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FACULTY OF VETERINARY MEDICINE

INVESTIGATION OF FOOT AND MOUTH DISEASE OUTBREAKS AND ASSESMENT OF RISK FACTORS IN OROMIA, AMHARA AND SOUTHEREN NATIONS, NATIONALITIES AND PEOPLES (SNNP) REGIONAL STATES OF ETHIOPIA

BY

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DEDICATION

This work is dedicated to my wife Hirut Zeleke and my twin sons Robel Yoseph and Nobel Yoseph

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ABBREVIATIONS

CFT	Complement Fixation Test
ELLISA	Enzyme Linked Immunosorbent Assay
FAO	the Food and Agricultural Organization of the United Nations
ICTV	the International Committee of Taxonomy of Viruses
OIE	International Animal Health Organization
PCR	Polymerase Chain Rection
RNA	Ribonucleic acid
SAT	South African Territories
SNNP	Southern Nations Nationalities and peoples
ssRNA	Single stranded RNA
Vgp	Viral genome protein
VNT	Virus Neutralization Test
VP1—VP4	Viral proteins1-4
WRL	World Referral Laboratory for epidemiology and research on FMD
DZARC	Debre Zeit Agricultural Research Center
Vgp	Viral genome protein
RT-PCR	Reverse Transcription Polymerase Chain Reaction
USAID	United States Aid for International Development
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
UK	United Kingdom
DNA	Deoxyribonucleic acid
Masl	Meters above sea level
OP	Oesophageal-Pharyngeal
CE	Cytopathic Effect
Ig	Immunoglobulin
$US	Dollars of United States
MM	Millimeters
NAHDIC	National Animal Health Diagnostic and Investigation

PP	Percentage positivity
Nm	Nano meter
µl	Micro liter
CI	Confidence interval
OR	Odds ratio
^{O}C	Degree Celsius
AAU	Addis Ababa University
FVM	Faculty of Veterinary Medicine
SPSS	Statistical Package for Social Sciences

ABSTRACT

Seven foot and mouth disease outbreaks were investigated in Oromia, Amhara and SNNP regional states of Ethiopia, November2007 to March 2008. The objective of the study was to determine the epidemiological characteristics of FMD outbreaks and to assess the associated risk factors. Morbidity rates ranging from 22.14 (Torben ashe) to 47.56 (DZARC) and mortality rates 2.33 (Coca) to 0.61(DZARC) were observed. Two viral serotypes namely O and SAT1 were isolated from tissue and oesophageal-pharyngeal (OP) fluid samples submitted to WRL, Pirbright. The isolation of the later serotype (SAT1) is the first of its kind in Ethiopia. Clinical, serological and virological evidences have shown that small ruminants are readily affected by FMD. However, there was a highly significant difference between cattle and these species in manifesting the characteristic clinical signs of the disease, being sub clinical in shoats. Additional risk factors were host characteristics like age and breed of cattle. Morbidity and mortality rates were higher for younger (<2years) and lower for older (>4 years) cattle. Age specific morbidity rates were 46.37% and 27% for these two age groups in Chigit and mortality rates were 8.7% and 1.5% for cattle of the same category in Coca outbreak. In DZARC farm breed specific morbidity rates were 68.4% and 0% for crosses and Borans respectively. Thus susceptibility to FMD varied with species, age, breed and type of viral serotype involved. Furthermore, the absences of working control policy, lack of livestock movement control, involvement of various hosts and the high rate of contact at communal points were found to be the major risk factors of FMD in Ethiopia.

The present situation of FMD in Ethiopia requires an immediate intervention. Therefore, implementation of a profound control strategy involving livestock movement control, strategic vaccination of specific risk groups, consideration of SAT1 serotype, and further investigation on potential risk factors was recommended.

Key words: Epidemiological data, Foot and mouth disease, Foot and mouth disease virus (serotype), Outbreak,Investigation, Rate, Risk factors, Small ruminants

1. INTRODUCTION

Ethiopia has 44 million cattle, 23 million goats, 23 million sheep, 6 million equines, 2 million camels and 42 million chicken playing crucial roles in the livelihoods of the majority of rural people (Leforban, 2005). They provide milk, meat, manure, hides and skin, animal power and financial income (Bogale, 2005). However, the reward derived from rearing livestock is sub-optimal due to various constraints such as lack of proper management and presence of livestock diseases. At present, foot and mouth disease (FMD) is one of the major livestock diseases of socio-economic importance in the country (Sahle, 2004).

Foot and mouth disease is an extremely contagious, acute viral disease of all cloven-hoofed animals and pigs, characterized by fever, and vesicular eruptions in the mouth and on the feet and teats. It is caused by a virus of the genus *Aphthovirus*, family *Picarnaviridae* which has 7 immunologically district serotypes, namely, O, A, C, South African Territories (SAT) 1, SAT2, SAT3 and Asia1 (OIE, 2004). Cattle, sheep, goats, pig and buffaloes are susceptible to FMD. Besides, many species of cloven-hoofed wild animals such as African antelope (Thomson *et al.*, 2003), warthog and bush pig (Hedger *et al.*, 1972), Impala (Thomson, 1996), elephants, llamas and deer (David *et a*l., 1993) may be infected.

FMD is a highly infectious disease transmitted by direct contact, aerosol, and mechanical carriage, on fomites or through animal products (Radostits *et al.*, 2000). Its morbidity rate can approach 100% but mortality is generally less in adults. Greatest losses occur due to dramatic reduction in milk production, retarded growth, and draught power and from abortion and treatment costs (Quinn *et al.*, 2005). In addition, in many instances FMD is the only constraint to opening up lucrative markets of live animal and animal products in the world (Kitching, 1999). Classical example is the 2001 epidemic in United Kingdom (UK), which caused a loss of more than 270 million pounds. Hence, FMD affects the livelihoods of both simple farmers and large sophisticated farming practices and the national and international economies of countries (Bogale, 2005). This was the reason for the Food and Agricultural Organization (FAO) to recognize Pirbright as the World Referral Laboratory (WRL) for epidemiology and research on FMD, early in 1958 (Uppal, 2004)

By one time or another FMD was widely reported from most parts of the world (Pereira, 1981). Today, many countries have either eliminated it by compulsory slaughter of infected animals and movement control or have reduced its incidence greatly by extensive vaccination programs (Radostits *et al.,* 2000). North America, Central America, Australia, New Zealand, Chile, Japan and most of European countries have been recognized as free (Sahle, 2004). However, still the virus is circulating in about two thirds of the International Animal Health Organization (OIE) member countries and the disease is endemic in Africa, Asia, South America and The Middle East (Quinn *et al.,* 2005).

In Sub-Saharan African countries, where six of the seven serotypes (except Asia 1) are present, FMD is an endemic disease cycling in domestic animals and buffaloes (Vosloo *et al.,* 2004). Some countries have controlled the disease by separating infected buffaloes from livestock and by limited use of vaccination (Thomson *et al.,* 2003). Morocco, Swaziland, Lesotho, Zimbabwe, Namibia, Botswana and Republic of South Africa have been considered free of FMD by OIE in1999 (Kitching, 1999).

In Ethiopia, FMD is a notifiable disease and the Federal Veterinary Service sends monthly and annually official reports to OIE. The endemic disease is characterized by expanding boundaries and increasing total incidences (Wondwossen and Tariku, 2000). Recently it had become the major constraint hampering export of livestock and livestock products to the Middle East and African countries; the Egyptian trade ban of 2005/2006, in which Ethiopia lost more than 14 million $US, being a recent memory (Leforban, 2005). Such losses and the strict requirements of international trade warn Ethiopia to control the disease; and to make use of the untapped industry it should rely on one. As indicated by Putt *et al.,* (1988), the choice and implementation of any control measure requires prior identification of specific risk factors of host, agent or the environment. Shale (2004) emphasizes the limitation of data available on most of the factors in Ethiopia. The epidemiological role of small ruminants and wild animals is not defined. The severity of FMD outbreaks and their effect on animals belonging to different breed and age groups has not been determined. Among the ample outbreaks posing remarkable losses, only few are reported. Furthermore reported ones are not fully investigated and only small number of samples is submitted to WRL, Pirbright. While livestock are at risk from endemic as well as exotic serotypes and antigenic variants of FMD

circulating in neighboring countries, records available for the last twenty years reveal the occurrence of O, A, C, and SAT2 FMD viruses in Ethiopia (Gelaye *et al.,* 2001). Prevalences exceeding 20% have been reported in different parts of the country (Sahle, 2004; Rufael, 2006). However, at present there is no formal FMD control policy in Ethiopia (Bogale, 2005).

Content to the prevailing reality, attempt made towards the control of FMD in Ethiopia has a lot of tasks to accomplish the main being those generating information on the above gaps. Then it is true that well managed FMD outbreak investigations will come up with reliable inputs.

Therefore, the objectives of this study are:

- To investigate FMD outbreaks and determine their characteristics in different parts of Ethiopia
- To isolate the viral serotypes causing the outbreaks
- To assess the epidemiological risk factors associated with the disease

2. LITERATURE REVIEW

2.1. Definition

Foot-and-mouth disease (FMD) is an extremely contagious, acute viral disease of all cloven-hoofed animals and pigs, characterized by fever, loss of appetite, salivation and vesicular eruptions in the mouth, on the feet and teats and sudden death of young stock (Quinn *et al.,* 2005).

2.2. Etiology

2.2.1. Classification

The virus causing FMD was defined in 1963 by the International Committee of Taxonomy of Viruses (ICTV) as belonging to the genus *Aphthovirus* of family Picornaviridae. The name Picornaviridae is derived from the Latin words 'pico', which means small and 'rna' which refers to the RNA genome. The generic name *Aphthovirus* is derived from the Greek word 'aphta' which means vesicles in the mouth (Matthews, 1979*).* The genus *Aphthovirus* is divided into seven serotypes on the basis of *invitro* cross-protection tests and serological tests. These are O, A, C, South African Territories (SAT1), SAT2, SAT3 and Asia1. Each type is further subdivided into sub types on the basis of quantitative differences in cross-protection and serological tests (OIE, 2004).

2.2.2. Morphology

The foot-and-mouth disease virus is characterized by consisting of naked nucleocapsids approximately 22-25nm in diameter and possessing icosahedral symmetry (Robert and Bruce, 1981). The capsid consists of 60 capsomers each consisting of four proteins (VP1-VP4). The single stranded RNA genome is 8500 nucleotides in length and encodes a single open reading frame (Bittle *et al.,* 1982). The single stranded RNA (ssRNA) has a molecular weight of $2.6x10^6$ and acts as a messenger for the synthesis of virus proteins in the infected cell (Quinn *et al.,* 2005). Viral proteins (VP1-VP3) are exposed on the surface whilst VP4 is located internally at the pentameric apex of the icosahedrons. The VP1 is the most antigenic protein involved in cell attachment and carries an immunologically important GH loop (Barteling, 2002).

2.2.3. Genomic organization

The FMD viral genome consists of a positive sense single stranded RNA (ssRNA), approximately 8500 base long with a poly A tail at its 3' end and a viral genome protein (Vgp) at its 5' end (Bittle *et al.,* 1982). Four poly proteins (L1, P1, P2 and P3) are translated and processed into the different structural and non- structural proteins by viral encoded proteases (L^{pro}, 2A, Oligopeptide and $3C^{pro}$).

The L protein represents the leader protein, where two initiation sites (AUG codons) have been identified in FMDV, namely Lab and Lb (Sangar *et al.*, 1988). The P1 gene product is the precursor of the capsid proteins 1D, 1B, 1C and 1A. Firstly, the intermediate P1 precursor is processed with the help of viral protease $3C^{pro}$ to produce VPO, VP1 and VP3 where the products combine to form empty capsid particles. The mature virion is produced after the encapsidation of the virion RNA that is accompanied by the cleavage of VPO to VP2 and VP4. The P2 (2A, 2B, 2C) and P3 (3A, 3B, 3C, 3D) regions encode for non-structural proteins that are involved in viral RNA replication and protein processing (Belsham, 1993).

2.2.4. Physicochemical characteristics

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). FMDV is inactivated when exposed to pH below 6.5 or above 11. It can also be inactivated by a number of chemical substances at the acidic and alkaline pH ranges. However, it is stable between pH 7 and 9 at 4^0C and -20^0C (Wilks, 1992). In milk and milk products, the virion is protected, and can survive at 70^0C 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 NaOH or KOH and 4% Na_2C0_3 are effective disinfectants for FMDV contaminated objects, but the virus is resistant to alcohol and phenolic quaternary ammonium disinfectants (Russell and Edington, 1985). 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). 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. epithelial fragments (Donaldson, 1987).

2.2.5. Genetic variation

During replication FMDV undergoes a high rate of mutation. This is mainly due to lack of replication error checking mechanisms. RNA viruses, which exhibit such a deficiency, mutate at the rate of one nucleotide base change per 10^3 bases per replication cycle (Holland *et al.,* 1982). It is also estimated that a mutation rate of up to 10^{-8} – 10^{-9} nucleotide substitution per year during an epizootological cycle of FMD viruses can occur. Therefore, new variants of FMD viruses are continuously arising after each replication cycle, which constitutes an intratypic population of FMDV with different degree of genetic relationships (Domingo *et al.,* 1990). Modifications of the genomes of viruses isolated from persistently infected cattle and buffalo and during replication in cell culture have also been reported to occur. Generally, changes in the nucleotide compositions of the capsid genes are responsible for the genetic or antigenic variability of the virus (Meyer *et al.*, 1994; Vosloo *et al.,* 1996). It has been shown that genetic recombination occurs between viruses of the same serotype as well as between serotypes. Mutation through recombination could result in the exchange of genetic material that could lead to the generation of new antigenic variants that may escape immune pressure (King *et al*, 1982).

2.2.6. Antigenic variation

Among the capsid proteins, VP1 is the most antigenic protein and carries the domain mainly responsible for antigenic heterogeneity and cell-virus interaction (Rueckert, 1996). Changes to the genes encoding capsid proteins can result in antigenic variation and evolvement of new subtypes (Haydon *et al.,* 2001*).* Thus, not only is there no cross protection between FMDV serotypes, but also vaccination with one antigenic variant of serotype does not necessarily protect an animal when challenged with a different virus of the same serotype (Sangare, 2002). Initially over 60 different subtypes were identified by world Referral Laboratory (WRL), but it quickly became apparent that there is a continuous spectrum of intratypic antigenic variants, making a difficulty to identify specific subtypes (Quinn *et al.,* 2005).

2.2.7. Serotypes and subtypes

Serotype and subtype differentiation within viruses are based on the complete or partial lack of cross protection between FMD viruses. Currently, there are seven serotypes of FMDV, namely O, A, C, South African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. Within these types over 60 subtypes have also been described and new subtypes occasionally arise spontaneously. The importance of subtype is that a vaccine may have to be tailored 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. Epidemiology

2.3.1. Distribution

At one time or another, FMD used to occur in nearly all parts of the world (Quinn *et al.,* 2005). Today, many countries have eliminated it by compulsory slaughter of infected animals and movement control. Others have greatly reduced its incidence by extensive vaccination programmes (Radostits *et al.,* 2000). North America, Central America, Australia, New Zealand, Chile, Japan and most of European countries have been recognized as free and Argentina have not had outbreaks since April 1994 (Sahle, 2004). However, the virus is circulating in about two third of the OIE member countries and the disease is endemic in Africa, Asia, South America and The Middle East (Quinn *et al.,* 2005). In sub-Saharan Africa, FMD is endemic, six of the seven serotypes (except Asia1) being present in most of the countries with marked differences in distribution and prevalence (Vosloo *et al.,* 2002). Morocco, Swaziland, Lesotho, Namibia, Botswana, Zimbabwe and the Republic of South Africa have been considered free by the OIE in 1999 (Kitching, 1999).

The classical European types, A, O and C are present worldwide, while the SAT types (SAT1, 2 and 3) and Asia1 are restricted to sub-Saharan Africa and Asia, respectively (Kitching, 1999). The C serotype appears to have disappeared from the whole world with the exception of Kenya, where it occurred last in 2000 (Kitching, 2002a). Historically this serotype is limited to the occasional

records in the North (Tunisia), East (Kenya, Ethiopia, and Uganda) and South (Angola) (Pereira, 1981).

Table1: FMD viruses commonly isolated from different parts of the world

Continent	Virus serotypes
Europe (historically)	A, O, C
Asia	A, O, C, Asia 1
Africa	A, O, C, SAT 1, SAT 2, SAT 3
South America	A, O, C

Source: Quinn *et al.,* (2005)

2.3.2. Susceptible hosts

Foot-and-mouth disease affects over 70 species of domestic and wild cloven-hoofed animals (Hedger, 1981*).* It naturally infects and causes disease in cattle, pigs, sheep, goats and many wild ruminants like African antelope (Thomson *et al.,* 2003), warthog and bush pig (Hedger *et al.,* 1972), impala (Thomson, 1996), elephants and llamas (David *et al,* 1993), although apart from the African buffalo, their involvement in the epidemiology of FMD in the domesticated species is uncertain (OIE, 2004). The susceptibility of cloven-hoofed species can vary with breed of animal and strain of the virus. FMD is considerably less obvious or sub-clinical in breeds of cattle, sheep and goats indigenous to Africa and Asia, where it is endemic. FMD is not zoonotic, and only a few possible cases of infection of humans have been described (Hyslop, 1973), but man is an important mechanical carrier of the virus. In FMD outbreaks sheep, pigs and cattle act as maintenance, amplifier and indicator hosts (Radostits *et al.,* 2000).

2.3.3. Carriers of FMD virus

Animals in which the FMD virus persists in the oropharyngeal region for more than 28 days post infection are called carriers (OIE, 2004). Carrier ability of animals depends on the ability of the virus to persist in the pharyngeal area of animals, which signifies a special virus-host relationship while the duration of the state of persistence varies from species to species. This is up to five years for African buffaloes, 3 years for cattle, 9 months for sheep and 3-6 months for goats (Bastos *et al.*, 2000; Condy *et al.,* 1985). This may provide a mechanism for the maintenance of the virus in nature and the cause of acute episodes of disease and may also contribute to the emergence of new antigenically variant viruses (Domingo *et al.,* 1992).

2.3.4. Sources of infection and transmission

FMD is highly contagious and can be transmitted by direct contact between infected and susceptible animals (Woodburry, 1995). The viruses can survive in dry blood and defragmented epithelium in the environment for varying periods of time. The source of the 1967/68 outbreak of FMD on the British mainland was attributed to infected sheep meat imported from Argentina (Leforban and Gerbier, 2002). Similarly, the 1997 epidemic in Taiwan was suspected to be due to illegal imported meat from China (Kitching, 1999). In addition, vehicles and fomites have been responsible for transmission of the disease (Sellers, 1971). A person in contact with infected animals can serve as a source of infection for 24 hours post infection, and dogs, cats, horses and birds can transmit the disease mechanically (Hyslop, 1973; Bavor, 1997). Cattle, sheep and goats infected with FMD can excrete between 10^3 to 10^8 infectious virus units per day as an aerosol, while that of infected pig can be as high as 4×10^6 infectious units per day (Mann and Sellers, 1990; Kiching, 1992). Cattle are very susceptible to infection by the respiratory route and a dose of 20 $TCID_{50}$ (Tissue culture infective dose) of virus is sufficient to establish infection (Donaldson *et al.*, 1987). During the 1981 FMD outbreak virus spread from France to UK over 250 km across the sea. Given suitable conditions, it can also spread 60 km across the land (Kitching, 1992).

At present there are computer models which can predict the most likely windborne spread of the virus from infected herds and allow the examination of a variety of control strategies (Sanson *et al.*,

1991). Outbreaks of FMD can occur because of viruses escaping from research and vaccine production centers (Fraser, 1991) and the semen of infected bull can be a source of infection by artificial insemination (Radostits *et al.,* 2000).

2.3.5. 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).

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 while different subgroups (genotypes, topotypes) were defined by difference of 15 to 20% (Knowles and Samuel, 2003). Since 1987, the analysis of the genetic distance and phylogenetic resolution of the sequence of VP1 encoding gene have provided crucial epidemiological information covering different degree of genetic relationships between field isolates as follows: virus isolates from the same epizootic differ by ≤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 ≥ 20% (Sahle, 2004).

The evolutionary changes of viruses are determined by comparing genomic material from more than one virus with each other. The basic process in the evolution of DNA/RNA sequence is the substitution of one nucleotide for another over evolutionary time. Changes in nucleotide sequences are used in molecular evolutionary studies both for estimating the rate of evolution and for re-constructing the evolutionary history of organisms (Graur and Wen-Hsiung, 2000).

2.4. Clinical signs

The incubation period of FMD is 3-14 days and excretion of the virus in excretions and excretions usually begins before the appearance of visible clinical signs (Kitching, 2000a). 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 its degree of immunity. The signs can range from a mild or inapparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2004).

2.4.1. Clinical signs in cattle and pigs

Acutely infected cattle become febrile (about 40^0C), and dull, after which they stop feeding and reduce milk production. Then there appears abundant salivation, the saliva hanging in long, ropy strings, a characteristic smacking of the lips, and leads to nasal discharge (Quinn *et al.,* 2005). This is accompanied by vesicle formation on the dorsum of the tongue, hard palate, dental pads, lips, gums, muzzle, coronary bands and interdigital space with consequent lameness (Woodburry, 1995). The vesicles usually rupture within 24-48 hours leaving shallow erosions and animals become reluctant to eat and move. Other signs are licking of the feet or shifting weight from one leg to the other, holding one hoof off the ground, lagging behind the herd, lying down and reluctance to rise (Sahle, 2004).

Vesicles may also be seen on the teats of lactating cows. Young calves may die before the appearance of clinical signs due to virus infection of the developing heart muscle and the production of severe myocarditis (Radostits *et al.,* 2000). In calves mortality can exceed 50%, but in general, mortality is less than 1% in adult animas. Morbidity can approach 100% (Woodburry, 1995). Most animals recover within 2-3 weeks, but secondary complications may delay the recovery of feet and teat lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight and breeding problems. Pregnant animals may abort and chronic panting syndrome characterized by dyspnoea, anorexia, and hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD (Burrow *et al.,* 1981).

In swine the initial signs are fever (about 40.6^0C), anorexia, reluctance to move and squeal when forced to move. Vesicles form on the coronary band, heels, and snouts and in interdigital space. Foot involvement is usually severe but oral lesions are not too common. There is no drooling but sows may abort and piglets may die with out showing any clinical signs (Radostits *et al.,* 2000).

2.4.2. Clinical signs in small ruminants

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

2.5. Pathogenesis

Studies on the pathogenesis of FMD indicate that the main route of infection is the respiratory tract. Initial virus multiplication takes place in the pharynx and subsequently spreads to lungs and other tissues, virus being detected in blood, milk, pharynx, vagina and rectum before appearance of clinical signs (Quinn *et al.*, 2005). The virus is then distributed throughout the body, to reach best sites of multiplication such as the epithelium of oropharynx, oral cavity, feet, the under and heart. Viral excretion commences about 24 hours before the onset of clinical disease and continues for several days. Virus infectivity titers reach high levels at early stage (about 1 week) of the disease and subsequently show a gradual decline coinciding with the development of virus neutralizing antibody response (Murphy *et al.,* 1994).

2.5. Diagnosis

The diagnosis of FMD is based on clinical, epidemiological and laboratory observations. Rapid diagnosis, in each case, is of paramount importance, especially in countries that are usually free of infection, so that quarantine and eradication programmes can be implemented as quickly as possible (OIE, 2004*).*

2.5.1. Tentative diagnosis

A presumptive diagnosis is justified when a rapidly spreading disease characterized by vesicular lesions on the feet and in the mouth is observed affecting cattle, pigs, sheep and goats (Pereira, 1981). In cattle, FMD should be considered whenever salivation and lameness occur simultaneously and when a vesicular lesion is seen or suspected (Radostits *et al.*, 2000). However, when the disease is limited to hosts of a single species it is difficult or impossible to differentiate clinically from other conditions such as Vesicular Stomatitis, Vesicular Exanthema of pigs, Vesicular Disease, Blue tongue, Pox, Foot rot, Rinderpest, Malignant Catarrhal Fever and the Bovine Herpes infection (Quinn *et al.*, 2005).

2.5.2. Laboratory diagnosis

In FMD laboratory, virus can be isolated on cell culture or the virus antigen detected using ELISA. There are techniques that detect the presence of antibodies to the structural proteins or non-structural proteins. The presence of viral genomic material can be detected using PCR assays. Since FMD is highly contagious and economically important the laboratory diagnosis and serotype identification should be done in a virus-secure laboratory (OIE, 2004).

Laboratory specimens

For laboratory diagnosis of FMD, appropriate samples include vesicular fluid and epithelium from early or recently ruptured vesicles (OIE, 2004). When epithelium tissue is not available, e.g. in advanced or convalescent cases and infection is suspected in the absence of clinical sign, samples of

esophageal-pharyngeal fluids (OP) fluid is collected by means of a probang and used for virus isolation (Bogale, 2005). Other samples include blood with anticoagulant, serum, lymph nodes, thyroid gland, kidney, heart and adrenal gland from postmortem (Radostits *et al.*, 2000).

Virus isolation

The isolation and characterization of the virus is the "golden standard" test for the diagnosis of FMD. The suspensions of field samples suspected to contain FMDV are inoculated into cell culture. In most laboratories primary cell lines are used for virus isolation and IBRS-2 (Instituto Biologico Rim Suino, De Castro, 1964) and BHK (baby hamster kidney) for virus propagation. Primary pig, calf, or lamb cells can be used instead. The specimen is incubated at 37 and examined for cytopathic effect (CPE) 24-48 hours after inoculation.

Serological Tests

Other tests used for the detection of either antigen or antibody may be used e.g. the Complement Fixation Test (CFT), Virus Neutralization Test (VNT) and Enzyme Linked Immunosorbent Assay (ELISA) (Anonymous, 1996). Liquid phase blocking ELISA detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals (Hamblin *et al.*, 1986a). On the other hand, the detection of antibody to the poly protein 3ABC proteins is useful indicator of FMDV infection with any of the seven serotypes of the virus. Antibody to this 3ABC is only found in virus-infected animals but not in vaccinated animals (Mackay *et al.,* 1998).

The Polymerase Chain Reaction (PCR) can be used to amplify the genome fragments of FMDV in diagnostic material. It is an extremely sensitive and rapid assay that allows the detection of genetic material even in the absence of infectivity for tissue culture or laboratory animal. This was demonstrated from samples of esophageal-pharyngeal scrapings of carrier cattle taken at 180 and 560 days post infection (Laor *et al.*, 1992; Murphy *et al.,* 1994), in aerosols (Suryanaryana et *al.*, 1999), in cell culture isolates (Laor *et al.*, 1992; Callens and De Clercq, 1997), skin and tongue epithelium (Laor *et al.*, 1992) and blood (Bastos, 1998).

2.7. Immunity

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The level of immunity against FMD coincides with development of neutralizing antibodies. Infection with one serotype produces complete protection against homologous virus, but little or no protection against heterologous viruses (Fraser, 1991***).*** 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 IgG-1 antibodies have developed. The localized antibody response, specific to anti FMD IgM and IgA antibodies in the pharyngeal fluid of cattle develops 7 days after exposure to the virus, while IgG activity reaches 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). There is also a cellular response mediated by T-helper and T-cytotoxic cells that plays a role in immune response to FMDV infection (Sanz Parra *et al.,* 1998).

2.8. Control, prevention and eradication

The official attitude of a country regarding control of a disease depends on how seriously the disease affects the country, on the financial and technical ability of the country and what its neighbors are doing (Boagle, 2005). The procedures commonly followed are control by eradication and control by vaccination or a combination of the two (Radostits *et al.,* 2000).

2.8.1. Endemic regions

For most of the world, regular vaccination and movement restriction of animals are the ways of life and vaccine production is a major industry. The development of *in vitro* methods, have increased the production capacity of inactivated FMD vaccines to the point of meeting worldwide demands (Quinn *et al.,* 2005). Vaccination policies 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 and vaccination programme must ensure that no less than 85% of the cattle population is vaccinated at intervals sufficiently short to maintain constant immunity (Bogale, 2005*).* Therefore bi-, tri or polyvalent inactivated vaccines containing the representative strains of the serotypes circulating in the region, must be used (Gonzalez *et al.*, 1992).

In ruminants primary vaccination induces immunity that lasts for about 3-6 months. Therefore, frequency of vaccination varies from once to three times a year in different countries (Radostits *et al*., 2000). For proper protection, calves originating from vaccinated and unvaccinated dams should be vaccinated at about 6 months and 2 weeks of age, respectively (Garland, 1999). Also, it is important that vaccines be officially approved after quality control tests including identity of virus types, potency and innocuity, and stored and administered under conditions that ensure preservation of potency. Under the best conditions, vaccination can markedly reduce disease incidence, but when used alone, it can at best maintain endemicity at low levels. Once this point is achieved, however, the possibility of combining vaccination and slaughter polices become feasible and a change of strategy towards eradication can be envisaged (Pereira, 1981).

The control strategies followed by many African countries include the slaughter of infected animals and animals at risk, control of contact between infected wildlife and domestic animals by fencing and ring vaccination, control of cattle movement and the introduction of strict quarantine measures (Sahle, 2004*)*

2.8.2. Disease free regions

In disease free regions the main objective is to prevent the introduction of infection. Here, rapid diagnosis is essential, followed by quarantine of the premises (including movement of personnel and vehicles) followed by slaughter and disposal of the carcass (by burning or burial) and decontamination of the premises (Fraser, 1991). This measure, also called ‘stamping out’ requires the slaughter of in-contact and other herds in which there is no clinical evidence of the disease and payment of full compensation paid to livestock owners (Radostits *et al.,* 2000). The United Kingdom

(UK), Ireland, countries of Scandinavia, Canada and USA were able to control FMD by this method. 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).

2.9. Economic importance

Foot-and-mouth disease has considerable economic consequences, which can be attributed to both direct and indirect costs. The main effect of the disease is due to its high infectiousness (Radostits *et al.,* 2000) and direct economic loss via loss of milk production, loss of draught power, retardation of growth, abortion in pregnant animals, treatment costs and death of calves, lambs, kids and piglets. The indirect losses are attributed to the disruption in trade of animals and animal products, the effect of which is higher than the acute illness (Woodburry, 1995). Thus loss in animal production and international trade restrictions imposed following an outbreak make FMD a major concern. The 1967/68 FMD epidemic in England, in which 211825 cattle, 108345 sheep 113766 pigs and 51 goats were slaughtered, caused a loss of over 35 million pounds. The control of FMD outbreak in Taiwan, during 1997 required about 378.6 million USD (Kiching, 1999*)*. In 2001, England also faced an FMD outbreak with huge economic consequence for culling, disposal and compensation that ranged about 270 million pounds. Similarly, the Egyptian trade ban of animal and animal products, in 2005/2006, caused Ethiopia a loss of more than 14 million $US (Leforban, 2005).

Since the year 1921, types O, A and C foot and mouth disease viruses have been isolated from people in Europe, Africa and South America (Hyslop, 1973). However, since infection is uncommon in human beings, FMD is not considered a public health threat (Bogale, 2005).

2.10. Foot-and-mouth disease in Ethiopia

In Ethiopia, foot-and-mouth disease is a notifiable disease and the Federal Veterinary Service sends monthly and annually official reports to OIE (Leforban, 2005). In the past, outbreaks used to occur in the pastoral herds of the marginal lowland areas of Ethiopia. However, this trend has been changed and currently the disease is frequently noted in the highlands of the country. During the period extending from 1990 to 2000, the incidence of FMD outbreaks had increased by 1.3 to 1.5 folds. Extensive livestock movement and the high rate of contact among animals at communal grazing areas, watering points and commercial markets were associated to the increasing incidences (Sahle, 2004).

Four of the seven serotypes of FMD viruses are endemic in Ethiopia. The occurrence of serotypes O and C was confirmed in 1957. Then in 1969 and 1988 serotypes A and SAT2 were identified, respectively (Pereira, 1981; Roeder *et al.,* 1994). Knowledge available on the epidemiology of FMD in Ethiopia including spatial and temporal distribution and importance of various hosts is limited. Moreover, there are no policies and guidelines enforcing the prevention and control of FMD in Ethiopia (Sahle, 2004; Bogale, 2005).

2.11. Epidemiological investigation of disease

A description of a disease problem should specify the disease and population at risk, give information on the distribution of events in time and space, and include an attempt to quantity disease events (Putt *et al.,* 1988).

2.11.1. Disease diagnosis and determination of population at risk

This phase of epidemiological investigation enables the confirmation of disease presence. On the other hand, studying the distribution of the disease within host populations by species, breed, age and sex can identify population at risk. Descriptions of population densities and movements are also of great value (Thrusfield, 2005).

2.11.2. Distribution of disease events in time and space

This generally involves looking for the "clustering" of disease events in time, space or both. The clustering of disease events in space can often be demonstrated by the use of conventional mapping techniques. This type of clustering may indicate the presence of a particular determinant or determinants in an area. Clustering in space occurs, naturally in the case of contact transmitted diseases, but it may also be a function of host-population density (Pfeiffer, 2002). The distribution of disease events in populations in time and space can be described by three basic descriptive terms: endemic, epidemic and sporadic.

Endemic

An endemic disease is a disease that occurs in a population with predictable regularity and with only minor deviations from its expected frequency of occurrence. In endemic diseases, disease events are clustered in space but not in time. A disease may be endemic in a population with predictable regularity. Additional terms can be used to describe endemic diseases according to their frequency of occurrence. Thus Hyperendemic, mesoendemic and hypoendemic are used to describe endemic diseases that affect a high, moderate and small proportion of the population at risk, respectively (Thrusfield, 2005).

Epidemic

An epidemic disease is a disease that occurs in a population in excess of its normally expected frequency of occurrence. Here, disease events are clustered in time and space. A disease may be epidemic even at a low frequency of occurrence, provided that it occurs in excess of its expected frequency. A large epidemic affecting several countries or even one or more continents is termed as pandemic (Pfeiffer, 2002).

Sporadic

A sporadic disease is one that is normally absent from a population but which can occur in that population, although rarely and without predictable regularity. Many epidemics of infectious

diseases occur in a regular cyclical fashion over a prolonged period of time. This is because with an increasing frequency of occurrence of disease in a host population, then the number of susceptible host decreases as individuals within that population become infected and then either die or recover and become immune to re-infection (Thrusfield, 2005).

As the number of susceptible hosts decreases, so does the opportunity for disease transmission this, in turn, means that the frequency of occurrence of new cases of the disease declines. A period of time, then lapses during which new susceptible individuals are born into the host population. The number of susceptible hosts in the population thus increases, and the opportunities for the disease agent to find susceptible host are enhanced. As a result the frequency of occurrence of the disease may increase and a new epidemic may occur (Putt *et al.,* 1988).

2.11.3. Quantification of disease events in population

As indicated in Thusfield, (2005) data used to quantify disease events in a population are often dichotomous in nature and they are frequently presented in the form of an epidemiological rate. In epidemiology, a rate can be defined as the number of individuals having or acquiring a particular characteristic (normally an infection, a disease or a characteristic associated with a disease) during a period of observation, divided by the total number of individuals at risk of having or acquiring that characteristic during the observation period. The expression is then multiplied by a factor, normally a multiple of 10, to relate it to a specified unit of population.

Rates are commonly expressed as decimals, percentages or events per standard units of population e.g. per 1000, 10000 animals etc. This produces a standardized measure of disease occurrence and therefore allows comparisons of disease frequencies over time to be made between or within populations. In a rate, the numerator is always included in the denominator and the period of observation should always be defined. In addition, to make valid comparisons of disease between or within populations the denominator should be properly calculated. In veterinary epidemiology two main types of rates are used namely morbidity and mortality rates. They are mostly commonly used in Africa (Pfeiffer, 2002).

Morbidity rates

Morbidity rates are used to measure the proportion of affected individuals in a population or the risk of an individual in a population of becoming affected. They include incidence, attack, prevalence and proportional morbidity rates. Incidence rate is the number of new cases of a disease occurring in a specified population during a specified time period, divided by the average number of individuals in that population during the specified time period. This is a way of measuring the risk that a susceptible individual in a population has of contracting a disease during a specified time period (Putt *et al.*, 1988; Thrusfield, 2005).

When calculating incidence rates, problems frequently arise in estimating the denominator. Because of births, deaths, sales, movements etc. livestock populations rarely remain stable over periods of time, and such fluctuations in the denominator will obviously affect the calculation of the incidence rate. There are various ways of estimating the denominator in incidence rate calculations. These normally involve measuring the population at various intervals during the study period and averaging the results, for example measuring the population at the beginning, middle and end of observations and averaging them. The method used in calculating the denominator should always be specified when comparisons of incidence are being made and the same method should be used throughout (Pfeiffer, 2002).

The attack rate is the total number of cases of a disease occurring in a specified population during a specified time period, divided by the total number of individuals in that population at the start of the specified time period. The denominator, therefore, remains constant throughout the period of observation. Strictly speaking, the definition of the attack rate requires that all cases of disease not just new cases are included in the denominator. They are normally used, however, to quantity the progress of a disease during an outbreak. In most instances there would have been no cases of the disease in question prior to the onset of the outbreak, so that all the cases are new cases and the attack rate becomes a modified form of incidence rate, sometimes referred to as a commulative incidence rate (Thrusfield, 2005).

Mortality rates

Mortality rates measure the proportion of animals dying in a population, the most commonly used ones being crude death rate and cause specific death rate. Crude death rate is the total number of deaths occurring in a specified population during a specified time period, divided by the average number of individuals in that population during the specified time period. The denominator can be estimated in the same way as that for an incidence rate. The method of calculating the denominator should always be defined and the same method used throughout to enable meaningful comparisons to be made. Case fatality rate is the number of deaths from a specified disease in a specified population during a specified period, divided by the number of cases of that disease in that population during that time period (Pfeiffer, 2002).

Differences in the characteristics of host populations due to age breed and sex can be expressed by calculating rates that take these specific characteristics in to considerations. Age-specific incidence rate is defined as the number of new cases of a disease occurring among individuals of a specified age group in a specified population during a specified time period, divided by the average number of individuals in that specified age group in that population during that time period. Similarly, breed-specific incidence rate is defined as the total number of new cases of a disease occurring among individuals of a specified breed in a specified population during a specified time period, divided by the average number of individuals of that breed in that population during that time period. The same procedures can be applied to other morbidity and mortality rates. Thus, age and breed specific mortality rates can be calculated (Thrusfield, 2005).

2.12. Risk factors of FMD

Risk factors for foot and mouth disease may include factors that may change the level of risk (e.g. new serotypes or biotypes, or changing epidemiological or livestock husbandry patterns), and factors that may impinge on the national veterinary services to respond effectively to the disease threats (Kitching, 1999). These factors are associated with the three elements host, agent and the environment (Putt *et al.,* 1988).

2.12.1. Host factors

Multiple host factors

Of the domesticated animal species cattle, sheep, goats, pigs and buffalo are susceptible to FMD (OIE, 2004). Besides many species of cloven-hoofed wild animals, African buffalo (*Syncerus caffer*), Impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*), warthog (Phacochoerus aethiopicus) and elephants have role in the epidemiology of the disease (Vosoo *et al.,* 2002). Beer, antelope and wild pigs may also become infected (Thomson *et al.,* 2003). Buffaloes are believed to be the ultimate source of infection for livestock in Southern Africa due to their ability to both maintain and transmit the disease (Sangare, 2002). Although they do not show clinical disease, they excrete virus throughout the acute phase of the disease during which time they can infect other susceptible species (Sahle *et al.,* 2007; Vosloo, 2006).

The co-existence of different host species affects the incidence and distribution of FMD. Accordingly, studies by Sellers and Parker (1969) revealed that whereas cattle are the main disseminators of disease, sheep are important as maintenance hosts and pigs as amplifiers of infection (Cited by Pereira, 1981). The role of small ruminants has repeatedly been indicated; examples include, the 1978 and 1983 type A epidemics in Morocco (Donaldson, 1999), the 1994 type O epidemic in Greece (Tsaglas, 1995), the 1989-92 North African epidemics in Tunisia, Algeria and Morocco (Samuel *et al.,* 1999) and the 2001 epidemics in Turkey, UK and Ireland (Uppal, 2004).

Presence of carrier state

Following recovery from the acute stage of infection, virus disappears from all secretions and excretions with the exception of esophageal-pharyngeal fluid. The carrier state in cattle may last up to 3 years. For buffaloes, this period has been shown to be up to 5 years that within a herd of buffalo, the virus may be maintained for 24 years or longer (Condy *et al.,* 1985). This post-infection carrier state is about 9 months for sheep and 3-6 months for goats (Quinn *et al.,* 2005).

Intrinsic host factors

The most important intrinsic host factor in the epidemiology of FMD is the immune status of exposed population. The presence of high proportion of unprotected susceptible population is a potential epidemiological risk factor. Susceptibility to infection and consequently disease incidence may also be related to race (breed), age and nutritional state of host populations (Pereira, 1981). Thus FMD is more severe in European breeds of cattle, while it is mild in African or Asian breeds (Quinn *et al.,* 2005). Even if the morbidity rate of FMD in susceptible population can approach 100%, mortality is common more in young stock *(*Radostits *et al.,* 2000).

2.12.2. Agent factors

There are seven serotypes of FMDV, namely A, O, C, SAT1, SAT 2, SAT 3 and Asia 1. Infection with any one serotype does not confer immunity against the other (OIE, 2004). Furthermore, antigenic variation with in a type occurs as a continuous process of antigenic drift without clear-cut demarcations between sub types (Vosloo *et al.,* 1996). Foot and mouth disease virus does also posses biotypical strains and topo-types (Samuel and Knowles, 2001). Thus the presence of multiple serotypes and continuous generation of new variants is considered as one of the major problems in the control of FMD by vaccination (Sahle, 2004; Vosloo *et al.,* 2004*).*

2.12.3. Environmental factors /Extrinsic factors

Disease incidence and distribution are related to the type of husbandry and population density (Pereira, 1981*).* Extensive livestock production enhances contact transmission of disease (Vosloo *et al.,* 2004) and herd size, has been described as risk factors in FMD by Hugh-Jones (1972) (Cited by Bogale, 2005). Climatic factors, such as low temperature, high humidity and poor sunlight favor the wind borne transmission of FMD in the temperate climate than in the tropics, were outbreaks occur more frequently during dry seasons (Pereira, 1981).

In Africa, use of vaccination for the control of FMD has been less uniform. It has been applied to control epidemics in countries previously free of disease such as Morocco, Algeria and more generally in Eastern and Southern countries where disease is endemic. Elsewhere vaccination has been used, if at all, sporadically to control the invasions of disease from neighboring countries (Pereira, 1981). In Ethiopia this is limited to periurban dairy farms (Bogale, 2005). So, the absence of relatively higher herd immunity is a severe epidemiological risk of FMD.

The absence of movement control for animals across national or international borders may spread disease over vast distances (Vosloo *et al.,* 2004). To this effect the molecular epidemiology of O, A and C foot and mouth disease virus isolates of Ethiopia, Kenya and Eritrea reveal marked genetic relationship suggesting that cross-border livestock movement is a major cause for disease dissemination (Sahle, 2004*)*

3. MATERIALS AND METHODS

3.1. Study areas

The study was carried out in 3 regional states in Ethiopia – Oromia National Regional State, Amhara National Regional State and the Southern Nations Nationalities and peoples (SNNP) Regional State. A total of seven FMD outbreaks were investigated three in Oromia and two from each of Amhara and SNNP regions.

The three outbreaks in Oromia occurred in three districts; Ada, Girar Jarso and Yabelo found in East Shoa, North Shoa and Borena administrative zones respectively. While the first two districts are located on the Central Highlands of Ethiopia, where a mixed type of farming practiced, Yabelo is a lowland district inhabited by Borena pastoralists in Southern Ethiopia bordering -the Republic of Kenya. Unlike the former group that receives an average annual rainfall greater than 1500mm, Yabelo is restricted to a range of 500-600 mm perannum.

In Amhara regional state, two outbreaks were investigated in Ankesha Guagusa and Jawi districts of Awi zone. Districts lie 1600 meters above sea level (masl) and 1200 (masl) and receive annually a rainfall of 1600mm and 500mm respectively. A mixed farming system is practiced in common. They are located in North Western part of Ethiopia bordering Oromia and Benishangul Gumuz regions.

The rest two outbreaks were investigated in Surma and Maji districts, located in Bench Maji zone of SNNP Regional state. Both are lowland districts, but unlike Surma of pastoralists, in Maji mixed agricultural activity is exercised. Surma borders the Republic of Sudan, where the pastoralists in both countries move in any direction, with their herds, in search of feed and water.

Specifically, the study was conducted in 7 villages of the above districts. These were Debrezeit Agricultural Research Center (DZARC) (in Ada), Torben Ashe (Girar Jarso), Did Hara (Yabelo), Coca (Surma), Chigit (Maji), Ayehu (Ankesha Guagusa.) and Ilala (Jawil). Except DZARC, which is a semi-intensive dairy farm, the rest epidemiological units are villages of rural animal husbandry.

Livestock production is an integral part of the people in all of the three regions serving as source of food, draught power, income generation and social prestige (Bogale, 2005). Fig. 1 and Table1 present additional information.

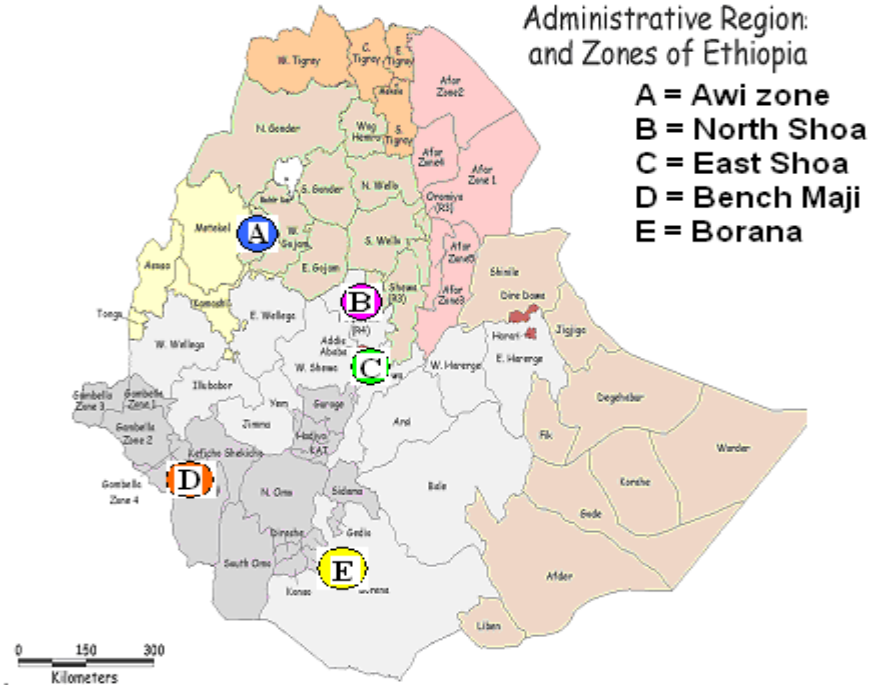

Figure 1: Outbreak investigation areas

Table 2: Description of study areas (districts)

No	Region	Zone	District	Village	Location		Altitude (masl)	Temperature OC	Rain fall mm/y	Farming system	Distance from AA (Km)
					Latitude	Longitude					
1	Oromia	East shoa	Ada	DZARC farm						Intensive	45
2	Oromia	North shoa	Girar Jarso	Torben Ashe	$9^{0}45'77^{11}N$	$38^{0}45'76''$	2853	18-27	2000	Ext-Mix farming	110
3	Oromia	Borena	Yabelo	Did Hara	$4^{0}30'N$-$3^{0}30'N$	$37^{0}45'$-$38^{0}38''$	1500	24-34	500	Mixed Farming	600
4	SNNPRS	Bench Maji	Surma	Coca	$6^{0}84'12''$ N	$35^{o}26'28''$	1250	28-36	500-600	Pastoral	774
5	SNNPRS	Asossa	Maji	Chigit	$6^{o}59'90''$ N	$35^{0}31'61''$	1480	26-34	750	Pastoral	614
6	Amhara	Awi	Ankesha Guegsa	Ayehu	$10^{0}51'15''$ N	$36^{0}53'45.4''$	1600	26-30	1600	Mixed	447
7	Amhara	Awi	Jawi	Ilala			1200	26-35	500	Mixed	668

Table 3:.Livestock population of the study areas (Farm /districts)

No	District/ Farm	Livestock population		
		Cattle	Sheep	Goats
1	DZARC	164	105	0
2	Girar Jarso	90901	32440	15951
3	Yabelo	250,866	20000	10000
4	Surma	97950	370	13600
5	Maji	22386	5513	8097
6	Ankeshe Guagsa	148999	52283	15717
7	Jawi	68403	11805	17832

3.2. The study population and sampling technique

The study population consisted of cattle and small ruminates that were affected or in-contact in the seven FMD outbreaks. The investigation of outbreaks in three regions, five administrative zones or seven districts was included primarily for they occurred and because of temporal feasibility to handle. Cattle and small ruminants manifesting clinical signs of FMD or those in close contact to the outbreak were included in the study. Except for affected animals, cattle and small ruminants of any sex but greater than six months were considered. In DZARC and Tobren Ashe cattle (Crosses of Holstein and Barka) were involved in addition to the indigenous breeds. About 234 cattle, 193 sheep and 203 goats were sampled.

3.3. Study design

Before the beginning this proper information channel was organized. Regional animal health diagnostic and investigation laboratories were informed to report FMD outbreaks that would occur to the National Animal Health Diagnostic and Investigation Center (NAHDIC). When an active outbreak was reported, a field investigation was conducted right at the location. In each village, the same procedures were followed: epidemiological information was gathered by interviewing village leaders, livestock owners, district animal health

workers and development agents. Clinical and epidemiological information was recorded on forms prepared for the purpose and sample of animals was clinically examined and specimens collected for diagnostic testing.

Serum samples were analyzed by FMD-3ABC-ELISA at NAHDIC. In addition, tissue and OP fluid samples were submitted to WRL, Institute of Animal Health, Pirbright, England, where they were detected by RT-PCR and serotyped by cell culture / ELISA.

3.3.1. Epidemiological data collection

As indicated by Putt *et al* (1988), the investigation process involved tentative diagnosis of outbreaks and determination of animals exposed, affected or killed by the disease. Relevant data was gathered by interviewing village leaders, livestock owners (including village leaders), animal health workers and development agents. By careful counting, information was recorded on a designed format. Spatial and temporal data was registered for each outbreak including, specific location, altitude, and length of time since outbreak. For the purpose 15 animal health workers, 6 development agents and 60 livestock owners (including village leaders) were involved in seven outbreaks. This data was collected to determine the characteristics of FMD outbreaks and to assess associated risk factors.

3.3.2. Clinical examination

For each outbreak sampled animals were clinically examined for presence of typical vesicular lesions in the mouth, on the feet and teats and lameness. Rectal temperature was taken for active cases. Small ruminants in contact to the outbreak-affected cattle were similarly examined. In seven outbreaks 234 cattle, 193 sheep and 203 goats were clinically observed. This was performed to identify the clinical picture of FMD in cattle and small ruminants.

3.4. Sample collection

3.4.1. Serum samples

Whole blood was collected from the jugular vein of FMD affected and/or in-contact 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 NAHDIC, Sebeta for analysis. In the laboratory, the serum was stored at -20^0c until ready for laboratory investigation (OIE, 2004). A total of 234, 193 and 203 serum samples were collected from clinically affected or in-contact cattle, sheep and goats respectively.

3.4.2. Tissue samples

Epithelial tissue was collected from un-ruptured or fresh-ruptured vesicles and placed in a bottle with transport medium composed of equal amount of glycerol and O.O4M phosphate buffer with antibiotics (OIE, 2004). Then identification number, species, sex, age, village and tissue were labeled and transported in an icebox to NAHDIC, Sebeta. Then it was stored at -70^0C until submitted to the WRL, Pirbright. In five outbreaks twenty-six tissue samples were collected from the gum, tongue and interdigital space of cattle (Table 4).

3.4.3. Oesophageal-pharyngeal (OP) fluid

Samples of OP fluid were collected by probang from sheep and goats. About 2ml of transport medium was added to a container of 5 ml. Then the OP fluid was collected by a probang and poured into a 20ml bottle. The fluid was then examined and about 2ml of it containing cellular material was added to the bottle with transport medium (OIE, 2004*).* Then identification number, species, village, sex and age were labeled and transported in icebox to NAHADIC. Until submitted to the WRL, the samples were kept at – 70^0C (Table 4).

Table 4: Summary of samples collected in 5 outbreaks and submitted to WRL, Pirbright for viral identification.

Outbreak	Species	Type of Sample	Number of	
			Samples	Pool
Torben Ashe	Bovine	Tissue	5	3
Did Hara	Bovine	Tissue	1	-
	Ovine	OP fluid	5	1
	Caprine	OP fluid	10	1
Coca	Bovine	Tissue	5	1
	Caprine	OP fluid	19	3
Chigit	Bovine	Tissue	3	1
	Ovine	OP fluid	1	-
	Caprine	OP fluid	5	1
Ayehu	Bovine	Tissue	12	1
	Ovine	OP fluid	30	4
	Caprine	OP fluid	8	2
Total	Bovine	Tissue	26	7
	Ovine	OP fluid	36	6
	Caprine	OP fluid	42	7

3.4.4. Processing of specimens for shipment to WRL

After collection all samples were labeled, catalogued and stored under prescribed conditions at NAHDIC. Tissue sample and probang fluids were further processed before shipment to WRL, England. Tissue samples were sent either singly or in pool of two to three samples, but most of the probang samples were sent in pool of 5-8 samples. These were prepared in 2 ml cryotubes and sealed with Para film and kept cold. The air-shipment of specimens to WRL had such steps;

1. Arrangements were made with Ethiopian airlines accepting “infectious substances” as specimens for delivery to WRL, must be sent by airfreight direct to London Heathrow.
2. Packaging was made sufficient to allow the inclusion of dry ice as a refrigerant and sufficiently well insulated to keep specimens frozen for at least 48 hours.
3. A shipper’s declaration and other documents (including labels) were completed in accordance with shipping regulations and the instructions provided by WRL.
4. A contact person at the WRL was noticed of the flight details and the air way bill number for the shipment.

3.5. Laboratory Tests

3.5.1. Virus detection tests

Virus isolation

At WRL, Pirbright, tissue or probang samples are inoculated on to primary bovine thyroid cell cultures for isolation. Cytopathic effect is observed after 24-48 hours (OIE, 2004).

Serotyping ELISA

Supernatant fluids from cell cultures showing cytopathic effect, was tested for presence of FMDV, by serotyping ELISA.

Real-Time Polymerase Chain Reaction (RT-PCR)

Tissue and probang fluids collected in buffers were tested by real-time, RT- PCR using the Procedure of Reid et al., (2003) with both the original and newly modified 3 diagnostic primers and Taqman probe (cited in OIE, 2004)

3.5.2. Serological tests

FMD-3ABC-ELISA

The relative positivity of cattle and small ruminant serum was analyzed by FMD-3ABC-ELISA test. The test was performed as follows:

About 100µ1of prediluted samples (1:16 in diluent buffer A) and controls (1:100 in CHEKIT- FMD- 3ABC-ELISA sample diluents) were dispensed into the appropriate wells of the micro titer plate pre-coated with recombinant FMD- 3ABC viral antigen. The plates were covered with a lid and incubated for 60 minutes at 37^0C in a humid chamber. After incubation micro plates were filled with about 300µl CHEKIT-FMD-3ABC washing solution and washed three times then 100µl of ready to use CHEKIT-FMD-3ABC-Ant-: Ruminant-IgG-PO-Conjugate was dispensed into each well and incubated for 60 minutes at 37^0c in humid chamber. After washing the plate 100µl of TMB- substrate was dispensed in to all 96 wells and incubated at room temperature or optimally at 25^OC for 15 minutes. Finally, adding100µl CHEKIT-Stopping TMB- solution stopped the reaction and the result was read using a spectrophotometer at 450nm wavelength. The reader, connected to the computer loaded with ProComm and word package, was used to automate the reading of OD value. The percentage positively (PP) for test samples in relation to the positive and negative controls was calculated as follows:

$$\text{PP value} = \frac{\text{OD sample} - \text{OD negative}}{\text{OD positive} - \text{OD negative}}$$

Percentage positively value greater then 30% was used to identify positive animals

3.6. Data analysis

Descriptive statistics was used to calculate epidemiological rates. Results of clinical examination and serology were analyzed using Statistical Package for Social Sciences (SPSS).

4. RESULTS

4.1. Results of epidemiological characteristics

Epidemiological data was collected with respect to the number of animals exposed, affected and died and epidemiological rates were calculated in the seven outbreaks (Table5). Attack rate was the highest (47.56%), moderate (38.48) and lowest (22.14%) in DZARC, Coca and Torban Ashe outbreaks respectively. The highest mortality rate was recorded in Coca (2.334) and the lowest in DZARC (0.614). The case fatality rate was highest in Coca (6.05%), followed by Chigit (5.17%) and lowest in DZARC (1.28%).

Table5: Morbidity, mortality and case fatality rates

	Population at Risk	Affected	Dead	Attack Rate	Death Rate	Case Fatality Rate
DZARC Farm	164	78	1	47.56	0.61	1.28
Torban Ashe	935	207	3	22.14	0.32	1.45
Did Hara	3235	798	23	24.67	0.71	2.88
Coca	2448	942	57	38.48	2.33	6.05
Chigit	1726	561	29	32.50	1.68	5.17
Ayehu	1712	537	27	31.37	1.58	5.03
Ellala	1127	329	14	29.19	1.24	4.26
Total	11347	3452	154	30.42	1.36	4.46

4. 2. Results of clinical examination

A total of 234 cattle, 193 sheep and 203 goats were carefully examined for the presence of characteristic signs of FMD, during ampling. In each outbreak animals manifesting the characteristic signs of FMD: vesicular lesions in the oral cavity and on the feet and teats, lameness and rise in temperature were considered as clinically affected by FMD. Accordingly 124 cattle (53%) showed characteristic signs of FMD in the 7 outbreaks. On the other hand, clinical disease in small ruminants was observed only in 13 sheep and goats, which is 3.3%. In Torban Ashe and DZARC outbreaks there were no goats while there were no sheep in Coca outbreak (Table 6).

Table 6: Result of clinical observation

Outbreak	Cattle		Sheep		Goats	
	Examined	Affected N (%)	Examined	Affected N (%)	Examined	Affected **N(%)**
DZARC	25	10(40)	12	0	-	-
Torben Ashe	27	23(85)	50	2(4)	-	-
Did Hara	37	15(40)	36	0	27	0
Coca	42	19(45)	0	0	56	4(7)
Chigit	33	23(69.6)	15	1(6.7)	45	3(7.8)
Ayehu	48	23(47.9)	52	0	38	0
Ilala	22	11(50)	28	1(2.7)	37	2(5.4)
Total	234	124(53)	193	4(2)	203	9(4.4)

4.3. Serological results

Based on serological test by FMD- 3ABC- ELISA the overall positivity was 66.7%. In sheep and goats the overall positivity was 13.4% while the species-specific result was 9.3% for sheep and 17.2% for goats respectively. (Table7).

4.4. Results of virus Isolation

Tissue samples taken from three outbreaks; namely Torben Ashe, Dide Hara and Ayehu yielded type-O FMD viruses. But in Coca and Chigit, SAT1 serotypes were isolated. Similarly, the OP fluid samples taken from sheep (Chigit) and goats (Coca) were positive for SAT1 FMD virus. However, no virus was detected in OP fluid samples of Did Hara and Ayehu origin (Table 8). The isolation of SAT1 FMD virus from either tissue samples of bovine or OP fluid samples of sheep and goats is the first record in Ethiopia.

Table 7: Results of serology

Outbreak	Cattle		Sheep		Goats	
	Tested	Positive	Tested	Positive	Tested	Positive
DZARC	25	16(64)	12		-	-
T. Ashe	27	20(74.1)	50	5(10)	-	-
Did Hara	37	25(67.6)	36	2(5.6)	27	4(14.8)
Coca	42(	30(71.4)	-	-	56	10(17.9)
Chigit	33	19(57.6)	15	3(20)	45	8(17.8)
Ayehu	48	30(62.5)	52	2(3.8)	38	5(13.2)
Ilala	22	16(72.7)	28	6(21.4)	37	9(24.3)
Total	234	156(66.7)	193	18(9.3)	203	35(`7.2)

Table 8: FMD viruses isolated in five outbreaks

Outbreak	Species	Type of sample	FMDV type isolated
Torben Ashe	Bovine	Tissue	O
Did Hara	Bovine	Tissue	O
Coca	Bovine Caprine	Tissue OP fluid	SAT-1 SAT-1
Chigit	Bovine Ovine	Tissue Op fluid	SAT-1 SAT-1
Ayehu	Bovine	Tissue	O

4.5. Risk factors

4.5.1. Small ruminants

There is a highly significant difference in manifesting clinical signs of FMD between cattle and small ruminants ($P<0.05$; OR = 16.4, 95% CI = 9.331-27.924). Characteristic signs of FMD manifest 16.4 times more in cattle than in small ruminants. Also there occurred a highly significant difference in sero-conversion rate between cattle and small ruminants ($P<0.05$; OR=4.9, 95% CI= 3.816-6.502).

4.5.2. Age of cattle

Age specific attack rates were higher in the age group less than 2 years (53.65 in DZARC, 46.37 in Chigit) while the lowest values were recorded in age group above 4 years(22,27 in Dide Hara, 27 in Chigit) (Figure2). Similarly, age specific crude death rates were higher in younger cattle (8.7 in Coca, 2.3 in Dide Hara) as compared to older cattle (1.5 in Coca, 0.2 in Dide Hara) adults (Figure 3).

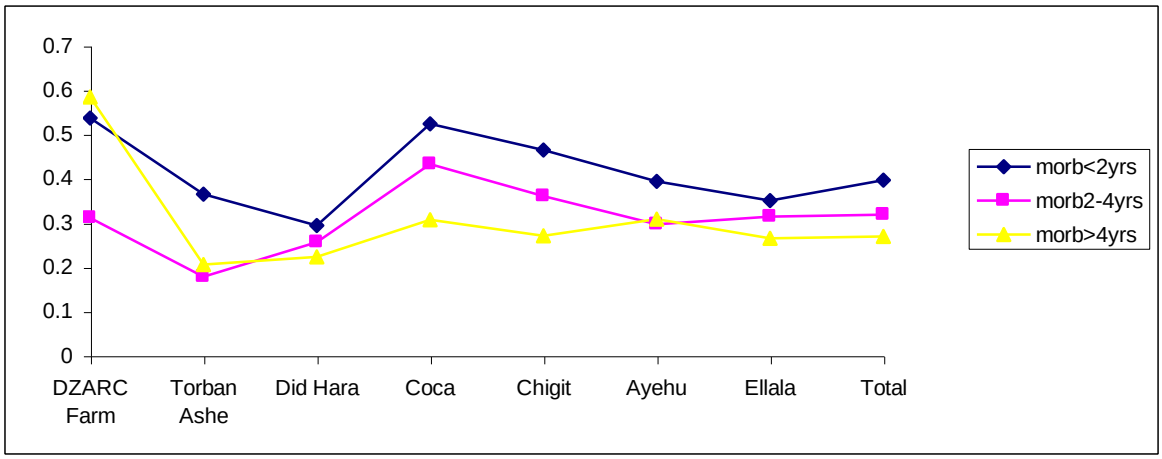

Figure2: Morbidity rates in different age groups of cattle

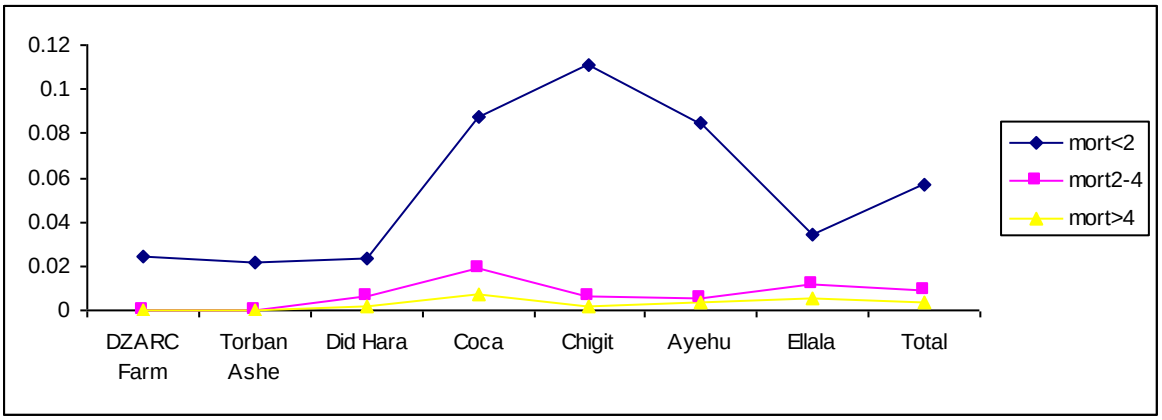

Figure 3: Mortality rates in different age groups of cattle

4.5.3. Breed of Cattle

In two of the outbreaks which occurred in DZARC and Torban Ashe, two different breeds of cattle involving the Holestien- Barka crosses and indigenous (Boran) were attacked by the disease. At DZARC farm from 164 cattle that are composed of 114 crosses and 50 Boran breeds one calf less than two years of age died. It was observed that the disease affected none of the 50 Boran cattle while 78 (68.4%) of the crosses became sick. In Torban Ashe, 390 crosses and 545 local breeds were exposed. In this outbreak 3 calves of less than two years, all cross breeds, died. Of 545 local breeds only 8 (1.5%) were affected while 199 of their crossbred herd mates were morbid at a rate of 51% (Figure 5).

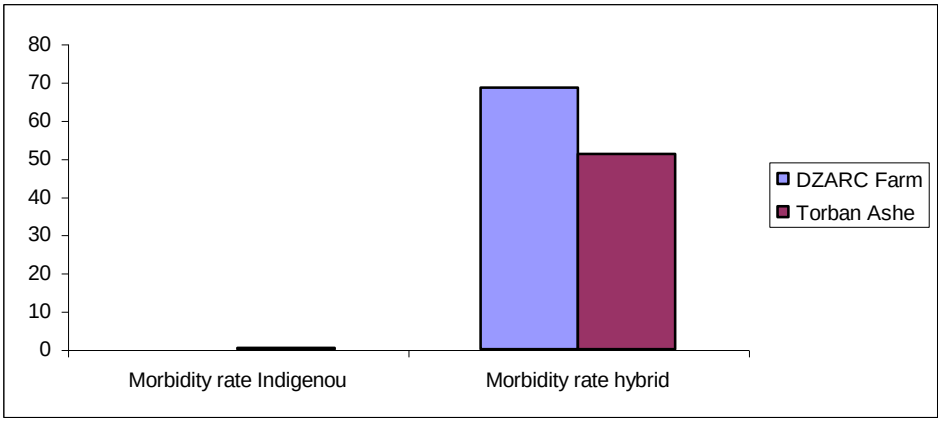

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Figure 4: Breed specific attack rates in DZARC and Torben Ashe

4.5.4. Miscellaneous risk factors

Absence of FMD control strategy

In the seven out breaks investigated by the current study, Pastoral herds (Coca and Did Hara), cattle in mixed farming mid and highlands (Torben Ashe, Ayehu, Chigit and Ilara) and in Semi-intensive management belonging to agricultural research center (DZARC) all were attacked. However, none of the outbreaks were controlled by vaccination. The record

of vaccination against FMD was restricted to DZARC farm that was given last in October 2005. In the other outbreaks no animal had been vaccinated before. This means that almost all cattle population of the country is susceptible to FMD, and that is why the disease is expanding by invading new territories (e.g Ayehu and Ilala). The absence of FMD control strategy at national level thus remains a major risk factor.

Absence of livestock movement control

Except in DZARC Farm, livestock movement was possible in any direction. Extensive movements were observed both within the country and across borders. The investigation for the likely sources of infection showed that spontaneous movement of infected animals precipitated almost all of the seven outbreaks. Such movements were responsible for spread of disease to previously unaffected areas (Ilala, Ayehu), which when occurred across border could introduce new strain of FMD virus (Coca). The FMD outbreak in DZARC was tentatively traced to the introduction of Boran heifers from an FMD endemic Borena area, two weeks before the outbreak. This specific outbreak disclosed the risk of introducing uncertified animals.

Contact with wild animals

Wild animals like buffaloes, antelopes and wild pig are susceptible to FMD. Even if major epidemiological roles have been associated to only buffaloes; many countries have successfully controlled FMD by game exclusion. The DZARC and Torben Ashe cattle had no contact with wild animals; however, cattle in the rest five villages had possible contacts with one or another of these risk factors.

Contacts at communal points

Unlike the DZARC farm, which had an enclosed grazing, supplement feeding and individual water source, cattle in the rest villages were reared by extensive livestock husbandry characterized by the use of communal grazing and watering sources. In pastoral

areas (Dide Hara and Coca) during the dry seasons, there was shortage of grass and water. The accumulation of huge livestock population from different areas, for the scarce grass and water sources was observed.

5. DISCUSSION

The aim of this study was to investigate FMD outbreaks and identify the risk factors associated, including casual serotypes and the role of small ruminants and to characterize specific outbreaks. Efforts were made to obtain the fullest possible epidemiological data of outbreaks and a wide range of samples were collected so that both virological and serological evidence of outbreak could be comprehensively assessed.

The clinical picture of FMD in cattle including fever, vesicular lesions in the mouth and on the feet and lameness enabled the diagnosis in all outbreaks. Consistency of clinical disease in small ruminants was very low. This finding is similar to that reported by Uppal (2004) in India or the works of Donaldson and Sellers (2000). Differences in morbidity and mortality rates were observed between different outbreaks due to breed composition, as in DZARC and Torben Ashe or involvement of exotic strain of FMD virus, as for Coca and Chigit outbreaks. This is an expected reality (Pereira, 1981; Radostits *et al.,* 2000; Quinn et al., 2005). Mersie *et al.,* (1992) reported similar result in Ethiopia.

Serotypes O and SAT1 FMD viruses were isolated from all outbreaks. This study showed domination of serotype O FMDV in most outbreaks. This is in line with the conclusions of previous works (Sahle, 2004; Rufael, 2006). Due to absence of livestock movement control, the possibility of introduction of exotic FMD virus, SAT1 in this case, has been suggested (Shale, 2004; Vosloo *et al.,* 2004).

The involvement of small ruminants in the epidemiology of FMD has been evidenced by clinical, serological and virological investigations. This is similar to the works in Morocco (Donaldson, 1999), in Greece (Tsaglas, 1995), in Tunisia, Algeria and Morocco (Samuel *et al.,* 1999) and in Turkey, UK and Ireland (Uppal, 2004). This study also showed the higher risk of FMD in younger and cross breed cattle. Age and breed difference in susceptibility to FMD is well documented in conventional veterinary literature (Radostits *et al.,* 2000; Quinn *et al.,* 2005).

The likely source of FMD outbreak in DZARC was the introduction of Boran heifers, 60% of which were seropositive while clinically healthy. Thomson (1996) and Alexanderson *et al.*,(2003) explained the role of carrier animals in FMD transmission. The absence of FMD control measures such as vaccination was among the major risk factor identified in this study. Similarly, studies in Israel have shown a morbidity rate of 30% and 0% in none vaccinated and vaccinated cattle respectively (Yadin *et al.,* 2004).

6. CONCLUSIONS AND RECOMMENDATION

Foot and mouth disease is an endemic disease of cattle in Ethiopia. It affects livestock owners though loss in milk production, draught power, market value, and death of young stock. As evidenced by occurrence of newly attacked foci FMD is expanding its territory. Its occurrence coincides with the dry season in different regions. There is no national FMD control strategy and almost the entire livestock population of the country is at risk of repeated incursions. The morbidity and mortality rates of the disease varied with the species, breed and age of animals as well as the type of FMD virus involved.

The isolation of SAT1 FMD virus in Ethiopia increased the number of endemic serotypes to five. This in turn worsens local impacts and becomes a major problem of disease control by vaccination. Type O FMD virus is still the major serotype in Ethiopia, causing huge losses. FMD is sub clinical in small ruminants and affects small proportions of the flock. But clinical, serological and virological evidences showed that they might play role in disease transmission.

Based on the findings of this study the following recommendations are forwarded:
The present situation of FMD in Ethiopia requires an immediate intervention. Therefore, the Ministry of Agriculture and Rural Development (MoARD) should design and implement an effective FMD control policy. There should be a legal guideline enforcing the control of livestock movement both with in the country and across the borders with the neighboring countries. The control endeavors involving vaccination should consider the presence SAT1 FMD viruses.

There risk of FMD in crossbred and younger cattle is relatively higher. Therefore the use of inactivated vaccine for this risk group is highly recommended. In addition, intensive and semi intensive livestock production facilities, like DZARC, should adopt an FMD pre-testing of replacement stocks. The determination of proper roles of different hosts, including small ruminants and wild animals needs further investigations.

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8. ANNEXES

Annex 1: Summarized History of Seven FMD Outbreaks investigated from November 2007 to March 2008

1. DZARC Farm

*Location***:** Ada district in East Shoa Zone of Oromia region;

Farming: Semi-intensive (Government owned)

Census: 164 cattle and 105 sheep.

Breeds: 114 cross breeds of Holstein Friesian and Barka and 50 indigenous (Boran) breeds. The sheep are indigenous Arsi breeds.

Outbreak history: the outbreak was reported on of Jan 2008. During the investigation 78 clinical cases of FMD (all in cross breed cattle) were observed. Most of the affected cattle had healing lesions at that time. However, no lesion was observed in both indigenous cattle and sheep. The most likely source of infection was the introduction of 35 indigenous (Boran) heifers (on 31 Dec, 2007) from an FMD endemic area-Borena, Southern Ethiopia.

Sampling and examination: 25 cattle (10 crosses and 15 new comers) and 12 sheep were sampled. All the ten cross breed cattle had exhibited lesions typical of FMD at that time. No suggestive signs were observed, however, on either of the indigenous cattle or sheep.

Previous history: There was an outbreak of FMD in 2005, when cattle were last vaccinated.

2. Tobrben Ashe outbreaks

Location: Girar Jarso district in North Shoa zone of Oromia regional state, $9^{0}45'77^{10}$N, $38^{0}45'76^{11}$E.

Farming system: extensive mixed farming system

Census: 935 cattle and 742 sheep

Breed: cattle (390 crosses and 545 indigenous breeds) and 742 local breeds of sheep.

Outbreak history: 207 clinical cases were counted when the outbreak was investigated. During the investigation there were few cases with freshly ruptured vesicles in the mouth and on the feet, from which five epithelial samples taken. But most of them had heading

lesions. About 199 of the cases were crossbreed cattle, while 8 were indigenous breeds of cattle. Clinical disease in sheep was not reported but during examination and sampling lameness was observed in two ewes, which later were proved by serology. This outbreak was suggested to have coincided with the introduction of a newly purchased crossbreed dairy cow, which was also affected.

Sampling and examination: Among the affected cattle about 16 were clearly identified by the usual signs. All of these cattle were crosses. Fifty sheep were closely inspected while sampling among which two were lame.

In this village the history of the last FMD outbreak dates back to 2004. The control of FMD by vaccination had never been attempted in the district.

3. Dida Hara

Location: Yabelo district in Borena zone of Oromia region: $4^0$30'N=$5^0$30'N, $37^0$45'E-$38^0$38'E

Farming: Pastoral, communal grazing and watering

Census: 3235 cattle, 1091 goats and 2474 sheep

Breed: all indigenous, cattle being Boran

Outbreak history: the outbreak of a locally endemic FMD was reported on 5 December 2007. Up to 22 December 200 a total of 798 cattle were known to be clinically affected among which 23 died. All age groups of cattle were affected, 162, 293 and 343 being included in the less than two (<2), 2-4 and >4 years categories respectively. Death of 13, 7 and 3 animals was also observed in that order. Most of the affected animals suffered from lameness, and inability to graze, lactating cows also stopped milk production. Animals with acute feet lesions were found with their feet soaked in a mud, as a traditional remedy. The most likely source of infection was a communal watering point, where cattle of different origin mix. Clinical FMD in small ruminants was neither reported nor observed.

Sampling and examination: Serum sample was taken from 37, 36 and 27 cattle, sheep and goats respectively. In addition OP fluid sample was collected by a probang from 6 sheep and 10 goats.

The outbreak of FMD had also been encountered in the similar season of 2006 (dry season). No vaccination had been given in the district before.

4. Coca

Location: Surma district in Bench Maji zone of SNNP regional state; $6^{0}84'12^{11}$N, $35^{0}26'28^{11}$E.

Farming: Pastoral, with communal grazing and watering points shared with livestock of neighboring districts and Democratic Republic of Sudan.

Census: 2440 cattle and 1060 goats.

Breed: all indigenous

Outbreak history: there was a report of FMD outbreak since September 2007. During investigation of the Coca case most of the affected cattle had healing lesions. About 942 cattle were clinically affected, with 57 deaths. An attack rate of 38.5% and crude death rate of 2.3% was calculated for cattle of age <2 years. Few animals of active oral lesions were observed and tissue samples taken from 5. There was a shortage of feed for the animals of Bume (Surii) pastoralists of Sudan. This problem urged them to cross Ethiopia's border and enter the Surma district of Ethiopian Suri pastoralists. The nutritionally stressed herds from Sudan consisted of cattle affected by Rokono (Suri-sore). Therefore, the most likely source of this outbreak was associated to the introduction of affected cattle from Sudan, which was also favored by the absence of cross border livestock movement control.

There was an outbreak of Rokono (FMD) in this village during 2005, when the serotype O was isolated. There is no record of FMD vaccination.

A total of 42 cattle and 56 goats were subjected to serum sample collection and accompanying clinical inspection. Four goats were observed with clinical sign –lameness. In addition 19 OP fluid samples were taken from goats and 5 tissue samples were collected from cattle.

5. Chigit

Location: 'Tum' area – Maji district in Bench Maji zone of SNNP, $6^{0}59'90^{11}$N $35^{0}31'61''$E.
Farming: Extensive-mixed farming system where communal livestock rearing is practiced.
Census: 1726 cattle, 213 sheep and 890 goats.
Breed: all indigenous.
History of outbreak: This outbreak was reported on 5 December 2007, while the Coca outbreak was under investigation. It was known that the outbreak started around 28 December 2007. The investigation revealed that there were about 561 clinically affected and 29 dead cattle until 7 December 2007. Most of the affected animals were in the acute active phase, with excessive salivation, vesicular lesions in the mouth and on the feet, and rectal temperature more than 40^{0}C. Similar to Coca two goats and a sheep were observed with vesicular lesion in the interdigital space of the feet and lameness. This outbreak was suggested to be precipitated by the uncontrolled movement of animals from communal grazing and watering areas in the viscinity.

Sampling and examination: A total of 32, 15 and 45 serum samples were taken from cattle, sheep and goats respectively. OP fluid samples were also taken from one sheep and five goats, while three tissue samples were taken from cattle. The outbreak of FMD ('Kalgut' means sore – in Bench) was encountered in this village during 2004; cattle have never been vaccinated against 'Kalgut' in the village.

6. Ayehu

Location: Ankesha Guagulsa district in Awi zone of Amhara regional state: $10^{0}51'15^{11}$N$36^{0}53'45.4^{11}$E.
Farming: Extensive mixed farming, animals sharing communal grazing and watering sources.
Census: 1712 cattle, 842 sheep and 224 goats
Breed: all indigenous
History of outbreak: During investigation there were 537 clincially-affected cattle. In the district the outbreak of FMD was encountered for the first time. Here it is called 'Maz' or

‘Afemaz’ – ‘Amharic –means ’sore in mouth’. Most of the affected animals had healing lesions, but there were also few cases with active lesion. The most likely source of this outbreak was the uncontrolled movement of affected cattle from other areas..

Sampling and examination: Forty-eight cattle were examined and serum taken from all, while 12 tissue samples were taken. No clinical cases of FMD was reported or observed in Ayehu in sheep and goat. However, 52 sheep and 38 goats in contact with the affected cattle were sampled, serum taken from all and OP fluid collected from 30 sheep and 8 goats. There was no record of both FMD outbreak and vaccination in the district.

7. Ilala

Location: Jawi district in Awi zone of Amhara region.
Farming: Extensive mixed farming.
Census: 1127 cattle, 412 sheep and 716 goats
Breed: all indigenous
History of outbreak: Outbreak was reported on 25 February 2008. Investigation revealed that there were 329 cattle affected until 30 March 2008. Most of the affected cattle had leaking lesions and no active lesion was encountered. In the district this is the first outbreak of FMD. There was a shortage of feed and water since the end of January 2008. The likely source of infection in this outbreak is an infected moving animal.

Sampling and examination: for serum analysis, 22 cattle were sampled. There were 2 goats and a sheep manifesting the expected sign- lameness. 28 sheep and 37 goats were examined

and serum collected. Neither the outbreak nor vaccination of FMD had occurred, in Jawi district, earlier.

Annex 2: Investigation of foot-and-mouth Disease outbreaks:

Epidemiological data collection format

1. General information

1.1. Area (spatial) information

Region _____ Zone _____ District _____ PA/Kebele _____

Distance from AA _____

1.2. Specific location of the outbreak

Latitude _____ Longitude _____ Altitude _____

2. Foot and mouth disease

2.1.Occurrence is: first time _____ common _____

2.2.Local name _____ Meaning _____

2.3.Season of occurrence:

Dry season _____ Rainy season _____ .Any season_____

2.4.The last outbreak of FMD in the village occurred in:

Before 2000 _____ 2000-2005 _____ In 2006 _____

None _____

2.5. How do you identify the disease? By signs in the mouth_____.On the feet_____. On the teats_____.

3. Outbreak history

3.1.First date of outbreak occurrence _____

3.2.Date reported _____

3.3.Total days since outbreak _____

3.4.Livestock population in the Farm/village:

Table: Total population at risk

Species	**Breed**	**Age Group**			**Total**
		<2 years	**2-4 years**	**>4 years**	
Cattle Sheep Goats					

3.5. Effect of the outbreak

3.5.1. Table: Total number of animals affected (sick):

Species	**Breed**	**Age Group**			**Total**
		<2 years	**2-4 years**	**>4 years**	
Cattle Sheep Goats					

3.5.2. Table: Total number of animals dead

Species	**Breed**	**Age Group**			**Total**
		<2 years	**2-4 years**	**>4 years**	
Cattle Sheep Goats					
Total					

3.5.3. What are the other effects? Reduced milk _____. Can't work_____

Reduced market value_____.

4. Information on risk factors:

4.1. Effect of the disease on sheep and goats

Present _____. Absent _____.Not known_____

4.2. Contact of animals with wild animals:

Present _____. Absent _____

4.3. The last control/prophylactic vaccination of FMD given in the farm/village:

Before a year _____2005-2000 _____Before 2000_____Never given--_____

4.4. Source of livestock feed in the farm/village:

Communal grazing _____

Enclosed grazing _____

Zero grazing _____

4.4. Source of water for animals:

Communal water points _____

Individual sources _____

4.5. Movement of animals:

Possible in any direction _____

Limited movement _____

Requires pre-testing for livestock diseases _____

4.6. Farming system:

Pastoral _____ Mixed farming _____

Semi intensives/intensive _____

4.7. Likely source of outbreak:

Introduction of infected animal /animals _____

Contact at communal points _____

Movement of infected animal/animals _____

Annex 3. Plate layout used for FMD-3ABC ELISA.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N	N	13									
B	P	P	14									
C	1	7	15									
D	2	8	16									
E	3	9	17									
F	4	10	etc									
G	5	11										
H	6	12										

N = negative control

P = positive control

1,2,3, etc = Test sample

9. CURRICULUM VITAE

1. Personal Details

Name: -	Yoseph Legesse
Sex: -	Male
Place of birth: -	Gursum, East Hararghe , Oromiya region
Date of birth: -	24/10/1972
Marital status: -	Married
Nationality: -	Ethiopian

Language: -	**speak**	**write**	**read**
Amharic	√	√	√
Somali	√	√	√
English	√	√	√
Afan Oromo	√	√	√
Harari	√	√	√

2. Educational Background

Primary School (Grade 1-6) Fugnanbira Primary School – (1981-1984)

Secondary school (Grade 7- 12) Gursum Senior Secondary School - (1985-1990)

Higher education – Addis Ababa University, Faculty of Veterinary Medicine – (1991-1996) Degree o

Doctor of Veterinary Medicine.

Post graduate: AAU, FVM, 2005-2008, MSc in Tropical Veterinary Epidemiology.

3. Work Experience

Employer – Oromia Agricultural Development Bureau

Date of Employment – October 1996

Duty area – Deder woreda, East Hararge

Major Duties: -

3.1 As field veterinarian and animal health team leader (Oct. 1996-Aug, 2002)

- Involved in livestock treatment and control
- Planning, coordinating, supervising, evaluating and reporting the overall activities of the veterinary team.
- Organized and delivered training on animal production and health issues to development agents, animal health workers, and farmers.
- Meat inspection and monitoring of slaughterhouse.

3.2 As head of woreda agriculture development office. (Sept. 2002- Apr. 2004)

- Leading role (planning, organizing, monitoring and evaluation of the overall activities.
- Preparation of training and workshop to farmers, staffs and other stakeholders and partners.

3.3 As Livestock and Fisheries Resources Development and Animal Health Team Leader (May 2004 . . .)

- Planning and implementation of animal production and health activities.
- Organizing various trainings on livestock production and health issues for development agents, animal health technicians and farmers
- Monitoring, evaluation and reporting

3.4 Experiences in Collaboration with NGOs

3.4.1 FARM AFRICA – Goat Development Program (1999-2001)

- Served as contact veterinarian
- Worked in women selection, verification, screening and group formation.
- Involved in goat purchasing and distribution
- Training and monitoring of community based animal health workers.
- Monitoring and reporting project activities.

3.4.2 OXFAM –GB Deder-Meta Development project

- Served as contact veterinarian
- Active project management committee member
- Involved in oxen purchase and distribution
- Evaluation and reporting of project activities

4 Participation

- Training of trainers on Community Animal Health Workers conducted by FARM AFRICA for 10 days at Asebe-Teferi in June 2000.
- Result Based Monitoring and Evaluation training workshop from 26-31 January 2004 organized by UN World Food Program at Harar.
- Goat Health Management training from August, 21-25,2000 in Debre-Zeit by FARM AFRICA
- Basic Goat Husbandry and Management at Debere- Zeit organized by FARM AFRICA.
- Planning workshop organized by Oromia Bureau of planning and Economic development, December-4, 2002
- Community based targeting and distribution work shop from December 13-14,2003 at Deder by OXFAM-GB
- Training on Rural Entrepreneurship from 5-9, March 2007, at Ambo Ethiopia.

5 Others

5.1 Computer literate

5.2 Publications

- Equine Helminthiasis and Management aspects around Diredawa, shinille and east Oromia , 1996 (DVM dissertation paper)
- Livestock Resource Data of Deder woreda, East Hararge, 1999
- Survey of Livestock Production and Health Problems in Deder woreda, 1999.
- Investigation of FMD outbreaks and assessment of risk factors in Oromia, Amhara and SNNP Regional States of Ethiopia.

6 Reference:-

1. Dr. Gezu Bekele, Tufts University, tell-0911 407631
2. Dr. Mesfin Sahle,NAHDIC, Sebeta, tell- 0911933248
3. Dr. Kelay Belihu AAU,FVM, Debre Zeit,tell- 0911174311

10. SIGNED DECLARATION

I, the undersigned, hereby declare that this thesis is my original work, has not been presented for a degree in any other University and all source of materials used for the thesis have been duly acknowledged.

Name: *Yoseph Legesse*

Signature: _____

Place: *Addis Ababa University*

Faculty of Veterinary Medicine

Debre- Zeit

Date of Submission: _____

This work has been presented with my approval as supervisor.

Name: *Dr.Yilkal Asfaw*

Signature: _____

Date: _____

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