of cattle, sheep and goats

occur, identified a high prevalence of antibodies against the virus infection associated with sheep and goats in the absence of clinical signs. This as well suggests that small ruminants have an important role in the epidemiology of FMD.

The potential risk of herd size variation in FMD occurrence showed that an increment of seropositivity with herd size expansion implies the importance of herd size in the epidemiology of the disease. Herds with large number of animals were found significantly more at risk of contracting FMD as compared to small and medium herd sizes. This could be attributed to crowding of animals caused by large herd size that can facilitate frequency of direct contact and hence enhances chances of transmission. The study conducted around South Omo Zone and Borana pastoral area reported a similar effect (Bayissa, 2009; Molla, 2009). Moreover, as already said for infectious disease in densely populated animals FMD may spread rapidly because of the high level of challenges from infected animals (Fraser, 1991; Seifert, 1996; Radostits *et al.*, 2000).

Age specific prevalence assessment has witnessed the ascending status of FMD magnitude with increasing of animal age. This may be due to the cumulative exposure of cattle population to the agent (Murphy *et al.*, 1999). Aged animals might have acquired the infection from multiple serotypes, and could have produced antibodies against those serotypes. The relatively low sero-prevalence in animal groups below two years old might be indicative of the existence of passive maternal immunity and low frequency of exposure (Bayissa, 2009; Nigussie, 2009). The significant prevalence variation observed between the sex groups of cattle may be related to the effect of the higher number of females included in the sample than males due to the fact that pastoralists are usually keeping few males only for breeding purpose.

Out of the total number of 338 cattle examined 124 (36.69%) animals manifested clinical signs and lesions suggestive of FMD. Legesse (2008) and Nigussie (2009) reported 53% and 28.2% clinically sick animals after conducting a number of outbreak investigations in different parts of the country, respectively. This is supported by the works of Kitching *et al.* (2005) and McLaws *et al.* (2006) who justified that variations in clinical severity and manifestation are associated with the strains and infection dose of the virus, species, individual susceptibility of the host and the farming system.

Characteristic signs and lesions suggestive of FMD were not observed in all clinically examined small ruminants. This is supported by the work of Barnett and Cox (1999) who verified that FMD in naturally acquired infection often takes on a milder form in small ruminants than cattle and pigs, and in many cases may be indistinguishable. However, it showed disparity with the findings of Legesse (2008) who reported 3.3% sick heads out of 396 clinically examined small ruminants from 7 different outbreak sites. The chance of getting the case may be increased in more number of outbreaks than only investigating a single epidemic.

During the study period FMD virus serotype SAT-2 was identified from bovine species. The virus was responsible for the occurrence of the investigated outbreak in the area. Previously serotype SAT-2 was isolated and reported from cattle in Addis Ababa (Dejene, 2004), Borana pastoral area (Gelaye *et al.*, 2005) and Benshangul-Gumz (Ayelet *et al.*, 2009). Recently the presence of serotype SAT-2 specific antibodies in cattle in Borana pastoral area was reported (Rufael *et al.*, 2008). Bastos and Sangare (2001) concluded that serotype SAT-2 occurs throughout sub-Saharan African countries and its frequent association with wildlife, particularly African buffalo has been indicated (Sangare, 2005; Sahle *et al.*, 2007).

The presently identified SAT-2 serotype was clustered to topotype XIII along virus isolates of Benshangul-Gumz and the Sudan isolates. This is in support of the findings of Ayelet *et al.* (2009), who reported SAT-2 virus belonged to topotype XIII along with the Sudan isolates. All these collective facts can obviously indicate that SAT-2 was introduced to the present study Districts in three possible ways; the first way of entry of the virus might be related to communal resource utilization and free movement of animals across the border because SAT-2 is endemic to the Sudan republic (Vosloo *et al.*, 2002; OIE, 2001). Secondly, the virus may be originated from Benshangul-Gumuz because of the two Regions' geographical close proximity. The third likely source of infection to the area is the persistence of the virus in wildlife and its probable circulation between the wildlife and domestic animals. But the virus has not been detected before because investigation was not conducted so far in the area.

The FMD virus genetic material (RNA) detected in goats further substantiated the serological evidence and showed the involvement of small ruminants in FMD occurrence in the study area. This agreed with the work of De Clercq *et al.* (1997) who confirmed the presence of FMD virus RNA in OP fluid specimen collected from sheep and goats. Barnett and Cox

(1999) concluded that sheep and goats are most likely involved in the transmission of FMDV during the early stages of either clinical or subclinical FMD infection, rather than when they are carriers, and the period of greatest risk of transmission is up to 7 days after contracting the infection.

The SAT-2 viruses isolated during the study had antigenic similarity with SAT2/ERI and SAT2/ZIM vaccine strains and hence using these vaccine strains could provide good protection for the current virus isolates. However, SAT2/ERI vaccine strain is found more protective than SAT2/ZIM vaccine strain.

The current questionnaire survey results have allowed us to generate relevant epidemiological information not shown by serological survey result. Hence, the long standing nature of the disease in the area and the communities' understanding about FMD was determined. Disease ranking descriptive summary indicated FMD was the fourth most important cattle health problem in the area. Comparable observation was reported in three pastoral areas of Ethiopia (Rufael, 2006; Jembere, 2008; Molla, 2009). All selected Districts had experienced FMD many times and, therefore, it was prevalent in the study area. Previously, Asfaw and Sintaro (2000), Sahle *et al.* (2004) and Leforban (2005) reported that FMD is endemic, widely distributed and frequently noted in different farming systems and agro-ecological zones of the country. Temporally, FMD most commonly occurs in the rainy season of the year in the study area. National disease outbreak report database investigation revealed that FMD can occur at any time but common during the wet season of the year. Studies conducted in Mali (Sangare *et al.*, 2004b) and in Ethiopia (Nigussie, 2009) also indicated that FMD is more prevalent in the cold and rainy seasons of the year.

Symptomatic disease identification experience of the herd owners showed that they can identify most of the clinical presentations of FMD very well. Most of the signs described by farmers for FMD were consistent with what was indicated in different reports (Catley *et al.*, 2004; Radostits *et al.*, 1994). In all the study Districts, livestock production system was extensive, animals used communal grazing land and watering points. Free animal movement was a common practice in the area. Introduction of new animals into individually owned herds following purchase for replacement, herd expansion and receiving cultural gifts was usually exercised without any screening for the health status of the new animal. Most of the herd keepers verified that these risk factors act as source of infection and FMD transmission

to their animals. This also implies that farmers of the study area also know some important epidemiological risk factors that could be associated with the disease. Similar opinion was reported by Mersie *et al.* (1992) who stated that the high incidence of the disease in Ethiopia may be associated with extensive movement of livestock and high rate of contact of animals at herd gathering sites. This was further substantiated by the work of MacPherson (1995) who stated that sharing watering points and grazing plots allow contact and intermingling of different herds that would possibly increase the risk of exposure and enhance the FMD virus transmission through the speculated routes.

Despite their existence in large number and regular contact with domestic animals the roles and the involvement of wildlife in the occurrence and transmission of FMD was not known by all the interviewed herds men. The effect of FMD on sheep and goats was uncommon; very few respondents recognized about the participation of small ruminants in the epidemiology of FMD. This finding was in agreement with Armstrong *et al.* (2005) who investigated that in sheep and goats the clinical sign can be very mild or absent, so FMD can spread unnoticed, even to experienced veterinarian. Vaccination against FMD was lacking in the area. Therefore, all cattle and small ruminant population of the study Districts are found at risk of contracting the infection any time.

6. CONCLUSION AND RECOMMENDATIONS

Foot and mouth disease is prevalent in the study Districts. It was verified historically, clinically, serologically and virologically. Livestock resources of the area have been frequently affected by the disease. The involvement of small ruminants in FMD occurrence was confirmed. SAT-2 serotype FMD virus was identified during the study. The virus lies on topotype XIII and geographically clustered along the Benshangul-Gumuz and the Sudan isolates. Except few, most vaccine strains found at WRL did not match with the current isolate which may necessitate the formulation of vaccine from its own local isolates. Factors such as herd composition or the involvement of multiple hosts (cattle, sheep and goats), herd size and individual animal age variability, existence of FMD potential reservoirs (African buffalo, Warthog, Bush pigs, Antelopes), lack of prophylactic vaccination, free cross border movement, communal grazing land and watering point resource utilizations and seasonal occurrence of flood were incriminated as major epidemiological risk factors.

Therefore, the following are offered as recommendations:

- At national level SAT-2 should be considered in vaccine formulation besides types A and O vaccine currently used.
- Regular vaccination should be coupled with the onset of seasonal flooding in the area.
- An extensive regular surveillance in small ruminants and further detailed investigation in wildlife are needed to determine their roles in FMD virus maintenance and transmission.
- Animals should be protected from wildlife park entry to minimize the possible risk of infection through creation of awareness to the community.
- Regional approach of FMD control programs across the border with the Sudan republic and other neighboring regional states is essential.

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8. ANNEXES

Annex 1: Proportional distribution of sampled cattle over the study Districts

District	No. of cattle	Percentage	Sample estimate	Sample size
Lare	34,726	40.6%	$0.406 \times 1,152^*$	468
Itang	23,000	26.9%	$0.269 \times 1,152$	310
Gambella	17,563	20.5%	$0.205 \times 1,152$	236
Abobo	10,234	12.0%	$0.120 \times 1,152$	138
Total	85,523	100.0		1,152

* Cattle sample size proposed for the study, three-fold (1,152)

Annex 2: Proportional distribution of sampled shoats over the study Districts

District	No. of sheep and goats	Percentage	Sample estimate	Sample size
Lare	22,233	40.8%	$0.408 \times 768^*$	313
Itang	16,055	29.4%	0.294×768	226
Gambella	7,500	13.8%	0.138×768	106
Abobo	8,692	16.0%	0.160×768	123
Total	54,480	100.0		768

*Sheep and goat sample size proposed for the study, two-fold (768)

Annex 3: Number of PAs/Kebeles randomly selected for the study within each District

District	Total number of PAs/Kebeles	30% of the total PAs/Kebeles randomly selected for the study
Lare	24	7
Itang	17	5
Gambella	13	4
Abobo	14	4
Total	68	20

Note: PAs/Kebeles with no livestock production practice were excluded and not considered for sampling in each district

Annex 4: Study animals age estimation on the basis of dental eruption

Species	Eruption of teeth	Age estimation in years
Bovine	One incisor	Less than 2
	Two incisors	From 2 - 3
	Three incisors	
	Canine Wear of teeth	Greater than 3
Sheep and goat	One incisor	Less than 2
	Two incisors	
	Three incisors	From 2 - 3
	Canine	Greater than 3

Source: (Merck veterinary manual, 1998)

Annex 5: Bovine blood sample and associated risk factors recording format

Region ____ Zone ____ District ____ PA ____ Village ____ Date ____ / ____ / ____

Animal		sex		Age in years			Herd size			Herd contact		Geo.ref.	
owner's name	ID. No.	M	F	< 2	2-3	> 3	< 50	50-100	> 100	Shoat	Wild life	Lat. N°	Long. E°
	1		x			x		x			x		
	2	x			x		x			x			
	.												
	.												
	1,152												

Annex 6: Sheep and goat blood sample and associated risk factors recording format

Region ____ Zone ____ District ____ PA ____ Village ____ Date ____ / ____ / ____

Animal		Species		sex		Age in years			Flock size			Flock contact		Geo.ref.	
Owner's name	ID No.	Ovine	Caprine	M	F	< 2	2-3	> 3	< 10	10-20	> 20	Cattle	Wild life	Lat. N°	Long. E°
	01		x		x		x		x			x			
	02	x		x				x			x		x		
	.														
	.														
	0768														

Annex 7: Tissue and oro-pharyngeal (OP) fluid sample collection sheet

Region ____ Zone ____ District ____ PA ____ Village ____ Date ____/____/____

Species	Sex		Sample				Geo. reference	
	F	M	Tissue	OP fluid	Number	Pool	Lat. N ⁰	Long. E ⁰
Cattle								
Sheep								
Goats								
Total								

Annex 8: Plate layout of SVANOVIR® FMDV 3ABC-Ab ELISA test

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	P	7	7								
B	N	N	8	8								
C	1	1	etc.	etc.								
D	2	2										
E	3	3										
F	4	4										
G	5	5										
H	6	6										

P = Positive control serum; N = Negative control serum; 1, 2, 3, etc. = Tested serum sample

Annex 9: Plate layout of CHEKIT FMDV 3ABC-Bo-Ov ELISA test

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	05										
B	P	06										
C	N	07										
D	N	08										
E	01	09										
F	02	etc.										
G	03											
H	04											

P = Positive control serum; N = Negative control serum; 01, 02, 03, etc. = Tested serum sample

Annex 10: Foot and mouth disease outbreak investigation recording sheet

1. Location of the outbreak:

Region	_____	Distance from Addis Ababa	_____
Zone	_____	Latitude N ^o	_____
District	_____	Longitude E ^o	_____
PA/kebele	_____	Altitude	_____
Village	_____	Date/month/year	_____

2. Animal owner's name _____

3. Number of animals clinically examined owned by individual farmers:

Species	Sex		Age categories			Total
	M	F	< 2 years	2-3 years	> 3 years	
Cattle						
Sheep						
Goats						

4. Number of animals affected (sick):

Species	Sex		Age categories			Total
	M	F	< 2 years	2-3 years	> 3 years	
Cattle						
Sheep						
Goats						

5. Clinical signs observed (tick):

Salivation	_____	Lameness	_____
Interdigital	_____	Udder lesion	_____
Oral lesion	_____	Others	_____

Annex 11: FMD epidemiological risk factors questionnaire sheet

1. Background information:

Name of respondent _____ Date _____

1.1. Region _____

1.2. Zone _____

1.3. District _____

1.4. PA/Kebele _____

2. Purpose of animal production (tick):

Species	Milk	Meat	Traction	Cash income	Others
2. 1. Cattle					
2.2. Sheep and goats					

3. Major problems of livestock production in the area (rank):

3.1. Disease _____

3.2. Feed and water shortage _____

3.3. Inadequate veterinary service _____

3.4. Others/specify _____

4. Most important livestock disease in order of importance (rank):

4.1. Trypanosomosis _____

4.2. Hemorrhagic septicemic (HS) pasteurellosis _____

4.3. Foot and mouth disease (FMD) _____

4.4. External and internal parasites _____

4.5. Contagious bovine pleura pneumonia (CBPP) _____

4.6. Others _____

5. Occurrence of FMD to the area: common /rare/not known

6. Season of occurrence of FMD: rainy season/dry season/any time

7. Clinical signs associated with FMD occurrence:

7.1. Salivation yes/no

7.2. Lesions on (mouth and foot) yes/no

7.3. Lameness and reluctant to move yes/no

7.4. Teat lesion yes/no

8. Impact of FMD:

8. 1. Mortality	high/low
8.2. Morbidity	high/low
8.3. Reduction of milk yield	present/absent
8.4. Reduction of traction power	present/absent
8.5. Reduction of sale price	present/absent

9. Effect of FMD on sheep and goats: present /absent/not known

10. Possible source of FMD infection:

10.1. Contact of animals at grazing and watering places	yes/no
10.2. Contact of animals at marketing places	yes/no
10.3. Introduction of new animals into herds	yes/no
10.4. Seasonal occurrence of flood	yes/no

11. Grazing land and watering point utilization: Communal/ private

12. Movement of animals:

12.1. Limited to the District	yes/no
12.2. Cross District boundary	yes/no
12.3. Cross regional boundary	yes/no
12.4. Cross national boundary	yes/no
12.5. Cross wildlife park boundary	yes/no

13. Disease in wildlife that can affect domestic animal: known/not known

14. Herd/flock contact history to wildlife: common/rare

15. Vaccination campaign in the village in the last 12 months: present/absent

16. Vaccination campaign in the village in this year: present/absent

17. Animal composition (tick):

- 17.1. Only herd of cattle
- 17.2. Herd of cattle and flocks of sheep and goats
- 17.3. Only flocks of sheep and/or goats

18. Farming system: pastoral/agro pastoral/ mixed farming

19. Local name of FMD _____ meaning _____

Thank you!

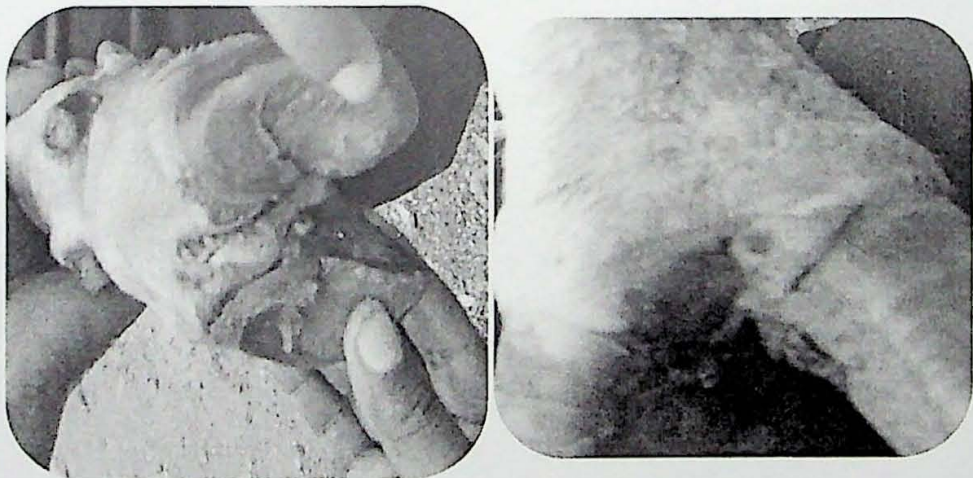
Annex 12: FMD clinical signs observed in cattle during outbreak investigation



Salivation due to acute FMDV infection



Gum and muzzle lesion



Hoof distortion/deformity and inter digital lesion



Interviewing the Nuer family holders through language translator



OP fluid sampling by probang



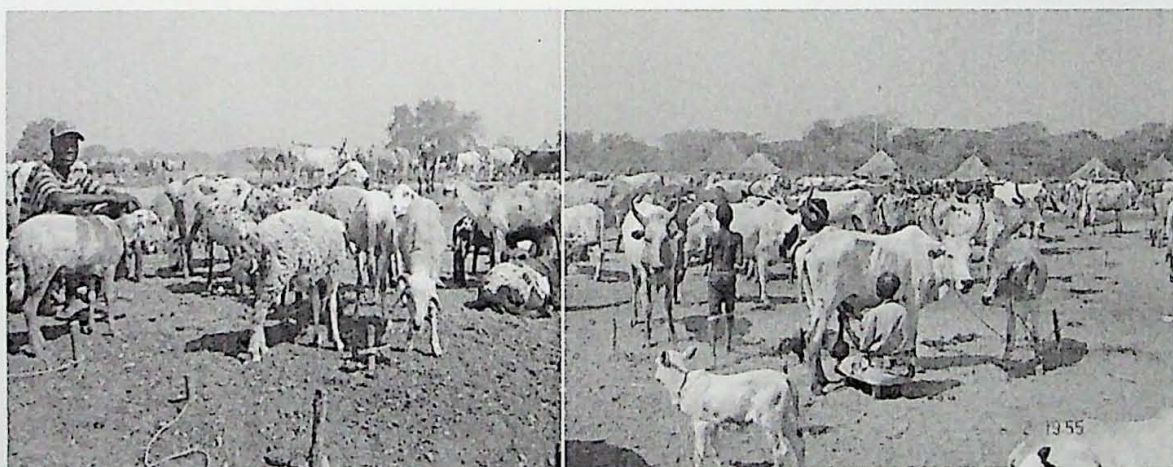
foot examination



Serum separating



Recording GPS reading



Different species and age groups tethered together in the same homestead (Lare-District)