ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

PARTICIPATORY APPRAISAL AND SEROPREVALENCE STUDY OF FOOT AND MOUTH DISEASE IN BORANA PASTORAL SYSTEM, SOUTH ETHIOPIA

BY

TESFAYE RUFAEL CHIBSSA

June 2006
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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University, in Partial fulfillment of the requirements for the degree of Master of Science (MSc) in Tropical Veterinary Medicine

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BOARD OF EXAMINERS:

1. Professor Phlippe Dorchies _____
2. Professor Stieg Einarsson _____
3. Dr. Mark Eisler _____
4. Dr. Maximillan Baumann _____
5. Dr. Mohammed Abdella _____

ACADEMIC ADIVISORS:

1. Dr. Asseged Bogale DVM, MSc, Ass.Prof._____
2. Dr. Mesfin Sahle DVM, MSc, PhD _____

DEDICATION

This thesis is dedicated to my wife Tamirat Tesfahun and my daughter Kaliti Tesfaye for their prayer, love, and encouragement.

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Ca	Antigen control
CBPP	Contagious Bovine Pleuropnumonia
CFT	Complement Fixation Test
CPE	Cytopathic Effect
EDI	ELISA Data Information
EDI	ELISA data information
ELISA	Enzyme Linked Immunosorbant Assay
FAO	Food and Agriculture Organization
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
ICTV	International Committee on Taxonomy of Viruses
IgA	Immunoglobulin A
IgG.	Immunoglobulin G
IgM	Immunoglobulin M
LSD	Lumpy Skin Disease
Masl	Meter above sea level
NAHRC	National Animal Health Research Center
NCR	Non-coding Region
NVI	National Veterinary Institute
OD	Optical Density
OIE	Office International des Epizooties
OP	Oesophageal Pharyngeal
OPD	Ortho-phenylene Diamine
PA	Participatory Appraisal
PAGE	Polyacrylamide Gel Electrophoresis
PAs	Pastoral Association
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PI	Percent Inhibition

PP	Percentage Positivity
PRA	Participatory Rural Appraisal
RNA	Ribonucleic Acid
RRA	Rapid Rural Appraisal
RT-PCR	Reveres Transcriptase Polymerase Chain Reaction
SAS	Statistical Analysis System
SAT	Southern African Territories
SORDU	Southern Rangeland Development Project
SPSS	Statistical Package for Social Sciences
SSI	Semi-structured Interview
ssRNA	Single stranded Ribonucleic Acid
TMB	Tetra-methylbenzidine
UK	United Kingdom
VNT	Viral Neutralization Test
VP1	Viral capsid protein one
Vpg	Viral genomic protein
WRL	World Reference laboratory
WRL	World Reference Laboratory
χ^2	Chi -square

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ABSTRACT

Participatory appraisal and conventional veterinary investigation methods were applied to validate Borana pastoralists' knowledge on cattle disease and to investigate the epidemiology of Foot and Mouth disease (FMD), respectively in Borana pastoral production system of Southern Ethiopia. Furthermore, determination of seroprevalence of FMD and assessment of associated risk factors (including serotyping of the circulating FMD virus) were conducted. The participatory methods used were clinical observation, matrix scoring, proportional piling, and seasonal calendars. Seroepidemiological investigations were performed using 3ABC ELISA and liquid phase blocking ELISA techniques.

The analysis of matrix scoring showed good level of agreement (W= 0.569-0.956) among the 12 informant groups for the disease indicators. Borana Pastoralists descriptions of cattle diseases much overlap with modern veterinary descriptions. The result of proportional piling indicated that about 41% of cattle population suffered from various diseases and 8.8% died in the past one year (FMD recorded the highest incidence of 16.1% and mortality of 1.1%). Calves recorded the highest mean incidence of clinical FMD (*Hoyale*; 18.5%), While lowest in chronic FMD (*Gandille*; 0.2%). Clinical FMD caused the highest mortality (2.8%) in calves compared to adults (0.3%). According to the seasonal calendar, the highest incidence of acute FMD (*Hooyale*) was recorded during the long dry season (*Bonna*) compared to cold dry season (*Hagayya*).

The overall seroprevalence of FMD was 21% (n = 920), herd infection rate, on the other hand, was 59% (n = 116). Significantly higher herd seroprevalence was recorded in Yabello district (61%), followed by Dirre (59%) and Moyale (52) districts. Similarly, Yabello district recorded the highest FMD seroprevalence (26.1%) on animal basis. From the various risk factors analyzed, Peasant Associations (PAs), herd size, sex and age were seen to be significantly associated ($P<0.05$) with the seroprevalence. Liquid phase blocking ELISA test revealed that 'O' type FMD virus is the major serotype (99.2%) circulating in the area, followed by 'A' (95.8%), 'SAT 2 ' (80%), and 'C' (67.5%).

The results of 3 ABC ELISA and Pastoralists' empirical knowledge of FMD showed a moderate agreement (kappa=0.45), indicating that FMD is an important cattle disease in Borana pastoral production system to warrant the institution of appropriate control strategy.

1. INTRODUCTION

Ethiopia has the largest livestock inventories in Africa, possessing more than 31 million cattle, 48 million small ruminants, 1.5 million camels and 7 million equines and 52 million chickens (Desta, 1999). Livestock ownership currently contributes to the livelihoods of an estimated 80 percent of the rural population. In the highlands, livestock are kept under settled or transhumant systems utilizing common pastures, many of which have a high clover content and crop residues. Such livestock includes some 9.3 million oxen providing draught power, for the mixed farming system that prevails. In the arid and semi-arid extensive grazing areas of the eastern, western, and southern lowlands, cattle, sheep, goats, and camels are managed in migratory pastoral production systems (FAO/WFP, 2005).

In the Greater Horn of Africa (GHA), pastoralists occupy large parts of arid and semi-arid lands of Ethiopia, Kenya, Somali, Djibouti, Eritrea, Sudan, Uganda, and Tanzania (FEWS NET, 2004). Together with agropastoralists, they comprise significant proportions of national populations in each of these countries. The Borena pastoralists in the study area manage their cattle, the dominant animal species, in a traditional pastoral system. The Cattle herd is split into two groups: the 'warra' herd is comprised of small number of animals specially milking cows and calves that are kept around the 'Olla's' (permanent encampments); and the 'forra' herd that encompasses the majority of animals which are driven long distances in search of good pasture and surface water, irrespective of national boundaries (Coppock, 1994).

Borana pastoralists' livelihoods depend predominantly on livestock and their products. They practice a transhumance nomadic system, which had been their traditional and primary survival strategy. However, physical infrastructure is poorly developed in areas where pastoralists live (FEWS NET, 2004). This has also been worsened by high prevalence rates of livestock diseases and poor veterinary infrastructures and services.

Borana breed, one of the multipurpose cattle breeds in Africa, was evolved, and is still widely reared by Borana pastoralists of southern Ethiopia. According to recent estimates, the population of this breed living in the Borana pastoral areas is estimated at 1.7 million (Desta, 1999).

Currently Ethiopia exports beef animals to Egypt, most of which originate from Borana pastoral areas. FMD is one of the diseases that can cause restriction on the trade of animals both locally and internationally, thereby threatening the livelihood of Borana pastoralists in particular, and national agricultural economy in general.

FMD, a viral vesicular disease of cloven-hoofed domestic and wild animal species, is one of the highly contagious infectious diseases that cause restriction to the trade of live animals and livestock products internationally (James and Rushton, 2002). It is characterized by fever, loss of appetite, salivation and vesicular eruptions on the feet, mouth and teats (Thomson, 1994). Morbidity is up to 100% in susceptible animal populations but mortality is low in adults. During centuries of evolution of FMD in the field, repeated opportunities for variation have led to the viral diversification which is, now a days, reflected in the co-existence of seven serotypes: A, O, C, SAT-1, SAT-2, SAT-3 and Asia-1 in the world (Sahle, 2004).

The disease spreads rapidly by movement of infected animals or mechanically on fomites such as clothing, shoes, vehicles, and veterinary instruments. The reasons for the rapidity of spread to fully susceptible populations is due to the highly infectious nature of the virus, the production of high titer in respiratory secretions and the large volumes of droplets and aerosols of virus shed by infected animals, the stability of virus in such droplets, the rapid replication cycle with very high virus yields and the short incubation period (Sellers, *et al.*, 1977).

FMD is a global disease that through the years has affected most of the countries. It occurs throughout the world, most commonly in Asia, Africa, the Middle East, and parts of South America. North America, Central America, Australia, New Zealand, Chile, Japan, and most of European countries have been recognized as free, and Uruguay and Argentina have not had an outbreak since April 1994 (Sahle, 2004). Due to poor reporting from the African continent, FMD is considered endemic in most of the African Countries with only Morocco (based on serological survey), Swaziland, Lesotho, Zimbabwe, Namibia, Botswana and the Republic of south Africa being considered free of the disease by the OIE in 1999 (Kitching, 1999).

In Ethiopia, outbreak of FMD frequently occurs in the pastoral herds of the marginal lowland areas of the country (Sahle *et al.*, 2004). This is mainly due to lack of effective vaccine, absence of livestock movement control and absence of systematic disease surveillance and reliable epidemiological data. It is however likely that the disease is underreported due to comparatively high tolerance of local breeds to the clinical episodes of the disease (Leforban, 2005). It seems therefore that FMD is more prevalent and has been one of the major causes for considerable economic loss of the rural communities in Ethiopia.

Providing veterinary services to the communities according to the western model has proven difficult due to lack of infrastructure and the veterinarian has limited experience in harsh environments of pastoral system. Thus, participatory approach (PA) methods become necessary. To this end, there is extensive body of traditional or Ethnoveterinary knowledge that pastoralists have known to possess and on which they rely to diagnose or treat many diseases (Kohler-Rollefson *et al.*, 2001). It is possible that the proper collection, collation, and analysis of data for diseases like FMD that are often under reported by conventional veterinary services would enable to formulate and implement sustainable control of FMD in Pastoral areas. Participatory rural appraisal (PRA) is a systematic data gathering activity carried out by a multidisciplinary team to reveal the unidentified facts about a community (Lielo *et al.*, 1995). Veterinarians and livestock workers have used and are presently using a variety of PRA methods to investigate animal health problems (Catley, 1999). The tools include interviewing, scoring and ranking, and visualization such as seasonal calendars, maps, Venn diagrams, and flow charts.

FMD is probably one of the most important livestock diseases in the world in terms of economic impact. The economic importance of the disease is not only due to the ability of the disease to cause losses of production, but also related to the reaction of veterinary services to the presence of the disease and to the restrictions on the trade of animals both locally and internationally (James and Rushton, 2002). Despite the wide spread and enormous economic importance of FMD in Ethiopia, clinical and serological studies to characterize the disease, under local Ethiopian conditions have never been exhaustive and the endemic level has not been established. The extent to which a disease is recognized as a problem is often dependent on the efficacy of the means for diagnosing it and observing its occurrence (Mochabo, 2003). Community participation

can play an enormous role in animal health services (Leyland, 1991). Therefore the main objectives of this study were:

- To validate Borana pastoralists knowledge on diagnosis of FMD and determination of the incidence, mortality and seasonality of the diseases
- To determine the seroprevalence of FMD and associated risk factors
- To identify the serotypes of FMD virus circulating in the Borana pastoral system

2. LITRATURE REVIEW

2.1. Definition

FMD is the most contagious viral disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. It is characterized by fever, loss of appetite, salivation and vesicular eruptions on the feet, mouth and teats (Thomson, 1994). It is a list A disease according to OIE disease classifications (OIE, 2004).

2.2. Etiology

2.2.1. Taxonomy

FMD virus was defined in 1963 by the International Committee on Taxonomy of Viruses (ICTV) as belonging to the genus *Aphthovirus*, family Picornaviridae. The name, picornaviride is derived from the Latin word 'Pico' meaning small and 'rna' meaning RNA, which refers to the size and genome type, of the virus while the genus name 'Aphthovirus' refers to the vesicular lesions produced in cloven-hoofed animals (OIE, 2004).

2.2.2. Physicochemical properties

Picornaviruses are small RNA viruses that are enclosed within a non-enveloped protein shell (capsid). The capsid consists of polypeptides, which are devoid of lipo-protein, and hence is stable to lipid solvents like ether and chloroform (Cooper *et al.*, 1978). The virus is pH sensitive; and is inactivated when exposed to PH below 6.5 or above 11. However, in milk and milk products, the virion is protected, and can survive at 70 ^{o}C for 15 seconds and pH 4.6. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes (Mckercher and Callis, 1983). Two percent solutions of NaOH or KOH and 4% Na_2Co_3 are effective disinfectants for FMD contaminated objects, but the virus is resistant to alcohol, phenolic and quaternary ammonium disinfectants (Sahle, 2004). The sizes of droplet aerosol also play an important role in the survival or drying out of the virus; droplet aerosol size of 0.5 - 0.7 μm is optimal for longer survival of the virus in the air, while smaller aerosols dry out. In dry

conditions the virus also survives longer in proteins e.g. in epithelial fragments (Donaldson, 1987).

2.2.3. Virus Morphology

Picornaviruses virions are icosahederal with no envelop and contain one molecule of infectious, positive sense, single stranded RNA (ssRNA), ranging from 7-8.5 kb in length. A viral genomic Protein (Vpg), which is enclosed by the 3B genome region, is covalently linked to the 5' end of the genome and a poly (A) tract of variable length is located at the 3' terminus. The diameter of 22 - 25 nm capsids is composed of 60 capsomers each consisting of four proteins (VP1-4). VP1-VP3 is exposed on the surface, whilst VP4 is located internally at the pentametric apex of the icosahedrons and contains a myristic acid molecule attached to the amino terminal glycine (Robert & Bruce, 1981).

2.2.4. Genomic organization

FMDV has single stranded, positive sense RNA that is approximately 8500 bases long and consists of a 5' non-coding region (NCR), a single open reading frame, and a short 3' NCR. It is polyadenylated, on the 3' end and has small virus encoded protein, Vpg, covalently attached to the 5' terminus. The major portion of the FMD genome consists of a single large open reading frame of 6996 nucleotides encoding a polyprotein of the 2332 amino acids (type O, Forss et al., 1984). Four distinct regions are distinguished for the polyprotein namely the L, P1, P2, and P3. Another characteristic, unique to FMDV, is that there are three species of Vpg encoded by protein 3B, termed 3B1, 3B2, and 3B3. All encoded Vpg variants have been shown to be attached to the 5' terminus of viral RNA (King *et al.*, 1982).

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

the virion RNA that is accompanied by the cleavage of VP0 to VP2 and VP4. The P2 (2A, 2B, 2C) and P3 (3A, 3B, 3C, 3D) regions encode for non-structural proteins that are involved in viral RNA replication and protein processing (Belsham, 1993).

2.2.5. Genetic vibration

The observed genetic variation in FMD viral genome is the result of a two-step process. Firstly, the replication of viral RNA is error-prone due to the absence of proofreading in the 3D-encoded RNA dependent RNA polymerase. Secondly, competitive selection is continuously acting on the genome. Thus, those mutants with a selective advantage in the prevailing environment will be better represented than those with a selective disadvantage (Sahle, 2004).

Mutation

Foot-and-mouth disease virus undergoes a high rate of mutation during replication. This is mainly due to a lack of replication error checking mechanisms. RNA viruses that 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} to 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 constitute an intratypic population of FMD viruses with different degrees of genetic relationships, previously described as the quasispecies phenomena (Domingo *et al.*, 1990). This may result in the generation of viral diversity. Changes in the nucleotide compositions of the capsid genes are responsible for the genetic or antigenic variability of the virus (Lews *et al.*, 1991; Meyer *et al.*, 1994). Thus, the generation of new variants is considered as one of the major problems in the control of FMD by vaccination.

Selection

One of the evolutionary mechanisms employed by RNA viruses is the profile mutant production, detailed above. The immune system of an infected animal, which presumably provides a power full selective force, is another driving force in viral evolution (Diez et al., 1990).

Recombination

Recombination is another important process driving viral biology and evolution. In RNA viruses, recombination involves the exchange of genetic material between two non-segmented RNA genomes resulting from polymerase 'jumping' during RNA synthesis. It has been shown that genetic recombination occurs between viruses of the same serotype as well as between serotypes. Intratypic recombination occurs more frequently than intertypic recombination and it appears that recombination events in FMD occur more readily in the 3' half of the genome, than in the capsid genome of the FMDV. Mutations through recombination could result in the exchange of genetic material that could lead to the generation of new antigenic variants that may escape immune pressure (King *et al.*, 1982).

2.2.6. Antigenic variation

One of the consequences of genetic variation through mutation, selection, and recombination is that new antigenic variants are constantly being generated. Not only is there no cross protection between FMDV serotypes, but vaccination with one antigenic variant of serotype does not necessarily protect an animal when challenged with a different virus of the same serotype (Sangare, 2002)). Attempts to characterize the extent of the antigenic variation with in the FMD serotype lead to the establishment of the techniques where by viral subtype could be identified. Initially over 60 different subtypes were identified by world Reference laboratory (WRL), but it quickly became apparent that there is a continues spectrum of intratypic antigenic variants, making a difficulty to identify specific subtypes (Asseged, 2005)).Changes to the genes encoding capsid proteins can result in antigenic variation and evolvement of new subtypes (Haydon *et al.*, 2001). This may give rise to immunological distinct variants that can re-infect individuals that have been previously infected by related viruses. The degree of cross protection among different

subtypes of the same serotype thus varies. Since there is continual antigenic drift in enzootic situation this is an important factor to consider when selecting vaccine strains (Grubman and Mason, 2002).

2.2.7. Serotypes and sub types

Currently there are seven serotypes of foot and mouth disease virus (FMDV), namely O, A, C, Southern African Territories (SAT) 1, 2 and 3, and Asia 1, which infect cloven-hoofed animals. Within these serotypes, over 60 subtypes have also been described using biochemical and immunological tests; and new subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used (OIE, 2004). At present, a sequencing of FMD virus is increasingly being used to establish intratypic variations of FMD viruses and classifying viruses in to genotypes and lineages (Sahle, 2004).

2.3. Epidemiology

2.3.1. Geographical distribution

FMDV has an essentially global distribution, with the exception of North America, Western Europe, and Australia. The FMD status of any particular country or region can be defined as endemic, epidemic (sporadic), or free. FMD-free regions can be defined by national borders (e.g., Australia, Indonesia), by supranational borders (Europe, North America) or by disease-free zones within non-free areas, which are maintained by movement control (e.g., Zimbabwe). Sporadic regions are characterized by repeated incursions of FMD viruses into regions where disease does not usually occur. The disease is either eliminated through control program or disappears naturally without intervention until the next introduction months or years later (Samuel and Knowles, 2001 b).

Some regions have eradicated FMD, often following mass annual prophylactic vaccination campaigns and through the stringent application of Zoosanitary measures following outbreaks.

Continental Europe falls mostly into this category. Some countries, such as the United Kingdom, have eradicated FMD without resort to vaccination. However, FMD is epizootic in several areas of the world and endemic in much of the developing world, including Africa, Asia, part of South America, and the Middle and Far East (Table 1). This situation exists despite continued efforts to control the disease and the extensive use of FMD vaccine throughout the affected areas of the world (Asseged, 2005). Western Europe has had recent outbreaks, which have all been successfully controlled. This includes the 2001 outbreak in the UK, which spread to Ireland, France, and the Netherlands, and separate outbreaks in Italy and Greece. Japan has also recently eradicated outbreaks (Leforban and Gerbier, 2002).

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

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

Source: Asseged, (2005

2.3.2. Host range

FMD is highly contagious and affects over 70 domestic and wild *Artiodactyla* species (Hedger, 1981). Of the domesticated species, cattle, pigs, sheep, goats, and buffalo are susceptible to FMD. In addition, many species of cloven-hoofed wild life, such as deer, antelope and wild pigs, may become infected, although, apart from the African buffalo, their involvement in the epidemiology of FMD in the domesticated species is not certain (OIE, 2004). The susceptibility of these animals can vary with breed of animal and strain of virus. The disease is considerably less obvious or sub-clinical in breeds of cattle, sheep, and goats indigenous to Africa and Asia, where FMD is endemic; and these animals are believed to have been the source of infection for

countries previously considered disease-free (Kitching, 2002a; Kitching and Hughes, 2002; Kitching and Alexandersen, 2002)

2.3.3. The role of carrier animals

Carrier, in FMD, is defined as an animal from which FMD virus can be isolated from the oesophageal pharyngeal (OP) area, more than 28 days after infection. Although it is well established that FMD virus persists in buffalo (up to 5 years), cattle (up to 3 years), Sheep (up to 9 months), and goats (between 3-6 month), the mechanisms underlying persistence and the immunological pathway that eventually leads to viral clearance are not well understood (Bastos *et al.*, 2000; Rossi *et al.*, 1988). This may provide a mechanism for the maintenance of the virus in nature and the cause of acute episodes of disease and may contribute to the emergence of new antigenically variant viruses (Domingo *et al.*, 1992; Kitching, 1998; Sahle, 2004).

2.3.4. FMD Serotypes in Africa

FMD is endemic in sub-Saharan African countries, except for Madagascar. Six serotypes, namely O, A, C, SAT-1, SAT-2 and SAT-3, are endemic in most sub-Saharan African countries with marked differences in the distribution and prevalence of serotypes (Kitching, 1998; Vosloo *et al.*, 2002). A and O are widespread throughout Sub-Saharan Africa, whilst type C appears to have disappeared from the world as a whole, with the exception of Kenya (Kitching, 2002a).

Historically type C is the rarest of the FMDV type to have occurred in Africa, having been recorded only in three countries, namely: Ethiopia, Kenya, and Angola. The last outbreaks of serotype C were reported in Kenya in 1996 and 2000, a country where numerous outbreaks due to the other serotypes, *viz.*, A, O, C, SAT-1 and 2 have also been reported. No other country has as wide a range of serotypes in the circulation (Kitching, 2002a). Type O is endemic in some countries of northern Africa, such as Egypt and Libya, while outbreaks due to this serotype have also been reported in Algeria, Morocco, and Tunisia. In central Africa, and West Africa, serotypes of O, A, SAT-1 and SAT-2 have been recorded since 1958, while most outbreaks were

attributed to serotypes A and SAT-2 (Vosloo *et al.,* 2002). The three SAT types are also prevalent in southern and eastern Africa, SAT1, and SAT2 circulate in West Africa and are the only serotypes to have made incursions into the Middle East with SAT-3 demonstrating the most restricted (Vosloo *et al.,* 2002),(Table 2). Due to poor reporting from the African continent, FMD is considered endemic in most of the African Countries with only Morocco (based on serological survey), Swaziland, Lesotho, Zimbabwe, Namibia, Botswana and the Republic of South Africa being considered free of the disease by the OIE in 1999 (Kitching, 1999).

Table 2. Topotypes of FMD serotypes O, A, C, and South African Territories Types (SAT-1, -2 and -3) in Africa

Sero-types	Topo-types	Representative country (ies)
SAT-1	I	South Africa, southern Zimbabwe, Mozambique
	II	Botswana, Namibia, western Zimbabwe
	III	Zambia, Malawi, Tanzania, northern Zimbabwe
	IV	Uganda
	V	Nigeria
	VI	Nigeria, Niger
SAT-2	I	South Africa, Mozambique, southern Zimbabwe
	II	Namibia, Botswana, northern and western Zimbabwe
	III	Botswana, Zambia
	IV	Burundi, Malawi, southern Kenya
	V	Nigeria, Senegal, Liberia, Ghana, Mali, Cote d'Ivoire
	VI	Gambia, Senegal
	VII	Eritrea
	VII	Rwanda
	IX	Kenya
	X	Democratic Republic of the Congo
	XI	Angola
SAT-3	I	South Africa, southern Zimbabwe
	II	Namibia, Botswana, western Zimbabwe
	III	Zambia
	IV	Northern Zimbabwe
	V	Uganda
O	I	South Africa
	II	Kenya, Uganda
	III	Algeria, Cote d'Ivoire, Guinea, Morocco, Niger, Ghana,
	IV	Burkina Faso, Tunisia
	V	Eritrea, Ethiopia, Tunisia, Egypt
A	I	Angola
	II	Mauritania, Mali, Cote d'Ivoire, Ghana, Niger, Nigeria,
	III	Cameroon, Chad, Senegal
	IV	Angola, Algeria, Morocco, Libya, Tunisia, Malawi
	V	Tanzania, Burundi, Kenya, Somalia, Malawi
	VI	Ethiopia
C	I	Sudan, Eritrea
	II	Uganda, Kenya, Ethiopia
	III	Kenya

Source: Sahle (2004)

2.3.5. The role of wild life

FMD has been reported in several species of wild life, such as African buffalo (*Syncerus caffer*), Impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*) species, Warthog (*Phacochoerus aethiopicus*), and elephants that has a role in epidemiology of the disease.

Buffalo 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. FMDV can persist in an isolated herd of buffalo for up to 24 years, whilst an individual animal can maintain the infection for up to five years. Further, more, buffalo have unequivocally been shown to be a source of infection for cattle under both natural and experimental conditions (Sangare, 2002).The mechanism facilitating SAT-type virus transmission of virus from buffalo appears to occur readily when, there is close contact between the two species during acute stage of infection and shedding large amounts of virus. Impala (*Aepyceros melampus*) is the most frequent infected species and act as intermediaries in disease transmission was recognized. Although studies have established that individual impala do not become carriers, it appears that the disease can persist in impala populations for between 6 and 13 months (Vosloo *et al.*, 2002). Kudu (*Tragelaphus strepsiceros)* were shown to be gradually infected, with the carrier state of between 106-140 days being demonstrated. Experimental infection of warthog (*Phacochoerus aethiopicus*) with SAT2 type virus resulted in severe clinical signs of infection, and transmission to in-contact animals. However, these animals do not excrete virus to the level of domestic pigs and are not believed to play an important role in the epidemiology of FMD in Africa. Rare case of FMD and have also been reported in Indian elephant (*Elephas maximus*) and in the African elephant (*Loxodo Africana*) (Thomson, 1994)

2.3.6. Molecular epidemiology

Phylogenetic analysis of the virus protein 1 (VP1) region of FMD virus has been used extensively to investigate the molecular epidemiology of the disease worldwide. These techniques have assisted in studies of the genetic relationships between different FMD virus isolates, geographical distribution of lineages, and genotypes. It is also used for the establishment

of genetically and geographically linked topotypes and tracing the source of virus during outbreaks (Knowles and Samuel, 2003; Sangare *et al.*, 2003).

Sequence differences of 30 to 55% of the VP1 gene were obtained between seven serotypes of FMD while different subgroups (genotypes, topotypes) were defined by differences of 15 to 20% (Knowles and Samuel, 2003). Since 1987, the analysis of the genetic distance and phylogenetic resolution of the sequence of VP1 encoding gene have provided crucial epidemiological information covering different degree of genetic relationships between field isolates (Sahle, 2004; Samuel *et al.*, 1999). The evolutionary changes of virus are determined by comparing genomic material from more than one virus with each other. At present, DNA sequencing and phylogenetic trees are widely used to illustrate the genetic relation ship between viruses (Sahle, 2004).

2.3.7. Mode of transmission

FMD virus can replicate and be excreted from respiratory tract of animals leading to airborne excretion of virus during the acute phase of infection, although, FMD virus may occur in all the secretions and excretions of acutely infected animals, including the expired air. Therefore, after an animal becomes infected by any means, the primary mode of spread is via respiratory aerosols from infected animals (requires proper humidity and temperature). When proper humidity and temperature are maintained, FMD virus can be carried up to 250 km across the sea and up to 60 km across the land. The prior condition has been held responsible for the FMD outbreak that occurred in France and then spread to UK in 1981 (Kitching, 1992) emphasizing the possible windborne spread of the virus under prevailing environmental conditions. At present, there are Computer models that can predict the most likely wind-borne spread of the virus from infected herds and allow the examination of a variety of control strategies (Sanson *et al.*, 1991; Sahle, 2004). Other important means of spread are by direct contact between infected and susceptible animals and indirectly by exposure of susceptible animals to the excretion and secretion of acutely infected animals. A person in contact with infected animals can have sufficient FMD virus in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals (Asseged, 2005).

2.4. Pathogenesis

The main route of infection in ruminants is through the inhalation of droplets, but ingestion of infected feed, inoculation with contaminated vaccines, insemination with contaminated semen, and contact with contaminating clothing, veterinary instruments, and so on can all produce infection. In animals infected via the respiratory tract, initial viral replication occurs in the pre pharyngeal area and the lungs followed by viremic spread to other tissues and organs before the onset of clinical disease. FMD virus is then distributed throughout the body, to reach best sites of multiplication sites such as the epithelium of Oro-phrynx, oral cavity, Feet, the udder and heart. Virus probably replicate in the mammary gland of susceptible cow, in the pituitary gland. Viral excretion commences about 24 hours prior to the onset of clinical disease and continues for several days FMD virus. The acute phase of the disease lasts about one week and viremia usually declines gradually coinciding with the appearance of strong humeral responses (Murphy *et al.*, 1999 Recovered cattle produce neutralizing antibodies and can resist re infection by the same subtype of virus for up to one year. It was suggested that heat intolerance was a sequel to FMD and was caused by damage to the endocrine system by. (Radostits *et al.*, 1994

2.5. Immune Response

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

exposure to the virus, while IgG activity reaches pick in serum only 14-21 days after infection (Mulcahy, *et al.*, 1990).

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

2.6. Clinical signs

When susceptible animals are in contact with clinically infected animals, clinical signs usually develop in 3 to 5 days (Kitching, 2002a), although in natural infection, the incubation period may range from 2-14 days. 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 in apparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2004).

In cattle, the initial signs are fever of 103-105° F (39.4-40.6° C), dullness, anorexia, and fall in milk production. These signs are followed by excessive salivation, smacking of the lips, grading of the teeth, drooling, serous nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats (Sahle, 2004; Woodbury, 1995). After vesicle formation, drooling may be more marked, and nasal discharge, lameness, or both may increase. Pregnant cows may abort, and young calves may die suddenly without developing any vesicle because of inflammation of the heart (Myocarditis) (Blood *et al*, 1994). Morbidity can approach 100%, but Mortality in adult animals is rare, although in young animals death can occur due to myocarditis and mortality can exceed 50% (Woodbury, 1995). Pregnant cows may abort (Blood *et al.*, 1994). The course of an FMD infection is 2 to 3 weeks although infection may delay recovery of mouth, feet and teat

lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight, and breeding problems. A lactating animal may not recover to pre infection production because of damage to the secretory tissue. A chronic Panting syndrome characterized by dyspnoea, anaemia, hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (Burrow *et al.*, 1981).

In sheep and goats, if the clinical signs occur, it tends to be very mild, and may include dullness, fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mild lameness may be the only sign. In lame animals, there may be vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort and nursing lambs may die without showing any clinical sign (Hughes *et al.*, 2002).

In swine, the initial signs are fever of 104-105° F (40-40.6° C), anorexia, reluctance to move, and squeal when forced to move. These signs are followed by vesicles on the coronary band, vesicles on the heals, vesicles in the interdigital space (foot involvement is usually severe), and vesicles on the snout. Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle and tend to be a "dry"-type lesion; there is no drooling; sows may abort; and piglets may die without showing any clinical sign (Blood *et al.*, 1994).

2.7. Pathology

In cattle, the diagnostic lesions are single or multiple vesicles ranging from 2 mm to 10 cm. These can occur at all sites of predilection. Usually gross lesions on the tongue progress in the following manner; a small-blanched whitish area develops in the epithelium; fluid fills the area and a vesicle (blister) is formed; vesicle enlarges and may coalesce with adjacent ones and then rupture, leaving an eroded (red) area. Gray fibrinous coating forms over the eroded area that becomes yellow, brown or green till the epithelium is restored (Woodbury, 1995).

The vesicle in the interdigital space is usually large because of the stress on the epithelium caused by movement and weight. The lesion at the coronary band at first appears blanched; then there is separation of the skin and horn. When healing occurs, new horn is formed, but a line resulting

from the coronitis is seen on the wall of the hoof. Animals that die may have grayish or yellowish streaking in the myocardium indicating degeneration and necrosis. These findings are known as "tiger heart". Skeletal muscle lesions occur but are rare (Woodbury, 1995)

2.8. Economic importance

FMD is probably one of the most important livestock diseases in the world in terms of economic impact. The economic importance of the disease is not only due to the ability of the disease to cause losses of production, but also related to the reaction of veterinary services to the presence of the disease and to the restrictions on the trade of animals both locally and internationally (James and Rushton, 2002). FMD, therefore, threatens the livelihoods of simple farmers, large sophisticated farming practices and the national and the international economies of the countries (Asseged, 2005).

The direct production effects in extensive production system include loss of milk due to udder involvement, and reduced draught animal power from lesions on the feet. FMD also causes lower rates of live-weight gain in growing animals due to reduced feed intake, and reduction in reproductive capacity by increased abortion rates of up to 10% in animals infected during pregnancy; the disease also causes up to 6% mortality in calves. Restrictions on animal movement and international trade can cause much more serious losses (James and Rushton, 2002).The loss in animal production and international trade restriction imposed following an outbreak makes FMD of a major concern for livestock owners. The control of outbreak (slaughter of infected and in contact, disposal of carcass in disease free zones) and the loss due to the ban on livestock exports costs several million US dollars for a single outbreak (Sellers and Daggupaty, 1990). A striking example is the recent outbreak of serotype O (the Pan Asian strain) in Great Britain, a country that had been free of FMD since 1981. This devastating epidemic of 2001 spread to Ireland, France and The Netherlands where the United kingdom alone were forced to slaughter about 4 million infected and in contact animals. The cost of this epidemic in the United Kingdom was estimated to be more than $29 billion (Samuel and Knowles, 2001a).

2.9. Diagnosis

Clinical diagnosis based on lesion identification, in the early stage of infection, FMD virus or viral antigens can be detected using several techniques. However, different serological methods are used to detect antibody against FMD virus and is the main indication that infection has taken place.

2.9.1. Field Diagnosis

In cattle, FMD should be considered whenever salivation and lameness occur simultaneously and a when a vesicular lesion is seen or suspected. Fever often precedes other clinical signs; therefore, febrile animals should be carefully examined. Early diagnostic lesions may be found before animals start to salivate, have a nasal discharge, or become lame. Clinical diagnosis can present many difficulties due to viral infections of the mucous membrane, which produce similar clinical signs. Differential diagnosis for FMD should include vesicular stomatitis, rinderpest, malignant catharal fever, the bovine herpes 1 infections, swine vesicular disease, vesicular exanthema of swine and bluetongue (Blood *et al.*, 1994).

2.9.2. Laboratory Diagnosis

Due to the highly contagious nature and economic importance of FMD, the laboratory diagnosis and serotype identification of the virus should be done in a virus-secure laboratory (OIE, 2004).

Specimens

Appropriate samples for FMD laboratory diagnosis are; Vesicular fluid usually contains the highest quantity of virus. Epitheliums from early vesicles and from recently ruptured vesicles are tissue of choice for virus isolation (OIE, 2004). When epithelium tissue is not available from ruminant animals e.g. in advance or convalescent cases and infection is suspected in the absence of clinical sign, samples of oesophageal-pharyngeal fluids(OP) is collected by means of a probang and used for virus isolation (Asseged, 2005). Other samples such as, blood with anticoagulant, Serum, and lymph nodes, thyroid gland, adrenal gland, kidney, and heart are good sources of specimens from postmortem.

Virus Isolation

The isolation and characterization of the virus is the "golden standard" for the diagnosis of viral diseases. The suspensions of field samples suspected to contain FMD virus are inoculated into cell cultures (primary pig kidney cells), incubated at 37 ^{0}C and examined for cytopathic effect (CPE), 24 to 48 hours post infection. No CPE confirms the absence of FMDV in the samples. Virus isolation is a very sensitive method, but laborious and expensive and there is the risk of the dissemination of the virus in the environment (Kitching *et al.*, 1989).

Antibody detection by liquid phase blocking ELISA

The liquid phase blocking ELISA detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals (Hamblin *et al.*, 1986 a). The test is based upon specific blocking of the FMDV sample. Rabbit antigen-specific antisera for the different serotypes of FMDV are passively adsorbed to polystyrene micro wells. Serial dilution of test serum is allowed to mix with the specific FMDV antigen; the test serum-antigen mixture is then transferred to an ELISA plate coated with FMDV trapping antiserum (rabbit FMD antisera). The presence of antibodies to FMDV in the serum sample will result in the formation of immune complex and consequently reduce the amount of free antigen trapped by the immobilized rabbit antiserum. In turn, fewer guinea pigs anti FMDV detecting antibodies will react in the next incubation step after the addition of enzyme labeled (HRP) anti-guinea pig Ig conjugate. Following incubation, the substrate/chromogen solution, containing H_2O_2 is added to each well, before being stopped after 15 minutes by addition of sulfuric acid. A change in colour development is read with spectrophotometer at 492 nm filters, in comparison to antigen Control (Ca), containing free antigen only. The diagnostic threshold for this assay is set at 50% inhibition (50PI). If either or both replicate PI values of test serum fall above 50 PI, then that test serum fall above 50 PI, and then that test serum is tentatively considered to be positive. If both replicate PI value of a test serum fall below 50 PI then the test serum is considered as negative (Ferris, 2004).

Antibody detection by 3 ABC ELISA

The detection of antibody to the polyprotein 3 ABC proteins is useful indicator of FMD virus infection with any of the seven serotypes of the virus (Mackay *et al.*, 1998). Antibody to the 3ABC is only found in virus-infected animals but not in vaccinated animals (Diego, 1997).

Briefly, the test is carried out as follows: Microtiter plates are supplied pre-coated with recombinant FMDV 3ABC viral antigen; dilutions of the samples to be tested are incubated in the well of these plates. Any antibody specific for 3ABC binds to the antigen in the wells and forms antigen-antibody complex on the plate well surface. Unbound material is removed from the wells by washing. Peroxidase labeled anti-IgG conjugate is added, which binds to the antibodies of the sample complex with the 3ABC antigen. Unbound conjugate is removed by washing, and the Tetra-methylbenzidinel (TMB) containing substrate is added to the wells. The degree of colour, which develops (optical density measured at 450nm), is directly proportional to the amount of antibody specific to 3ABC present in the sample. The diagnostic relevance of the result is obtained by comparing the optical density (OD), which develops in wells containing the samples with the OD from the wells containing the positive control.

Compared to the liquid phase blocking ELISA, 3 ABC ELISA allows differentiation between samples from infected (3ABC positive) and vaccinated (3ABC negative) animals (Hamblin *et al.*, 1986a,). The 3 ABC ELISA is also rapid test fore screening of large number of sera. In areas where more than one serotypes exist, the test is also cheaper compared to the conventional liquid phase blocking ELISA, which has the disadvantage that each serum sample must be tested against all existing serotypes (Sangare, 2002).

Nucleic acid recognition methods

The polymerase chain reaction (PCR) can be used to amplify the genome fragments of FMD virus in diagnostic material. Specific primers have been designed to distinguish between each of the seven serotypes and in-situ hybridization techniques have been developed for investigating the presence of FMD virus RNA in tissue samples (Woodbury, *et al.*, 1995).

Unlike many living organisms where the hereditary information is enclosed within a DNA genome, FMD virus has an RNA genome that can be sequenced directly, but RNA is unstable and is usually first transcribed into cDNA prior to performing the nucleotide sequence. Reverse transcriptase (RT) when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes. In epidemiological studies of FMD virus, nucleotide sequencing of the VP1 gene has been used extensively to determine the relationships between the field isolates. The technique is also routinely used to investigate genetic variation, molecular evolution in carrier animals, and to identify the source of infection in outbreak conditions (Vosloo *et al.*, 2002).

The molecular epidemiology of FMD is based on the comparison of genetic differences between virus isolates, and showing the genomic relationship between vaccine and field strains for all seven serotypes based on sequences derived from the 1D gene. Sequence differences of 30-55% of the VP1 gene are obtained among seven seroptypes while different subgroups (genotypes, topotypes) are defined by differences of 15-20% (Knowles and Samuel, 2003). Reveres-transcription PCR (RT-PCR) amplification of FMD virus RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform these comparisons (OIE, 2004).

2.10. Control

The official attitude of a country regarding control of a disease depends on how seriously the disease affects the country, the financial and technical ability of the country, and what its neighbors are doing. The degree of control of FMD thus varies as follows (Gonzalez *et al.*, 1992): Routine vaccination is used where the disease is endemic; in contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use strict movement controls and slaughter of infected and contract animals when outbreaks occur (OIE, 2004).

2.10.1. Endemic Areas

In endemic areas, the disease is generally controlled by vaccination and movement restriction of animals. Although the upper respiratory tract is a favored site for infection and replication, FMD vaccines are able to protect animals against the development of clinical disease when given by the parenteral route. One mechanism for this is the transudation of serum antibodies into the mucosae thereby preventing virus attachment to susceptible cells, aggregating and distorting virus particles and facilitating opsonisation, in which virus-antibody complexes are removed from the blood by scavenging phagocytes (Asseged, 2005). Vaccination against FMD virus is achieved with inactivated vaccines that should induce protective immunity against each type of antigens incorporated in the vaccine (Asseged, 2005). Therefore, when vaccinating animals, it is important that the vaccine contain the same subtype of virus as is in the area. This necessitates frequent checking of the serotype and subtype during an outbreak because FMD virus frequently changes during natural passage through various species. Protection induced by aqueous aluminum hydroxide vaccine can protect for 4-6 months while a double emulsion oil vaccine can protect for up to 1 year (Gonzalez *et al.*, 1992).

Intratypic variation of the field strains of FMD viruses must also be considered in the selection of seed virus for vaccine production (Grubman and Mason, 2002.). Immunity to one serotype provides protection only against the homologous viruses. In some cases, inactivated bi-, tri-, or polyvalent vaccine, which contains the representative strains of the serotypes that are in circulation in the region, must be used; therefore, active disease surveillance must be effective which needs a strong field service as well as proper laboratory facilities with efficient methods of detection and characterization of the virus.

The extent of vaccination coverage is of considerable importance to the protection of livestock populations, a concept called "herd immunity". When protective levels of immunity are achieved in the majority of individuals, the establishment or maintenance of the disease with in the population is unlikely to occur. For FMD, it is estimated that 80 to 85% of individuals must have protective levels of virus-neutralizing antibody to achieve herd immunity (Asseged, 2005).

In case of calves born to vaccinated dams, the first vaccination should be, delayed as long as possible to allow decline of material antibody, but, not beyond 4 months, as at that time a high proportion can be expected to respond effectively to vaccination whereas for calves born from non vaccinated dams, the first vaccination may be at 1 week of age (Garland, 1999).

2.10.2. Disease Free Areas

Stamping out

The more affluent FMD free nations, those with an economically significant live animal and animal product export trade, and those whose livestock are highly susceptible to FMD, have contingency plans to deal rapidly with confirmed FMD outbreaks (Sangare, 2002; Woodbury, 1995). In the first instance, 'stamping out' policy, consisting of the slaughter of all affected and in-contact susceptible animals would be instigated, together with associated zoosanitory measures including, the imposition of movements restriction, to control the outbreaks. Such measures might also extend to preemptively slaughtering other herds in which there is no clinical evidence of the disease, but which have been epidemiologically linked with an outbreak, and may therefore contain infected animals. The stamping out is done with full compensation paid for animals slaughtered. The success of 'stamping out' is recognized by the OIE in its guidelines on re- establishing trade following an outbreak (Asseged, 2005).

Emergency vaccination

Emergency vaccination, within an infected area, has gained more preference in recent years, in an attempt to reduce the amount of virus circulating and spreading beyond the restricted area. This so-called 'suppression' or 'dampening down' vaccination regime is now on the agenda of a European Commission working group setup to assist the Animal Health and Animal Welfare (AHAW) committee in establishing criteria for the eradication of certain infectious diseases including FMD. The use of emergency FMD vaccines has two clear objectives. Firstly, to provide protective immunity, as rapidly as, possible to susceptible stock, and secondly, to reduce the amount of virus released and thereby limit the spread of disease. (OIE, 2004; Asseged, 2005).

Protective vaccination

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

2.11. Foot and mouth disease in Ethiopia

FMD in cattle in Ethiopia was first recorded by Food and Agricultural Organization and World Reference Laboratory (FAO/WRL), which indicated that FMD serotypes O, A and C were responsible for FMD outbreaks during the period of 1957 to 1979 (Martel, 1974). The antibodies of SAT2 also were detected in 1971, in sera collected from cattle in the region now known as North Omo, southwestern Ethiopia (Roeder, *et al.*, 1994). According to the annual report of Animal Health Division of Ministry of Agriculture in 2000, the incidence of FMD outbreaks has increased by 1.3-1.5 folds since 1990 (Sahle *et al.*, 2004). Extensive movement of livestock, the high rate of contact among animals at commercial markets, in communal grazing areas and at watering points, were among the reasons forwarded for the increasing incidence of the disease in recent years (Mersie *et al.*, 1992).

However, it is important to note that only small percentage of the outbreaks is reported and typed in Ethiopia, therefore the above figure acutely under estimates the actual problem caused by FMD during the period (Sahle, 2004). During the period of 1988 to 1991, serotyping of FMDV was conducted at National Veterinary Institute (NVI), Ethiopia and WRL for FMD, United kingdom (UK) and serotypes O and SAT2 were identified. This is the first record of the presence of SAT2 FMDV in Ethiopia from sample collected from Borana, Southern Ethiopia (Roeder *et al.*, 1994). From record of outbreak investigation in cattle by NVI, between 1982-2000, three serotypes: O, A and SAT2 FMDV were identified (Gelaye, *et al.*, 2001). The serological survey on FMD indicates that Serotypes A, SAT1 and SAT2 were isolated from buffalo at Omo National

Park; O, A, SAT1 and SAT2 were isolated from cattle and O and A were isolated from Small ruminants in Ethiopia (Sahle, 2004). SAT1 antibody was detected for the first time in both buffalo and cattle.

In Borana pastoral area, FMD outbreak is known to occur two to three times per year, especially during the dry period when cattle have to move long distance in search of good pasture and surface water. Since Borana pastoral area lies bordering Kenya, the only country where five serotypes of FMD virus (O, A, C, SAT1 and SAT2) were recognized, it is a high-risk area with regard to transboundary diseases. The extensive seasonal cross border livestock movements further compound the problem.

2.12. Participatory Appraisal

It was recognized in the early 1970s that formal systems of inquiry were of limited value when working with rural communities in developing countries. Over use of questionnaire surveys, rural developmental tourism and poor cost effectiveness were identified as some of the key problem with formal method of data collection, particularly questionnaire survey (Chambers, 1983). In response to these problems, Rapid Rural Appraisal (RRA) was developed in 1980s, which rather than attempting to collect quantitative data on problems identified by researchers, tends to focus on pastoralists' perceptions of priorities and problems and is characterized by a reliance on qualitative data and, avoids statistical analysis.

In the late 80s, RRA evolved in to Participatory Rural Appraisal (PRA), which facilitated the participation of the communities in the analysis and solving of the problems, and encouraged project beneficiaries to plan and take action (Catley, 1997). It is now widely used by development projects in both rural and urban areas of the third world and some workers are investigating ways of combining participatory and formal approaches (Turton *et al.*, 1996).

Leyland (1991) has reviewed community participation and its role in animal health services. According to this review, there is substantial evidence that Participatory Appraisal (PA) methods do generate information that precisely describes local people perceptions on animal health

problems. The methods are relatively resource friendly and are easily flexible according to given circumstances and information needs (Catley, 1999). In addition, there is an increasing interest by research centers to involve rural communities in the definition of research problems and identification of their solutions (Catley and Irungu, 2000), since community-based programs look promising with regard to sustainability (Budd, 1999). PA has been used to good effect by community based animal projects and consequently, there is now potential for incorporating this method into conventional animal health data collection system (Catley, 1997).

2.12.1. Participatory Appraisal Tools

Participatory Appraisal and methods are useful in particular for veterinarians involved in community-based animal health services. However, it should be noted that participatory assessment is not only methods and tools, but also requires professionals to adopt a respectful, sensitive, and open approach to work with communities (Catley *et al.,* 2002). PA collects information using a toolkit comprising, interviewing, ranking, mapping, and scoring methods. In a typical survey, the combination of tools allows cross checking or "triangulation" of results while the researchers are still in the field. Results are also cross-checked by working with both men and women, and using informants with varying experience, skills, age, social status, or wealth. When investigating subjects such as livestock disease, local experts can be identified who are respected by their communities for possessing specialist knowledge (Catley, 1997). Several types of field data form the core of PA study that includes spatial, temporal, social, and technical data can be collected.

The semi-structured interview (SSI) has some relation with veterinary medicine in history taking and subsequent tentative diagnosis. The SSIs prompts one to have a checklist of some questions, followed by the follow-up questions for the informants for further information (Catley, 1999; Catley and Mohammed; 1996). For the visualization methods, mapping-involving construction of a map on the ground using locally available materials has been used in animal health surveys (Catley, 1999). For spatial data, maps and transects are used (Lelo *et al.,* 1995). For temporal data, the tools that have been employed are timelines, trend lines, and seasonal calendars. Ranking and scoring methods have been widely used whereby informants are required to

compare items or problems in pairs and decide the most important; the results are presented in a matrix with a total rank calculated (Catley, 1999). This scoring method, made visual when a matrix is drawn on the ground, with items along X-axis and indicators along the Y-axis, and stones used to score, has been used in northern Somalia to understand associations between different types of ticks and health problems (Catley and Aden, 1996).

Another visually oriented scoring method is proportional piling (Catley, 1999). The method involves the use of a large pile of counters like stones. The counters are usually 100 that the informants are asked to distribute on different items to show the relative sizes or importance.

Most of PA tools have been used to produce qualitative data and PA survey results are presented in a descriptive rather than numerical form. However, virtually any qualitative data can provide numerical data if transformation occurs at an early stage in data collection process, by defining the number of items to be scored and the number of stones to be used for the scoring of indicators. Specific indictors could be defined by the researchers and added to those produced by the informants. Replication of the standardized scoring tool would allow statistical analysis for non-parametric data.

2.12.2. Participatory appraisal compared to conventional methods

Several advantages and disadvantages of PA methods compared to conventional methods are implied (Catley and Mariner, 2002; Mugenda, 1999). The use of PA tools normally generate largely a qualitative data that are detailed, dynamic and defines local peoples' problems and solutions whereas the conventional (traditional) methods only yield data that are chiefly quantitative and stable (data whose facts do not change). Thus, the PA methods have been qualified as a bottom up approach compared to the top-down approach of the conventional approaches.

The PA approaches are holistic in nature whereby all aspects of the phenomenon in question are studied by use of multiple methods in a process referred to as triangulation. The focus of conventional methods is usually on selected predefined variables. The community has a greater

access, control, understanding, and analysis of information, when the PA tools are employed, but in the conventional ones, the community does not apparently own the information. In addition, this makes the project unsustainable, because researchers in conventional methods are seen as 'outsiders' and, decision-making is entirely from them. In contrast, the PA methods attempt to break the cultural barriers and researchers are seen as 'insiders', which in turn, lead to collection of useful information about the community, particularly with involvement of local people in decision-making.

Typically, quantitative data driven approaches are attempted, but prove to be untenable in large pastoral areas with relatively small and mobile human population, limited modern infrastructure, and insecurity. Other problems include luck of baseline data to perform random sampling procedures and the difficulty of following the herd during longitudinal studies (Catley and Mariner, 2002). The use of standardized PA tools, which produce quantitative data, is another option for veterinary epidemiologist for pastoral areas.

3. MATERIALS AND METHODS

3.1. General description of the study area

The research was conducted in three districts of Borana pastoral area of Oromia Regional State, located between $03^0$37' 23.8" to 05^0 02' 52.4" North and 37^0 56' 49.4" to 39^0 01' 101"East, in the Southern part of Ethiopia (Fig. 1). The altitude ranges from 970 masl in the south bordering Kenya to 1693 masl in the Northeast. The Borana pastoral system represents a vast lowland area, covering about 95,000 km^2 (Coppok, 1994). It borders republic of Kenya to the south, Somali Regional State to the east and, Southern Nation and Nationalities Regional State to the west and Gujji Zone to the North (Annex 1).

Source: FEWS NET

Figure 1: Map of pastoral zones in Greater Horn of Africa (GHA) countries showing location of the study area, Borana pastoral system, South Ethiopia

The climate is semi-arid, which receives annual average rainfall ranging from 500 mm^3 in the South to over 700 mm^3 in the North. Delivery of the rainfall is bimodal: 56% of the annual rainfall occurs from March to May and 27% from mid September to mid November (Coppock, 1994). Annual mean daily temperature varies from 19 °C to 24 °C with moderate seasonal variation. The Borana pastoral system is dominated by savannah vegetation containing mixtures of perennial and woody bush land. The major sources of water are ponds and deep wells during rainy and dry periods respectively (Helland, 1997). Livestock is an integral part of the Borana people that serve several purposes: as source of food, income generation and social prestige (Desta, 1999).

The livestock populations are approximately 1.7 million cattle, 2 million sheep and goats, 700,000 camels and 64,000 equines (Desta, 1999). The Borana pastoralists manage their cattle, the dominant animal species, in a traditional pastoral system. The cattle herd is split in to two groups: "Warra" herd, comprising of small number of animals, especially milking cows and calves, are kept around the Olla's (encampments); whereas "forra" herd, that encompass the majority of animals, are driven long distance in search of good pasture and surface water, irrespective of national boundaries (Coppock, 1994). This cattle keeping system highly exposes the animals to cross-border contagious diseases such as FMD.

3.2. Study population and sampling technique

Study animals were selected from approximately 290,000 Borana pastoral cattle population in three districts of Borana pastoral area namely: Yabello, Dire and Moyale. The three districts were selected from the existing six districts of Borana pastoral system based on their geographical location, proximity to livestock market, availability of watering wells and socioeconomic characteristics. From each district, about 20% of Pastoral Association (PAs) (15) were randomly selected, to be included in the study. From selected PA's, 10% of cattle herds (116) were selected randomly as a primary unit; then eight animals were randomly selected as a secondary unit, only from the selected herds, to be included in the study. Accordingly, 15 PAs, 116 herds and 920 animals were included in the study. This number was arrived at by first assuming the prevalence of 50%, to get the maximum sample size required to determine the prevalence in simple random

sampling and then inflating the number (384) by 2-3 folds, to account for the effect of randomness and representativeness in multistage sampling (Thrusfiled, 1995):

$$n = \frac{1.962 \times P_{exp}(1 - P_{exp})}{d^2}$$

Where,

n	=	required sample size
P_{exp}	=	expected prevalence
d^2	=	desired absolute precision

$$n = \frac{1.96^2 \times 0.5 (1 - 0.5)}{0.05^2} = 384 \text{ cattle}$$

Fifty of the selected herd owners (20 each from Yabello and Dirre, and 10 from Moyale districts), who were known to have a good Ethnoveterinary knowledge were chosen as key informants in a participatory appraisal.

3.3. Study Design

Both participatory appraisal and conventional veterinary investigation methods were applied to generate information on FMD. The participatory appraisal methods used were Clinical observation, Matrix scoring, Proportional piling, and Seasonal calendar (Catley, 2005; Catley *et al.*, 2001). Conventional investigation of FMD was conducted using 3ABC ELISA (to determine seroprevalence) and liquid phase blocking ELISA (to identify the serotypes). Geo-referenced data such as geographic location and altitude were collected using GARMIN 76 GPS.

3.4. Participatory disease appraisal

3.4.1. Clinical Observation

Clinical Observation of sick animals related to FMD was done during sample collection inorder to crosscheck, the perception of the livestock keeper with other participatory appraisal results. We visited 116 Borana herds during sera sample collection that eight animals with chronic FMD were found at different sites. The pastoralists identify the animals very well. Clinical signs

considered were, hair over growth, emaciation, reduced milk production, seek shade, and panting. Blood samples from these animals were collected for serological tests.

3.4.2. Matrix Scoring

During the participatory appraisal Semi-structured interviews (SSI) were used to gain an understanding of the local perception of cattle diseases. The groups of informants were identified by key informant to prioritize and rank the most important cattle diseases (Table 3). Pair- wise ranking of five cattle disease (FMD (Hooyale) and other four control diseases (CBPP (Sombessa), LSD (Sukii), Blackleg (Harka) and Mastitis (Nakarsa)) was conducted to identify locally perceived indicators (sings). The five diseases indicated above were presented using every day objects and placed along the tope X-axis of the matrix. Each of the five diseases in the matrix was scored against a list of 17 clinical signs or cause of the diseases. The indicators were illustrated along the Y-axis of the matrix (Fig. 2). For each indicator, informants were asked to score each disease by dividing piles of 25 stones against the five diseases. Matrix scoring was standardized and repeated with five in Yabello, five in Dire, and two PAs in Moyale (total 12 informants). Group sizes varied from 6 to 13 individuals. The level of an agreement across the groups was determined by the method of Siegel and castellan (1994).

Figure 2 Matrix scoring by men elders from Yabello district, Borana pastoral area

3.4.3. Proportional piling

Before performing the proportional piling to estimate the relative incidence and mortality caused by five most important cattle diseases during the past one year, informants were asked to classify the animals into different age groups, that fits into real cattle husbandry system: Waatiye/Jabiye (calves aged 0-2 years of both sexes); Raada/Jibicha (Weaner 2-3 years); Goromsa/Kurkura (young group 3-4 years); Hawicha/Korma (>4 years old).

Then every informant was allowed to maintain a pile of 100 stones for each age group, before splitting the pile of stones into two relative numbers of sick and healthy stocks during the past one year (Fig. 3). The pile of stones representing the sick cattle was then subdivided into the five major disease and other diseases to show the relative number of animals affected by different diseases during the year. Subsequently, each informant was asked to remove some of the already allocated stones representing the sick to indicate the number of dead animals/survivals during the year for each five prioritized and other disease of cattle. For FMD chronic disease was scored for animals that survived the acute attack. Age specific incidence and mortality rates and 95% confidence limits were calculated with parson's correlation coefficient.

Figure 3: Proportional piling by women informants in Moyale district, Borana Pastoral area

3.4.4. Seasonal Calendars

Seasonal calendars were used to describe the seasonal occurrences of the five most important cattle diseases identified in the matrix scoring. The methodology for constructing seasonal calendar was similar to matrix scoring. The season's local names of Borana long rain (Gana), short rain (Adolesa),cold dry (Hagaya) and long dry (Bona) were represented by local material on the X-axis and numbers representing diseases and causes placed along the Y-axis. This type of seasonal calendar was used with five groups of informants from Yabello, four from Dire and one from Moyale districts (10 groups of informants) (Fig. 4)

Rainfall data derived from seasonal calendar was compared with objective measures of rainfall collected by the Southern Rangeland Development Project (SRDU) to determine mean amount and proportion of total annual rainfall per Borana season.

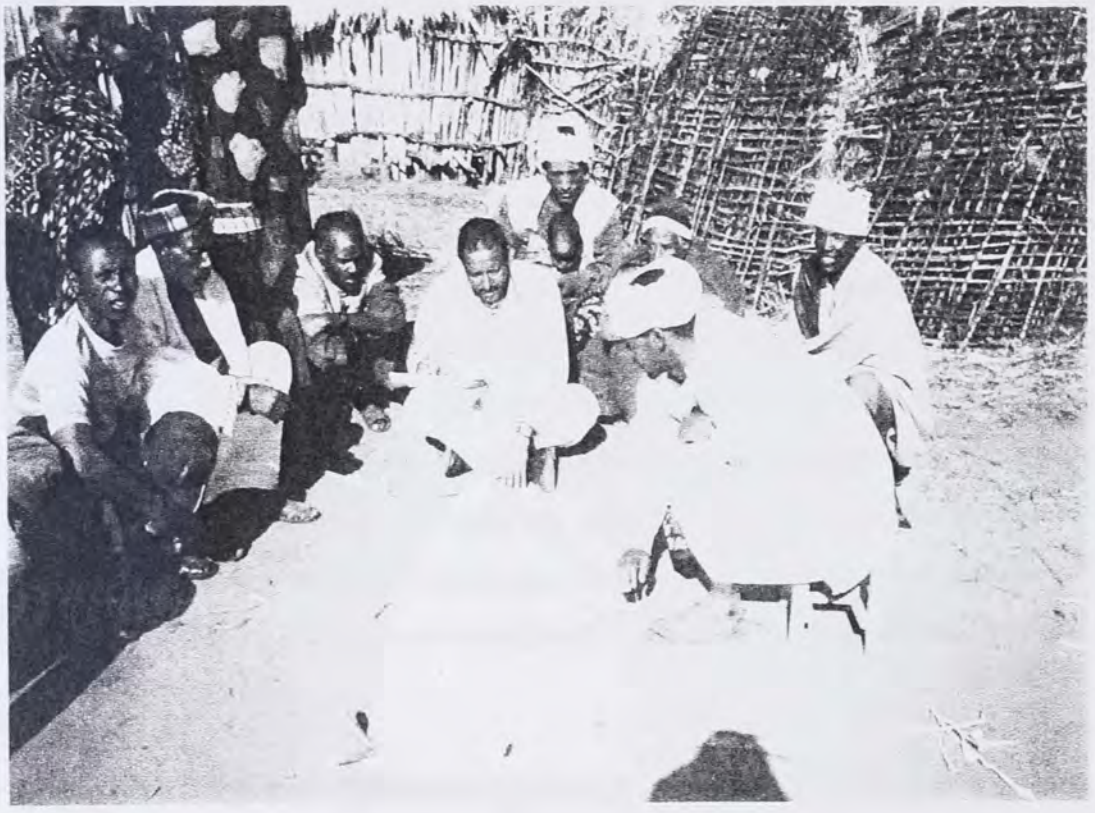


Figure 4: Seasonal calendar by men elders from Dire district, Borana pastoral area

3.5. Serum sample collection

Whole blood was collected from a jugular vein of randomly selected cattle into 10 ml sterile vacationer tubes and stored overnight at room temperature for serum separation. The serum was then transferred into a single sterile cryovial, bearing the names of the owner and PA, and herd number, age and sex, and transported in an icebox, to National Animal Health Research Center, Sebeta, for the analysis. In the laboratory, the serum was stored at -20 ^{0}C until laboratory investigation.

3.6. Laboratory Analysis of FMD

3.6.1. FMD-3ABC ELISA

About 100µl of prediluted samples (1: 16 in diluent buffer A) and controls (1: 100 in CHEKIT FMD 3ABC sample diluents) were dispensed into the appropriate wells of the microtiter plate pre-coated with recombinant FMDV 3ABC viral antigen (Annex. 2). The pleats were covered with a lid and incubated for 60 minutes at 37^{0}C in a humid chamber. After incubation microplates were filled with about 300µl CHEKIT FMD 3ABC washing solution and washed three times. Then 100µl of the ready to use CHEKIT-FMD 3ABC-Anti-Ruminant-IgG-Po-Conjugate was dispensed into each well and incubated for 60minutes at 37^{0}C in humid chamber. After washing the plate, 100µl of TMB-substrate was dispensed into each 96 wells and incubated at room temperature or optimally at 25 ^{0}C for 15 minutes. Finally, adding 100µl, CHEKIT-stopping TMB-solution stopped the reaction, and the result was read using a spectrophotometer at 450nm wavelength within 2 hours of adding the stopping solution. The Reader, connected to the computer loaded with ProComm and word packages, was used to automate the reading of OD value. The percentage positivity (PP) for test samples in relation to the negative and the positive controls was calculated as follows:

Equation .1 $$\text{PP value} = \frac{OD_{sample} - OD_{negative}}{OD_{posetive} - OD_{negative}} \times 100\%$$

The Cut off value provided by the Manufacturer was used to determine the percentage positivity,

3.6.2. Liquid phase blocking ELISA

Plates were coated with 50µl trapping rabbit antibody stock (Rabbit anti-FMDV serotypes O, A, C and SAT_2) diluted 1:1000 in coating buffer (carbonate/bicarbonate) into 96 wells of NUNC Maxisorp microplate and incubated at 4^0c over night. Simultaneously, 50µl of test and control sera (C^{++}, C^+ and C^-), diluted 1:16 in diluents buffer A (PBS and Tween 20) were added into wells of U bottom microplates according to lay out (Annex. 3) and 50µl of FMDV Antigen (serotypes O, A, C and SAT2) diluted at suggested working dilutions were added into all 96 wells of the perspective polypropylene U bottom microplates. Sera and antigen were mixed and incubated at 4^0C over night, washed with dilution of PBS at pH 7.4, three times; then, 50µl serum-antigen mixture was transferred from U bottom microplates to the appropriate wells of NUNC Mixsorp plate (Annex 4), and incubated at 37 ^{0}C for one hour, with continuous shaking. After microplates were washed, 50µl detecting antibody (Guinea pig anti-FMDV serotypes O, A, C and SAT2), diluted 1: 1000 in diluent buffer B (PBS, Tween 20 and skimmed milk powder) was added into all 96 wells of the respective microplates and incubated at 37^0C for one hour with continuous shaking. After washing the plates, 50µl of conjugate (Horseradish peroxides-conjugated rabbit anti-guinea pig immunoglobulin) diluted 1:200 in diluent buffer B was added into 96 wells of each microplate and incubated at 37 ^{0}C, for one hour with continuous shaking. Finally, the plates were washed and 50µl of substrate/chromogen (hydrogenperoxide (H_2O_2)/Ortho-Phenylenediamine (OPD) solution was added and incubated at ambient temperature for 15 minutes (briefly placed on the shaker to ensure even mixing) before 50µl of stopping solution (sulphuric acid (H_2SO_4) was added into all 96 wells of the microplates.

The ELISA reader was connected to the computer loaded with ELISA Data Information (EDI) Software, which is used to automate the reading of OD value and calculate the percentage inhibition (PI). The percent inhibition (PI) for control and test samples was calculated according to equation 2 and 3 respectively using manufacturer's manual (Ferris, 2004):

Equation. 2: Formula for calculation of PI on the control and qualify assurance acceptance.

$$PI = 100 - \frac{\text{Replicate OD of control x 100)}}{\text{Median OD of Ca}}$$

Equation 3: Formula for calculation of PI of test sera and Diagnostic interpretation.

$$PI = 100 - \frac{\text{Replicate OD of test serum x 100)}}{\text{Median OD of Ca}}$$

3.6.3. Antigen titration

Antigen titration was done to check the working dilution of each FMD antigen used in serotyping. Plates were washed three times between each stapes except after substrate added. The following procedures were used (Ferris, 2004):

Test plates were coated with rabbit trapping antibody stock of FMDV serotypes O, A, C, and SAT_2 diluted 1: 1000 in carbonate/bicarbonate coating buffer at PH 9.6. Then, 50µl of FMDV serotype O trapping antibody was dispensed into all wells of rows A and B of the microplate; FMDV serotype A trapping antibody to rows C and D; FMDV serotype C trapping antibody to rows E and F; and FMDV serotype SAT_2 trapping antibody to rows G and H and incubated over-night at 4°C. After washing the plates with PBS buffer solution three times, 50µl of diluents buffer A was dispensed into wells of rows A to H, columns 2 to 12, and 75µl to wells of rows A and B of column 1. Then 25µl of antigen serotype O was added to wells of row A and B of column 1, serotype A to rows C and D, serotype C to rows E and F, and serotype SAT_2 to rows G and H and mixed. About 50 µl of this dilution (1/4) was transferred from column 1 to 2, from 2 to 3 and soon, to repeat the mixing procedures as far as column 11; column 12 was left as a background control. The plates were then incubated at 37 ^{0}C for one hour with continuous shaking. After washing, 50µl of diluted 1:100 FMDV serotype O detecting antibody was added into all 12 wells of rows A and B of the microplate, serotype A detecting antibody to rows C and D, serotype C detecting antibody to rows E and F, and serotype SAT_2 detecting antibody to rows G and H. The plates were incubated at 37 °C for one hour with continuous shaking. After

washing with PBS, 50µl of anti species conjugate diluted 1:200 in diluents Buffer B was added into all 96 wells of the microplates and incubated for one hour at 37 ^{0}C with continuous shaking. After the pleats were washed and dried, 50µl of the substrate/chromogen solution was added into the all-96 wells of the microplates and incubated at ambient temperature for 15minutes, under continuous shaking, before 50µl of stopping solution was added and the plates read with spectrophotometer at 492nm filter. As indicated in the protocol, the dilution that gives an average OD value of 1.0 to 1.5 was selected and twice that concentration was used in the serum sample in the liquid phase blocking ELISA. Accordingly, the antigen working dilution used was selected for each serotype as follows:

O_1 Manisa	Lot 2590P031	Used at working dilution of 1:60
A_{22} Mahmatli	Lot2240P032	Used at working dilution of 1:60
C PHI7/84	6/8/99	Used at working dilution of 1:10
SAT2 Eritrea	Lot2330P036	Used at working dilution of 1: 60

3.7. Data collection and analysis

The participatory and laboratory investigation results were analyzed using Statistical Package for Social Sciences (SPSS, 2002) and Statistical Analysis System (SAS. 1998), respectively. Agreement among the scores of informant groups was assessed using Kendall coefficient of concordance (W) (Siegel and castellan, 1994). The level of agreement between FMD diagnosis by pastoralists and conventional diagnosis by way of 3ABC ELISA was analyzed by kappa statistic and positive predictive value (Thrusfield, 1995). Finally, logistic regression (SPSS, 2002 Version 11.5) was used to determine risk factors associated with seropositivity of the disease in the study area. In all the analyses, confidence level was at 95% and $P \leq 0.05$ was set for significance.

4. RESULTS

4.1. Participatory appraisal

4.1.1. Clinical observation

Eight chronic FMD cases were observed during the study with typical signs of rough and overgrowth of hair coats and emaciation (Fig. 5). The major clinical signs described by the farmers for animals with chronic FMD (*Gaandile)* were overgrowth of hair, dullness, isolation from the herd, abdominal breathing, panting, frothy discharge from the mouth, behavioral changes such as dysphagia, standing under shade during hotter hours of a day and reduced milk production, extended calving interval, and birth of weak calves with watery milk in the first week of birth. Heifers often develop chronic FMD after they recover from the acute attacks.

From eight animals identified as suffering from chronic FMD (*Gaandile*) by the pastoralists 50% were found positive by 3ABC ELISA, for the combined serotypes (O, A, C, and SAT2).

Figure. 5. A cattle with sign of chronic FMD, (*Gaandile*) at Dire district in Borana pastoral area.

4.1.2. Disease Matrix scoring

The result of semi-structured interviews (SSIs) revealed that Borana pastoralists were able to describe diseases and ranked them based on mortality, loss of production (milk), frequency of occurrence and the ease of or control. Table 3 shows diseases listed by pastoralists according to their order of importance.

Table 3 Summary of common cattle diseases prioritized by Borana pastoralists in the three districts of Borana area

Local Name	Scientific Name	Rank
Sombessa	Contagious Bovine Pleuropneumonia	1
Hooyale	Foot and Mouth Disease	2
Suuki	Lumpy Skin Disease	3
Haarka	Blackleg	4
Nakarsa	Mastitis	5
Silissa	Pasteurelloss	6
Dhukuba Gadamsa	Malignant Catharal Fever	7
Chirmale	Anthrax	8
Gaandile	Chronic Foot and Mouth Disease	9
Gandi	Trypanosomosis	10
Birtte	Babesiosis	11
Nyanye	Rabies	12

A pair wise comparison was used on five diseases of cattle, ranked high by pastoralists, to determine the disease indictors for use in matrix scoring; FMD (*Hooyale)* was the disease of interest while the other four were control diseases (Table 4).

Table 4. Summarized Matrix scoring of disease indicators in three districts of Borana pastoral area

Indictors	CBPP (Sombesa)	FMD (Hooyale)	LSD (Suuki)	Black leg (Haarka)	Mastitis (Nakarsa)
Coughing (W=0.956***)	25(17-25)	0(0-8)	0(0-0)	0(0-0)	0(0-0)
Salivation (W=0.885***)	0(0-6)	25(15-25)	0(0-0)	0(0-0)	0(0-0)
Abortion W=0.881***)	0(0-7)	25((18-25)	0(0-7)	0(0-2)	0(0-0)
Lameness (W=0.900***)	0(0-3)	13.5(6-24)	0(0-3)	7.5(1-13)	0(0-7)
Mortality (W=0.871***)	7.5(3-11)	3(0-5)	4(0-6)	10.5(7-22)	0(0-6)
Reduced milk production (W=0.543***)	0(0-8)	11(6-18)	3(0-9)	0(0-8)	10(2-13)
Loss of body weight (W=0.814***)	1(0-8)	16(8-25)	6(0-12)	0(0-2)	0(0-4)
Skin lesion (W=0.782***)	0(0-0)	7(0-10)	15.5(12-15)	0(0-5)	2(0-8)
Teat lesion (W=0.762***)	0(0-0)	0(0-5)	5(0-17)	0(0-0)	19.5(8-25)
Hair over growth (W=0.902***)	0(0-0)	25(17-25)	0(0-8)	0(0-0)	0(0-0)
Panting (W=0.778***)	3(0-15)	22(10-25)	0(0-5)	0(0-2)	0(0-0)
Seek shade (W=0.768***)	0(0-10)	20.5(14-25)	0(0-5)	0(0-4)	0(0-0)
Decrease fertility W(=0.840***)	0(0-0)	22(14-25)	2.5(0-11)	0(0-0)	0(0-0)
Decrease market value (W=0.624***)	0(0-9)	9(6-12)	7.5(4-13)	0(0-0)	5(0-13)
Disease affect wild life (W=0.569***)	0(0-0)	19(0-25)	0(0-7)	0(0-14)	0(0-5)
Transmitted by tick (W=0.9245***)	0(0-0)	0(0-0)	0(0-12)	0(0-0)	25(13-25)
Transmitted with contact (W=0.840***)	4.8(2-8)	6(5-10)	7(4-10)	0.5(0-4)	0(0-2)

Number of informants groups =12; W = Kendall's Coefficient of Concordance (*P<0.05; **P<0.01; ***P<0.001). W values vary from 0 to1; the higher the value the higher the level of agreement between informants groups. The number out of parentheses indicates median scores of the 12 groups and minimum and maximum limits are shown in Parentheses.

The analysis of matrix scoring showed good level of agreement among 12 informant groups (W= 0.569-0.956; P< 0.001). The groups of informants also indicated that salivation, lameness, abortion, decreased milk production, loss of body condition and mortality (in young animals) are the most important signs of FMD. In all groups informant scored the disease FMD by considering both Hoyale and chronic FMD (*Gaandile*).The disease Gaandile affected animals that seemed to recover from FMD but later developed signs of hair over growth, panting, seeking shade during sunny days and decreased milk production and infertility. Additional signs of FMD as observed by the pastoralists through SSI were lesion on foot and mouth, reduced appetite, birth of weak calves or still birth, and secretion of watery milk for about a week after calving.

FMD (*Hooyale*) and LSD (*Suuki*) in the matrix scoring received high scores for transmission by contact; women informants on the other hand scored mastitis (*Nakarsa*) as case strongly associated with tick infestation. The informants also indicated that FMD could affect other domestic animals such as sheep and goats, and wildlife such as Oryx, Kudu, and Gazelle.

4.1.3. Proportional piling result

Incidence

The Borana pastoralists categorize cattle into four age groups: calves, *Waatiye/Jabiye* (Both sex); Weaner, *Raada* (Female)/*Jibicha* (male); Young, *Goromsa* (Female)/*Kurkura* (Male) and Adult, *Hawicha* (Female)/Korma (Male). The disease incidence in specific age groups as estimated by the pastoralists is illustrated in Fig. 6 (a-f).

According to the result of proportional piling, acute FMD (*Hooyale*) had the highest incidence (18.5%) in calves (Jabiye) followed by adult male and female (Hawicha /Korma) (16%) (Fig. 6b). The Mean annual incidence of chronic FMD (*Gaandile)* varied from 0.2% in calves (Jabiye) to 1.8% in heifers (Goromsa) (Fig.6f). Pearson correlation coefficients for disease incidence by age groups were -0.124 (P>0.05) and 0.47 (P<0.001) for acute FMD, (*Hooyale*) and chronic FMD (*Gaandile*), respectively.

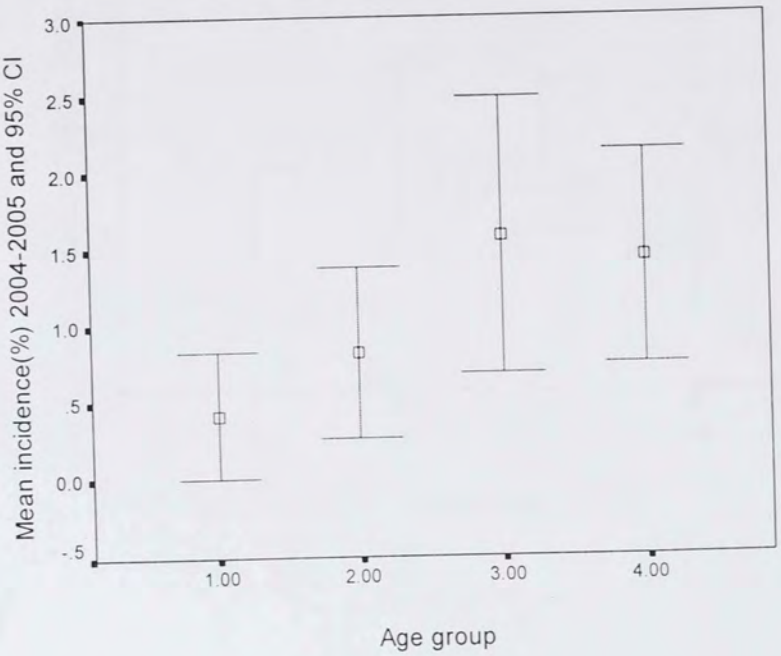
Figure 6 The mean annual incidence of important cattle diseases in different age groups in three districts of Borana pastoral area (Dec. 2004-Nov. 2005)

A. Contagious Bovine PleuroPnumonia/ *Sombesa*

Age groups:

1 = Calves *0-2 years*
2 = Weaner *2-3 years*
3 = Young *3-4 years*
4 = Adult *>4 years*

(N=50)

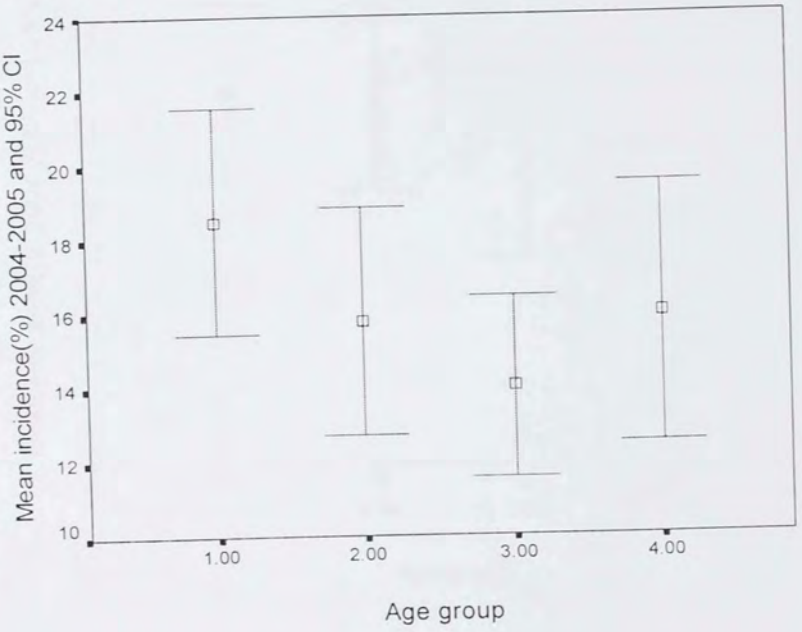


B. Foot and Mouth Disease/ *Hooyale*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)

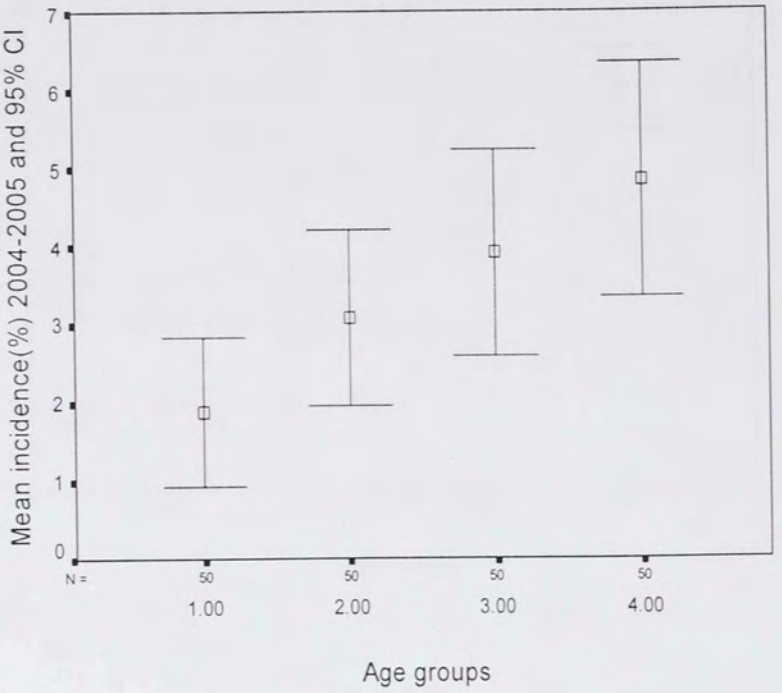


C. Lumpy skin disease/*Suuki*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)

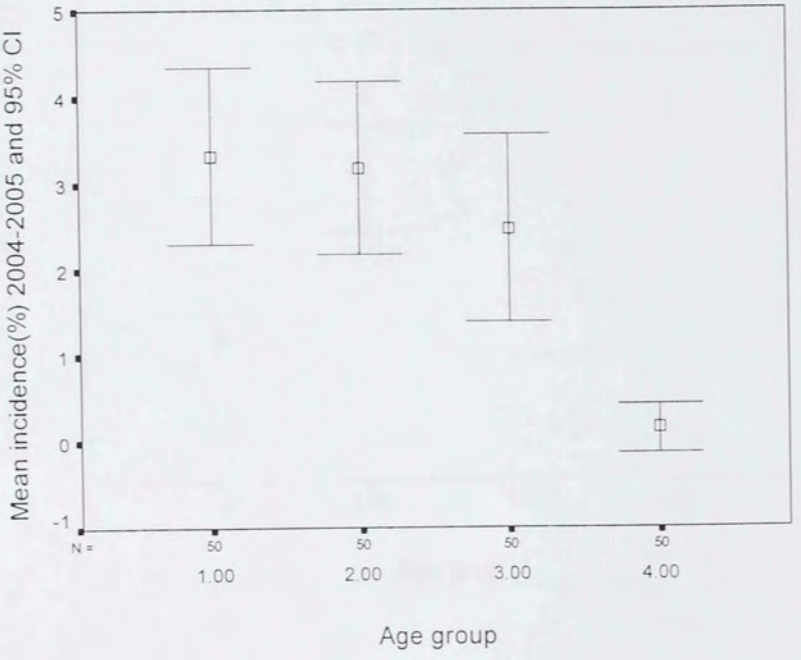


D. Blackleg /*Haarka*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)

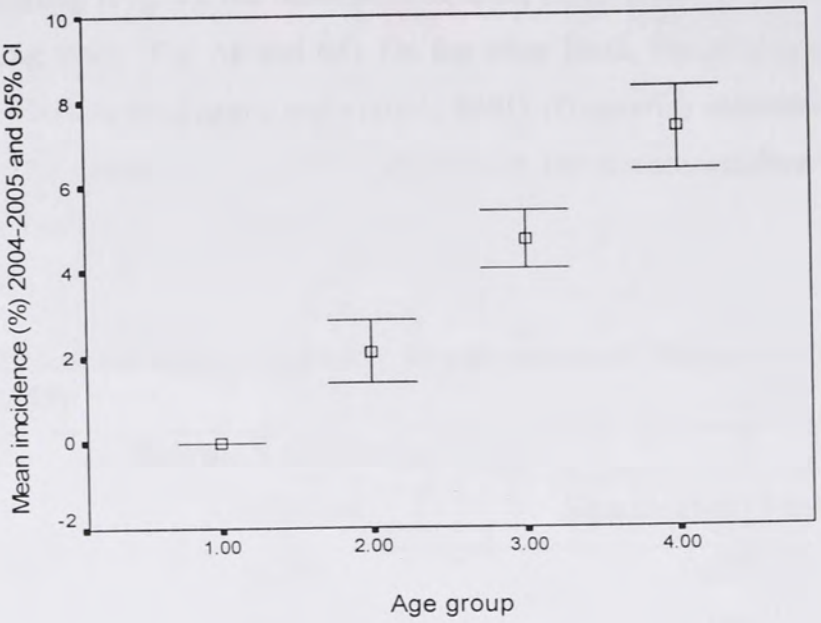


E. Mastitis/*Nakarsa*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)

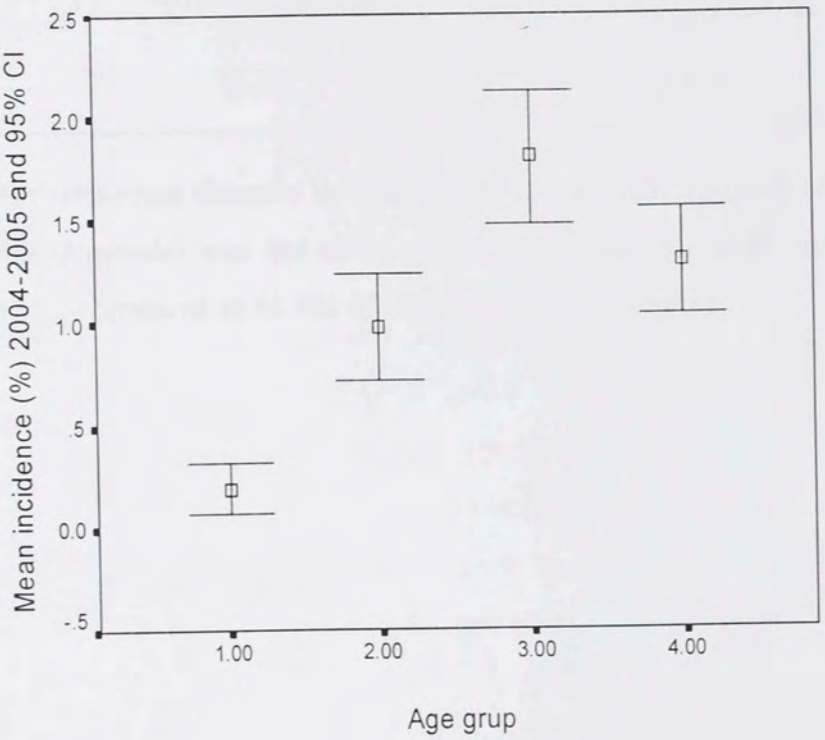


F. *Chronic FMD/Gaandile*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)



According to proportional piling (Fig. 6), the incidences of acute FMD (Hooyale) and Black leg (Haarka) were high in young stock (Fig. 6b and 6d). On the other hand, the incidence of CBPP (*Sombesa*), LSD (Suuki), Mastitis (Nakarsa), and chronic FMD (*Gaandile*) increased with age (Figs 6a, 6c, 6e, and 6f). The Pearson's correlation coefficients for disease incidence with age group were summarized in Table 5.

Table 5 Correlation coefficient for disease incidence by age groups in Borana pastoral area (Dec. 2004–Nov. 2005)

	Pearson's correlation	
Disease	**Coefficient**	**Significance (2-tailed)**
CBPP /*Sombesa*	0.196	0.005
FMD /*Hooyale*	-0.124	0.081
LSD /*Suuki*	0.232	0.001
Blackleg /*Haarka*	-0.391	0.000
Mastitis /*Nakersa*	0.775	0.000
Chronic FMD /*Gaandile*	0.47	0.000

Conversely, compared to other important diseases such as CBPP (*Sombesa*), Blackleg (*Haarka*), and Mastitis (*Nakarsa*), FMD (*Hooyale*) was the most commonly observed disease during the year; reportedly affecting 16.1%, compared to 41.6% of the total affected (Figure 7).

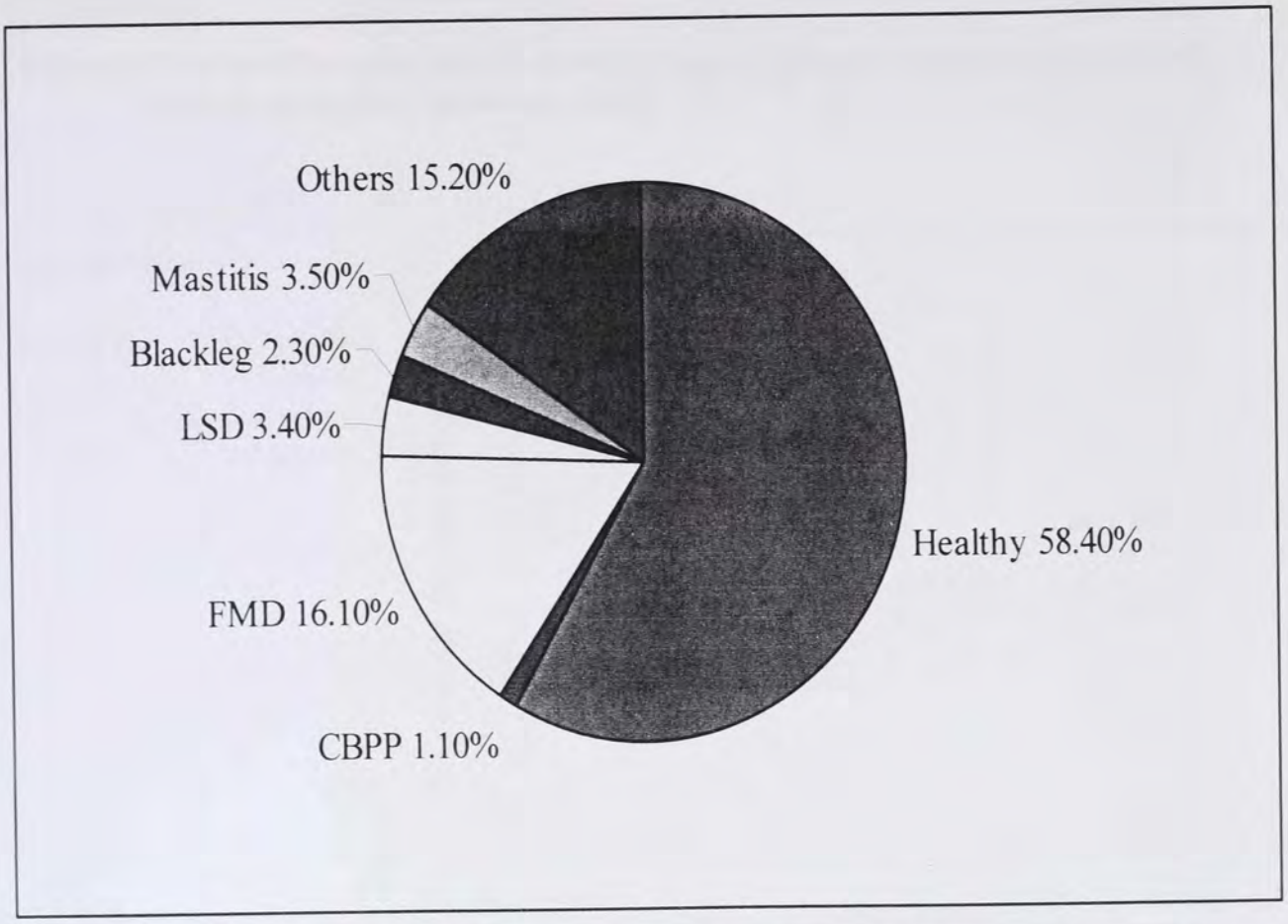


Figure 7. Mean incidence of cattle diseases, in relation to healthy cattle of all age groups in Borana pastoral area (Dec. 2004–Nov. 2005)

Disease mortality

The estimated mortality rate attributed to important cattle diseases in different age groups are shown in (Fig. 8). FMD (*Hooyale*) had the highest mortality (2.8 %) in Calves (*Jabiye*) and lowest (0.3%) in adult (*Hawicha/ Korma*) (Fig. 8b). Pearson correlation coefficients for disease mortality by age groups was -0.589 ($P<0.001$) for acute FMD (*Hooyale*) and Blackleg (Haarka). In all other disease conditions, mortality is negatively correlated to age groups ($P < 0.001$). Pearson's Correlation coefficients for age specific mortality is presented in Table 6.

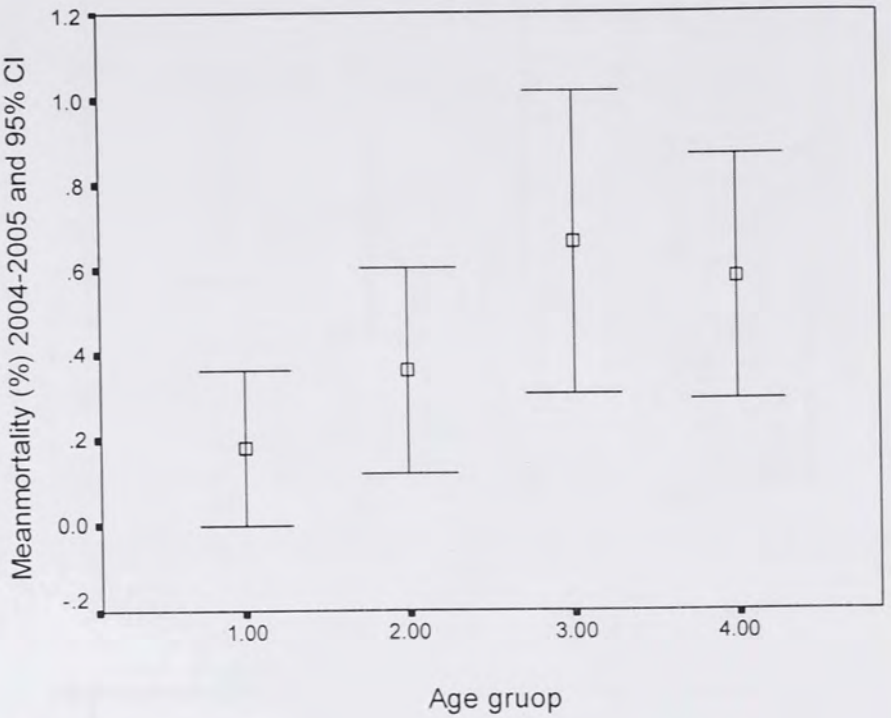
Figure 8 Case specific mean annual mortality rate of different age groups in Borana pastoral area (Dec. 2004-Nov. 2005)

A. CBPP/*Sombesa*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50

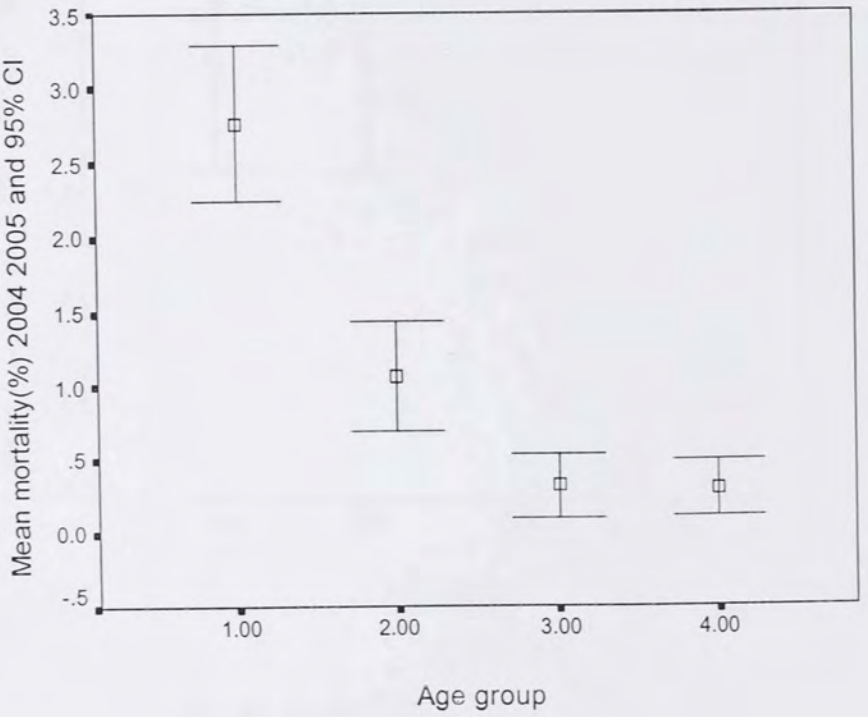


B. FMD/*Hooyale*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)



C. Lumpy skin disease/*Suuki*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

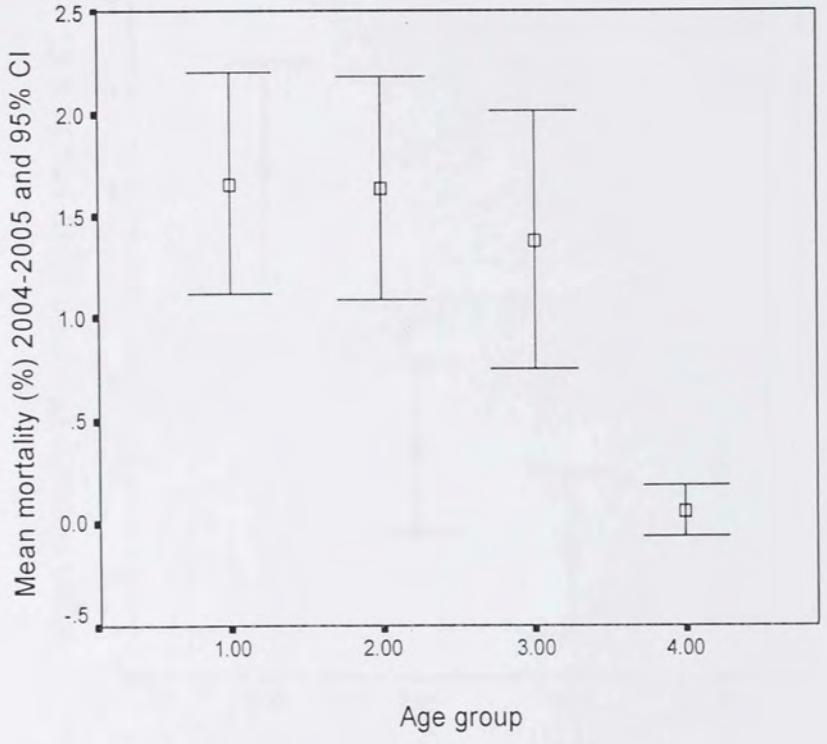
(N=50)

D. Blackleg/*Haarka*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)

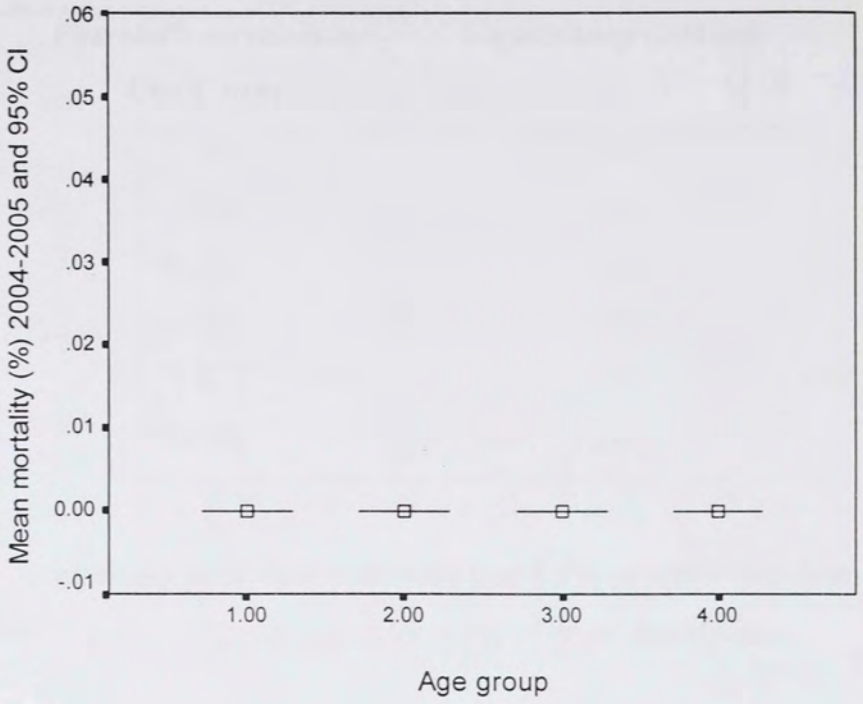


E. Mastitis/*Nakarsa*

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)



F. Other diseases

Age groups:

1=Calves *0-2 years*
2=Weaner *2-3 years*
3=Young *3-4 years*
4=Adult *>4 years*

(N=50)

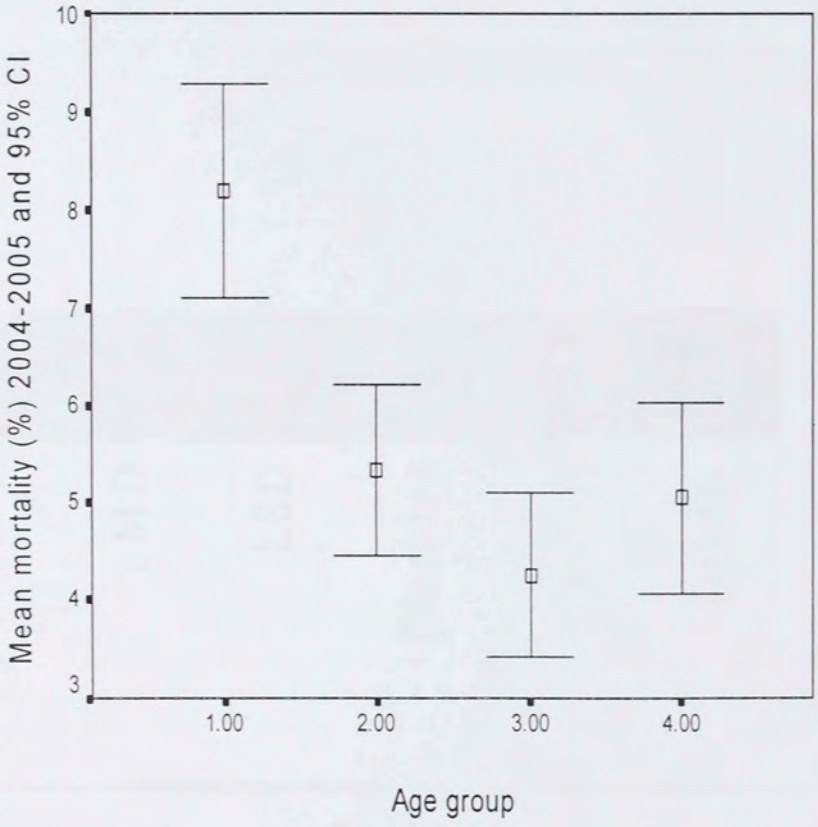


Table 6 Correlation coefficients for age specific mortality rate in Borana pastoral area (Dec. 2004-Nov. 2005)

Disease	Pearson's correlation Coefficient	Significance (2-tailed)
CBPP /*Sombesa*	0.185	0.01
FMD /*Hooyale*	-0.589	0.00
LSD /*Suuki*	0.023	0.75
Blackleg /*Haarka*	-0.381	0.00
Mastitis /*Nakarsa*	0	0
Others	-0.340	0.00

The results of proportional piling on mortality additionally showed that 8.8% of cattle died from various diseases in the past one year (Fig. 9). FMD accounted for 1.1% of crude fatality rate.

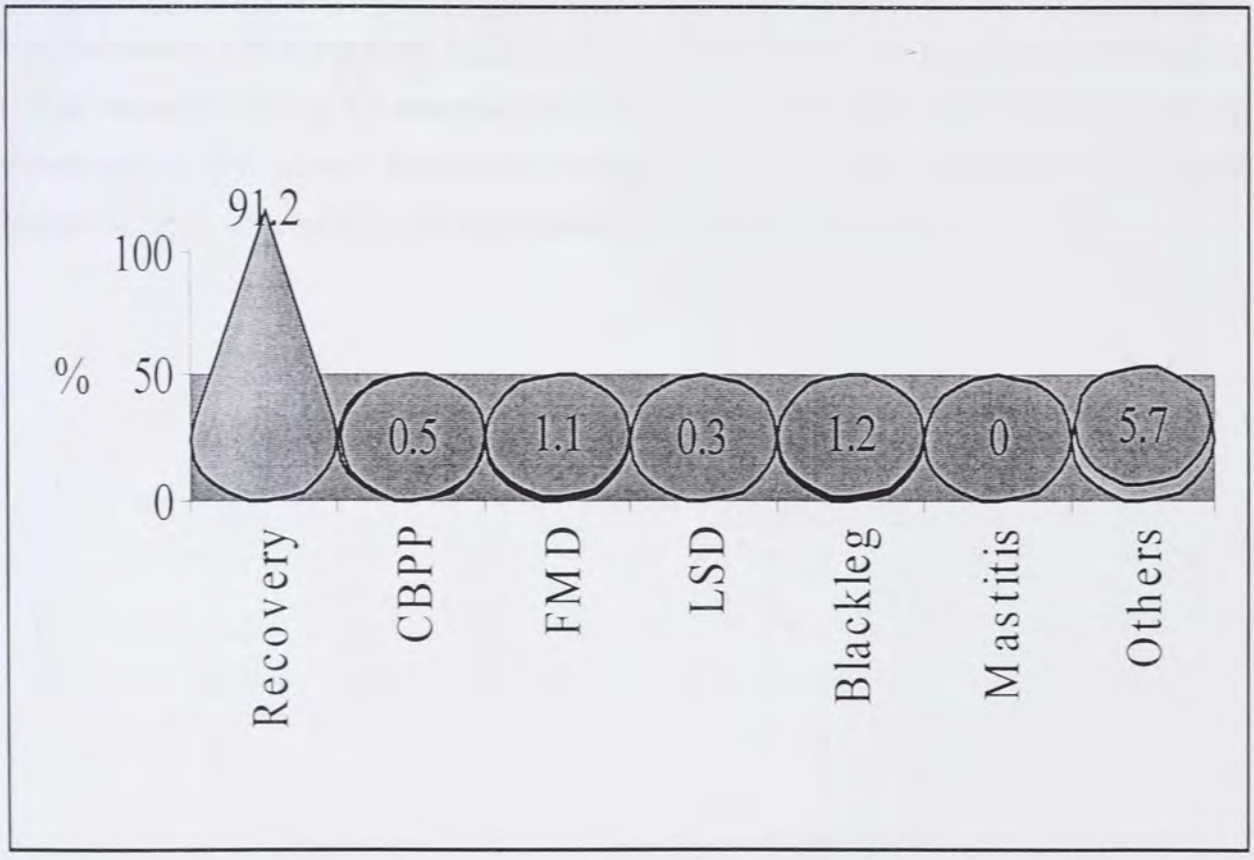


Figure 9 Mean case fatality rate relative to recovered cattle in Borana pastoral area (Dec. 2004-Nov. 2005)

4.1.4. Seasonal Calendars

The ten groups of Borana pastoralists divided a year in to four seasons: long rainy season (*Gana*), from March to May; Cold dry season (*Adoolessa*), from June to August; Short rainy season (*Hagayya*), form September to November; and Long dry season (*Bona*), from December to February (Annex 4).

Summarized seasonal calendar for livestock diseases, rainfall, tick infestation, cattle movement, and contact with wildlife was shown on (Table 7). Moderate to good agreement was seen among ten informant groups regarding seasonal occurrence of the diseases; such as CBPP (*Sombesa),* FMD *(Hooyale),* LSD *(Suuki),* Blackleg *(Haarka)* and Mastitis (*Nakarsa)*, and seasonal pattern of rainfall distribution (W= 0.397- 0.995). The incidence of FMD *(*Hooyale*)* was found to be high during dry season (Bona) than cold dry season (*Hagayya*). The lowest incidence was reported during rainy season.

The informants also have good agreement (W = 0.839) on seasonal animal movement, which occurs frequently during dry season in search of good pasture and water. This pattern of seasonal movement (in dry season) determines contact between domestic animals. However, regarding contact of cattle with wildlife, the agreement was moderate (W = 0.287).

Table 7 Summarized seasonal calendar on the occurrence of different diseases of cattle in Borana pastoral area (Dec. 2004-Nov. 2005)

	Borana seasons											
	Gana (Long rain)			Adoolessa (Short rain)			Hagayya (Cold dry)			Bonna (Long dry)		
	Months by Gregorian calendar											
	M	A	M	J	J	A	S	O	N	D	J	F
Rainfall (W=0.995)***	12(10-13)			2(0-3)			6(4-8)			0(0-2)		
CBPP (Sombesa) (W=0.700)***	2(0-5)			5.5(2-8)			1.5(0-4)			10(3-13)		
FMD (Hooyale) (W=0.938)***	1(0-3)			5(4-7)			1(0-3)			12.5(10-16)		
LSD (Suuki) (W=0.870)***	3(1-5)			12.5(9-18)			4(0-6)			0(0-2)		
Blackleg (Haarka) (W=0.919)***	13.5(9-18)			2(0-3)			4(0-8)			0(0-1)		
Mastitis (Nakarsa) (W=0.787)***	4.5(2-7)			3(2-4)			11.5(7-16)			0.5(0-6)		
Tick infestation (W=0.397)**	9.5(0-13)			3(0-7)			5(3-9)			2(0-10)		
Increased cattle movement (W=0.839)***	1.5(0-5)			5(3-6)			2(0-6)			11.5(9-14)		
Wildlife Contact (W=0.287)*	8.5(2-12)			4.5(0-10)			4.5(2-8)			2.5(0-10)		

N=10; W, Kendall's coefficient of concordance (*P <0.05; **P <0.01; ***P< 0.001). The number out side the bracket represents medians and minimum and maximum values are in the bracket. The agreement was termed weak, moderate and good if W-values were less than 0.26, between0.26 and 0.38 ($p < 0.05$) and greater than 0.38($p< 0.01$ to 0.001), respectively (Seigel and Castellan, 1994).

Seasonal rainfall pattern described by informants was validated by making comparison between seasonal calendars as described by the pastoralists and objective measures of rainfall (Table 8)

Table 8 Comparison of seasonal rainfall patterns as determined by pastoralists' seasonal calendar and objective rainfall data in Borana pastoral area (Dec. 2004-Nov. 2005).

	Data from seasonal calendar (N= 10 informants group)				Data from SORDU, Dec.2004-Nov.2005	
	Rainfall score			Median score as Proportion of total annual score	Mean rainfall (mm)	Rainfall as proportion of total annual rainfall
Seasons	Min	Median	Max			
Gana	10	12	13	60	199.9	62
Adoolessa	0	2	3	10	25.4	8
Hagayya	4	6	8	30	82.7	25.7
Bona	0	0	2	0	13.9	4.3

The pastoralists knowledge in the diagnosis of FMD and the result of 3ABC ELISA (Table 9) were in moderate agreement (k = 0.45) indicating a reasonably perfect (93% positive predictive value) recognition of FMD by pastoralists.

Table 9 Comparisons of knowledge of FMD diagnosis by pastoralists and their corresponding 3ABC ELISA result at herd level in Borana pastoral area

		Pastoralist Diagnosis of FMD			Positive predictive value (%) 95% CI of pastoralists diagnosis
		Positive	Negative	Total	
3ABC ELISA	Positive	27	2	29	93.1 (84.3-102.8)
	Negative	14	7	21	67 (49.3-90.2)
	Total	41	9	50	82 (72.0 - 93.4)

4.2. Laboratory analysis

4.2.1. Seroprevalence of FMDV using 3ABC ELISA

From 116 herds examined for the presence of antibodies to the 3ABC non-structural protein of FMD virus, 68 (59%) contained, at lest, one positive animal (Table10). The highest prevalence recorded in Yabello district (61%) was significantly different ($P<0.05$) from other districts (Table 10). On the other hand, from 920 animals examined, 193 (21%) were positive. The respective seroprevalence in the three districts (Fig. 10) shows statistically significant different.

Table 10 Seroprevalence of FMD in cattle herds in three districts of Borana Pastoral area

District	No of herd	No of Positive animal	No of positive herd	Herd level seroprevalence (%)	95% CI
Yabello	56	89	34	61	48.1 - 73.9
Dire	39	76	23	59	37.7 - 80.6
Moyale	21	28	11	52	39.1 - 64.9
Total	**116**	**193**	**68**	**59**	**50 - 68**

($\chi^2 = 9.09$; $P < 0.01$)

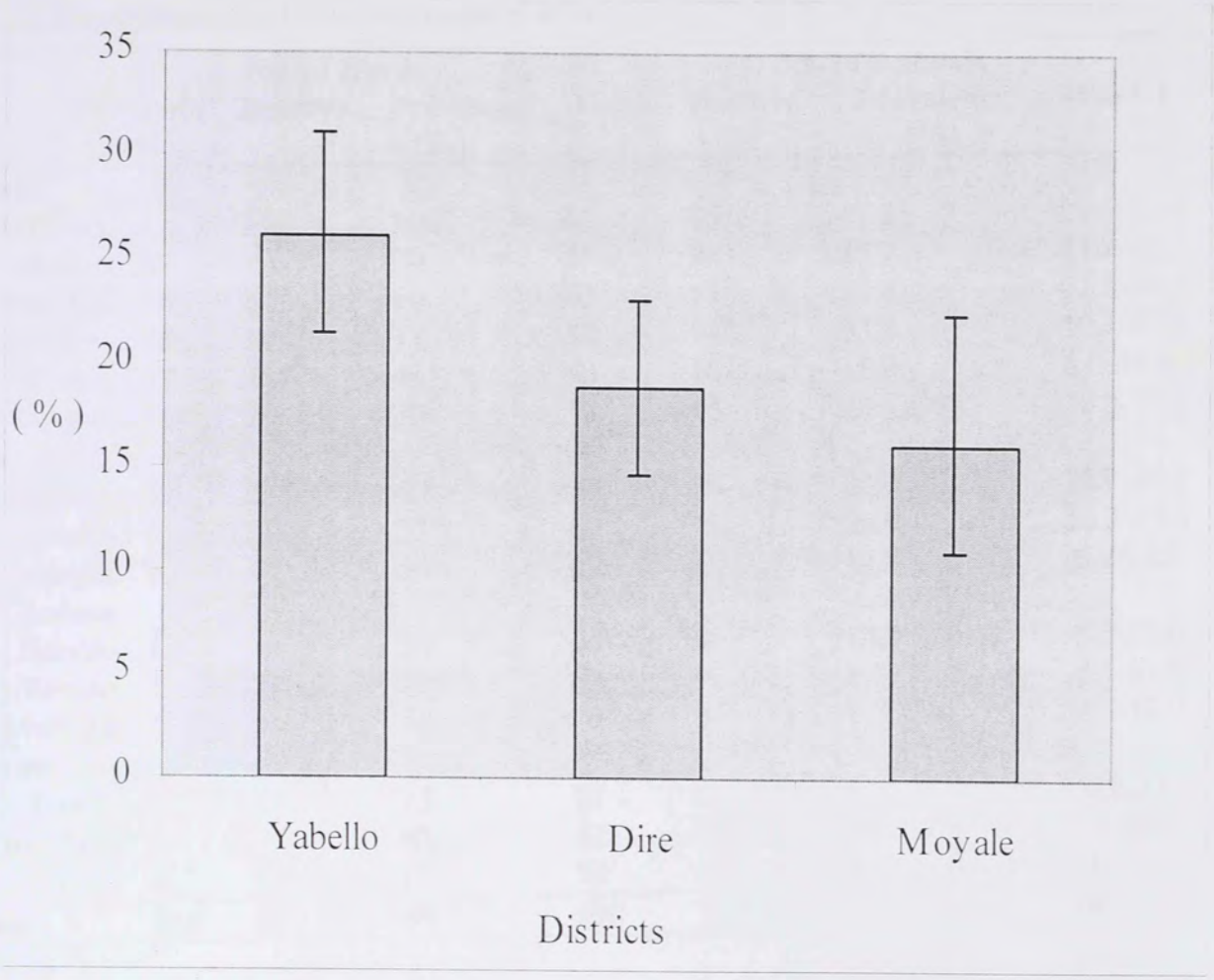


Figure 10. Seroprevalence rate of FMD at individual level in three districts of Borana pastoral area

The results at Pastoral Associations (PAs) level are given in Table 11. The highest herd seroprevalence was observed in Dida Tiyara (100%), Romso (100%), Dida Yabello (80%), and Garbi Minch (77%). On individual level, seropositivity was highest at Dida Tiyara (43.35%) Garbi Minch (33.3%), Magado (32.4%), and Medhecho (26.9%) PAs. This difference among the PAs was statistically significant ($\chi^2 = 47.2$; $P<0.001$).

Table 11. A summary of seroprevalence, at herd and animal level in cattle at different PAs of three districts of Borana pastoral area

PAs	No. of Herds			No. of Animals			
	Total	Positive	Prevalence (%)	Total	Positive	Prevalence (%)	95% CI
Yabello							
DidaTiyara	1	1	100	63	26	41.3	29.1-53.4
Dharito	5	1	20	35	9	25.7	11.2-40.2
Did/Yabello	10	8	80	63	15	23.8	13.3-34.3
Garbi/ Min.	13	10	77	42	14	33.3	19.1-47.6
Tedim	17	7	41	63	10	15.9	6.8-24.9
Areri	10	7	70	75	15	20	10.9-29.1
Dire							
Magado	5	3	60	74	24	32.4	21.8-43.1
Medhecho	6	2	33	67	18	26.9	16.3-37.5
Dembella	8	4	50	69	10	14.5	6.2-22.8
Bedana Harallo	11	4	36	64	9	14.1	5.5-22.6
Romso	5	5	100	69	9	8.7	5.1-21.0
Melbana	4	2	50	62	6	9.7	2.3-17.0
Moyale							
Dambi	7	1	15	53	3	5.7	0.6-11.9
Tile Mado	8	5	63	62	12	19.4	9.5-29.2
Tuka	6	4	67	59	13	22	11.5-32.6
Total	**116**	**68**	**59**	**920**	**193**	**21**	**18.4-23.6**

$\chi^2 = 47.2$; $P < 0.001$

Similarly, intrinsic host risk factors (age and sex) were seen to be significantly associated with FMD infection (Fig. 11, Table 12); herd size (Table 12) was also seen to be increasingly associated with FMD infection.

Table 12. Seroprevalence of FMD in cattle of different sex and herd size in Borana pastoral area

	No samples	Sero positive	Seroprevalence (%)	95% CI
Sex				
Male	281	44	15.7	11.4-20.0
Female	639	149	23.3	20.0-26.6
Herd size				
0-50	350	56	16	12.2-19.8
51-100	282	69	24.5	19.5-29.5
>100	288	68	23.6	18.7-28.5

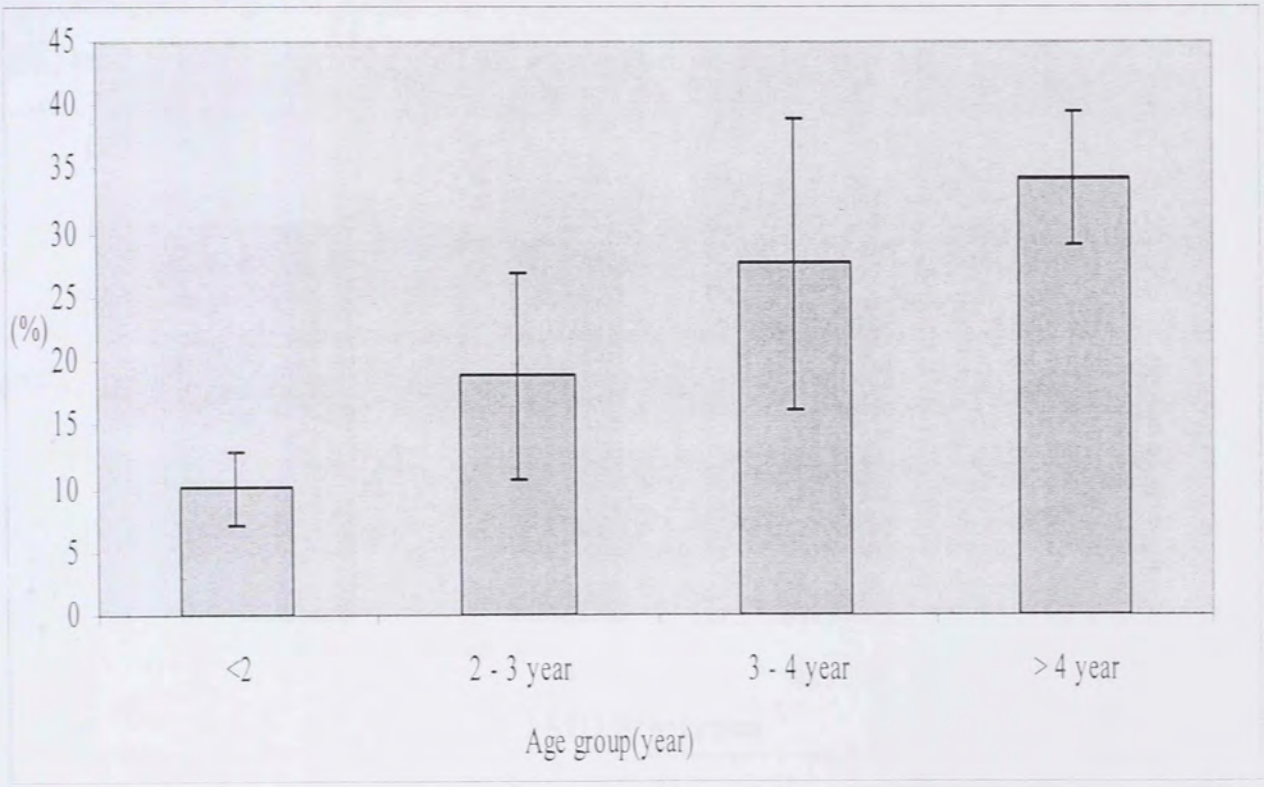


Figure 11. Seroprevalence of FMD in different age groups of cattle in Borana pastoral area

Table 13 Summary of the logistic regression test of the different risk factors of FMD in Borana pastoral area

Risk factors	In OR	DF	OR	95% CI (OR)	P-value
District	0.33	1	1.25	0.67-2.31	0.484
PA's	0.02	1	1.02	0.92-1.13	0.719
Herd size	-0.195	1	0.82	0.67-1.01	0.066
Age category	-0.52	1	0.59	0.52-0.68	0.000
Sex	-0.168	1	0.85	0.55-1.29	0.441

4.2.3. Serotyping based on liquid phase blocking ELISA

Out of 120 samples randomly selected from 193 positive sera for serotyping using liquid phase blocking ELISA, 99.2%, 95.8%, 80.0%, and 67.5% were positive, respectively for O, A, SAT2 and C serotypes (Fig. 12). There was a high degree of combinations of infections, and only one (0.8%) sample tested negative for all the serotypes examined (Table 14).

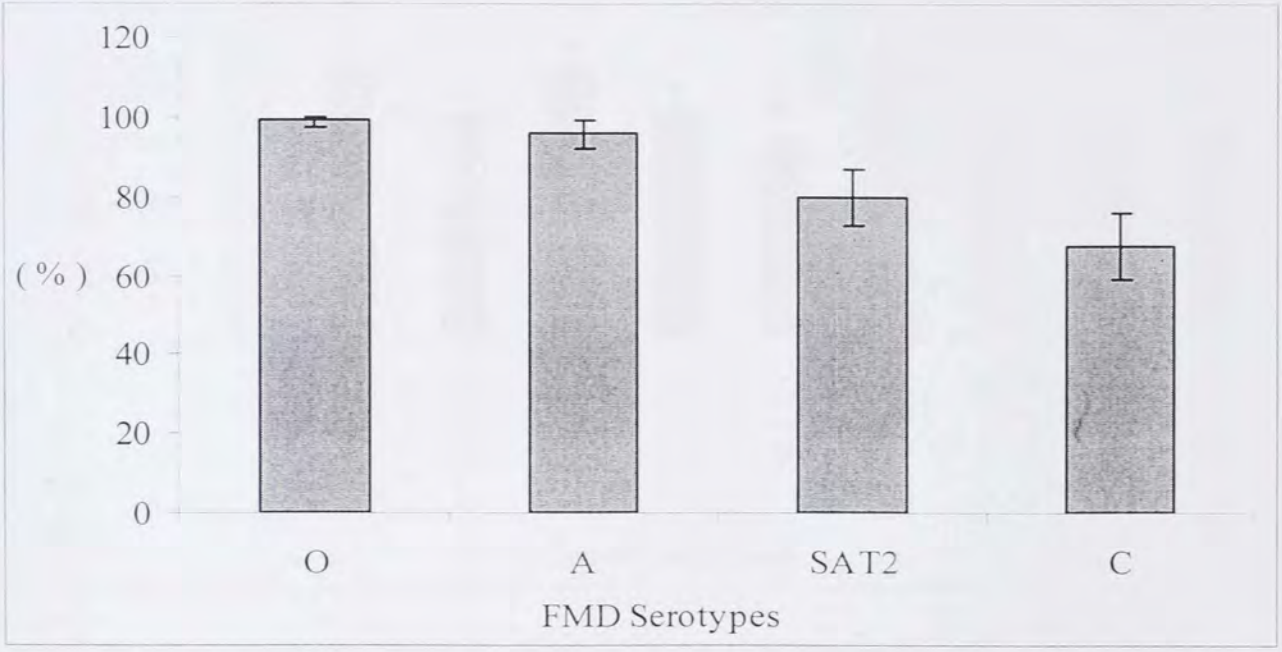


Figure 12 Proportion of different FMD serotypes identified in Borana pastoral area

Table 14. Exclusively combined serotypes of FMD virus antibody circulating in Borana pastoral area

Proportion	O	A	C	SAT2
71 (60%)				
24 (20%)				
10 (8.3%)				
9 (7.5%)				
4 (3.3%)				
1 (0.8%)				

Note: Shaded areas show the presence of each serotype in a combined infection

From all possible combinations of occurrence, the highest (95.8%) was recorded between O and A serotypes, followed by O and SAT2 (80%), A and SAT2, and O, A and SAT2 combinations (Fig 13).

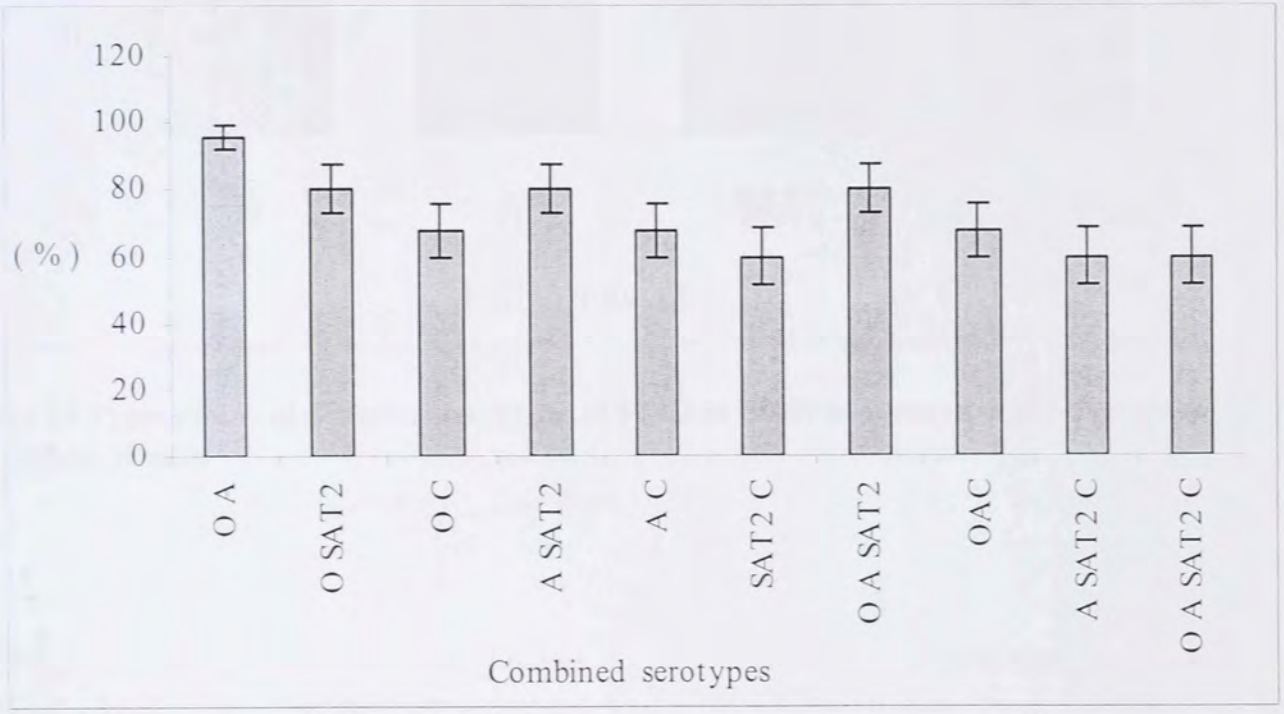


Figure 13 **Proportions of different combinations of FMD serotypes observed in cattle of Borana pastoral area**

Comparison of relative distribution of the different serotypes in the three districts of Borana area showed no statistically significant (P>0.05) difference (Figs. 14 and 15).

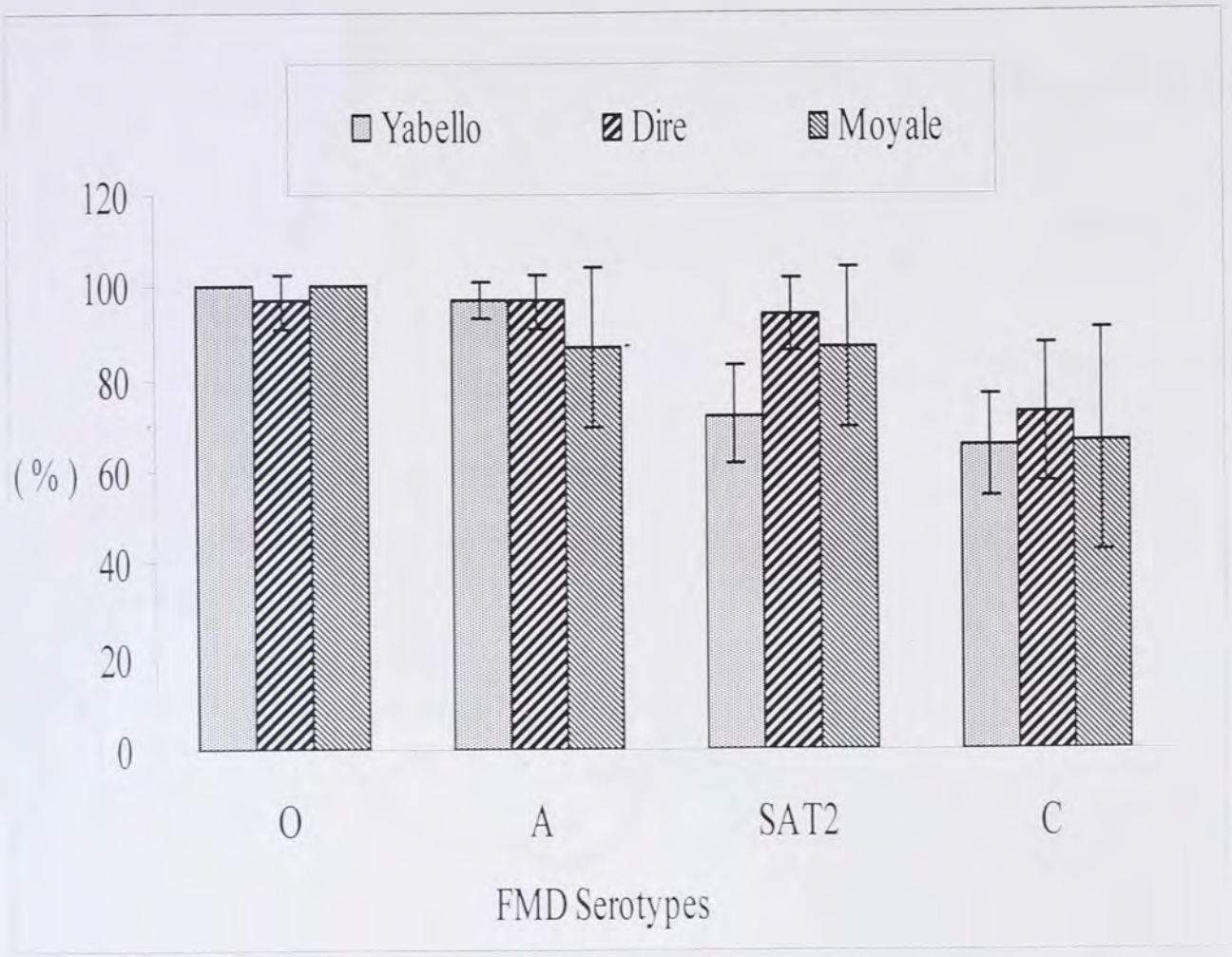


Figure 14 Proportions of different serotypes of FMD in cattle of three districts of Borana pastoral area.

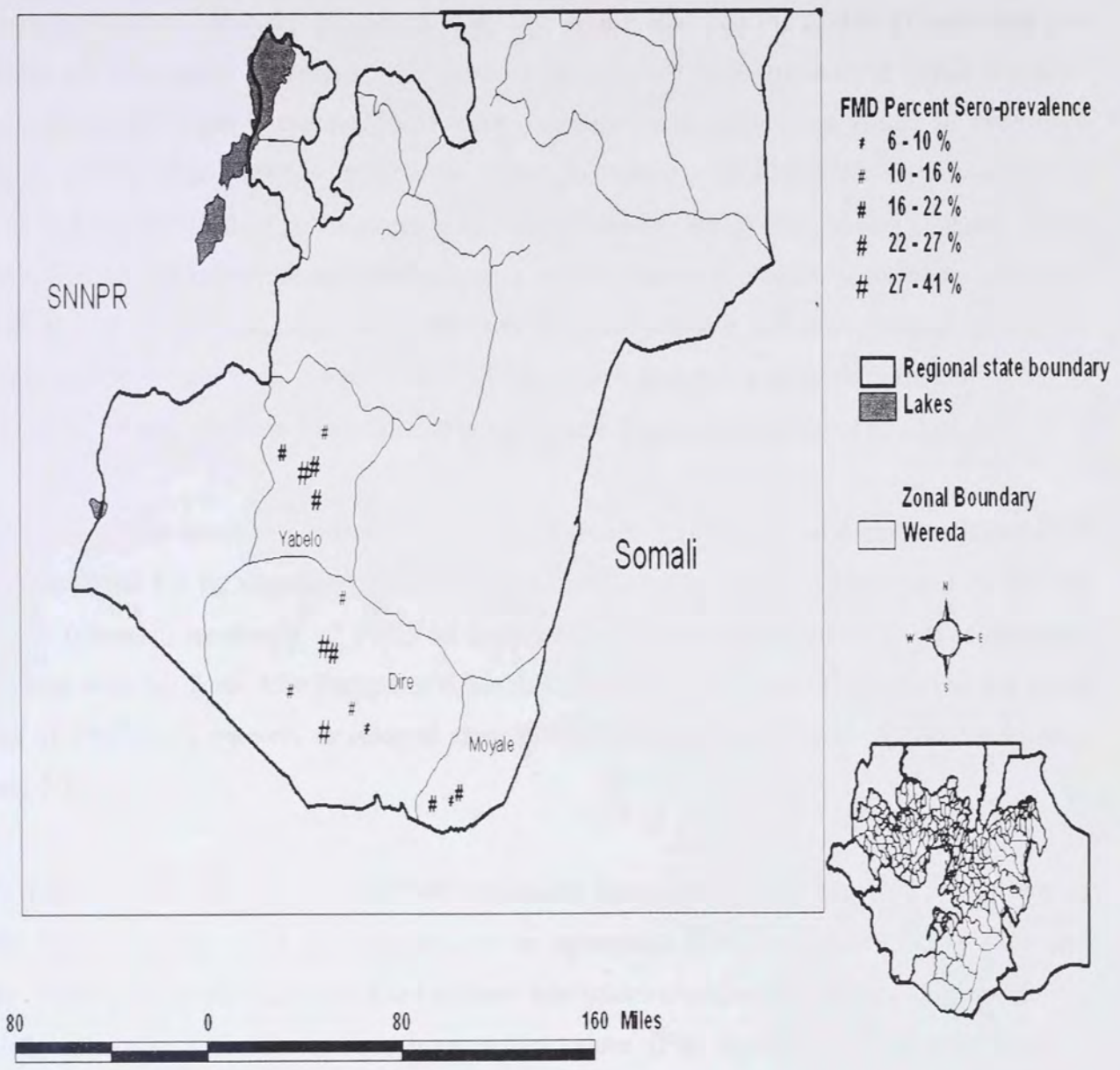


Figure 15 A map showing seroprevalence distribution of FMD in Pastoral association of three districts, Borana pastoral area

5. DISCUSSION

The present study has indicated that FMD is a second priority disease, next only to CBPP, in Borana pastoral area (Table 3). The result of disease sign matrix scoring (Table 4), indicated that the group of informants described well most of the clinical presentations of cattle diseases; indeed most of the signs listed for FMD were consistent with what is indicated in veterinary literatures (Catley *et al.*, 2001; Catley *et a*l., 2004; Rodostits *et al.*, 1994). In Ethiopia, similar signs have been reported in pastoral cattle of Afar (Tadesse, 2003) and Somali (Eshetu, 2003) Regional States. The moderate agreement (kappa = 0.45) between Borana pastoralists' way and serological test in the diagnosis of FMD (and a good positive predictive value of 93.1%) conforms well to the previous study (Catley, 2004), which reported a positive predictive value of up to 96% for Massai herders; indicating a strong disease diagnosing ability of pastoralists.

As indicated by the community, the incidence rates of acute (*Hooyale*) and chronic (*Gaandile*) FMD (16.1% and 1.1 %, respectively) was in agreement with the previous report of 17% (acute) and 1.1% (chronic) incidence of FMD in Sukuma herd, Tanzania (Catley 2004). Consistent finding was obtained from Afar Region of Ethiopia, in which 20% of animal affected by the acute episode of FMD progressively developed chronic FMD (*Halab*) with signs of heat intolerance (Eshetu, 2003).

The fact that the incidence of acute FMD (*Hooyale*) decreases as age increases while that of chronic FMD increases with age (Fig 6b,f) is in agreement with previous report (Catley and Irungu, 2000). Similar finding was reported from Afar pastoral region of Ethiopia (Eshetu, 2003). Coupled with the high mortality rate recorded in calves (Fig. 8b) experiential knowledge of informants conforms well to the conventional veterinary literatures (Rodostits *et al.*, 1994) that reported high mortality rate in calves.

Seasonal incidence of FMD (*Hooyale*) was found to be high during long dry season, (December to February) compared to cold dry season (June to July), the lowest incidence being during rainy season (Table 7). This seasonal pattern is associated with Borana herd management system that drive *forra* herd (66% of herd population) in search of good pasture and water. This system has

facilitated the contact among different herds from different locations, including contact between infected and non-infected ones. Chronic FMD (*Gaandile*), occurring following outbreaks of FMD during dry season, is consistent with high prevalence of chronic FMD reported from Tana River District, Kenya (Catley *et al.*, 2002b). The seasonal calendar, as evaluated by rainfall pattern (Table 8) was good indicator of the conventional calendar.

The overall seroprevalence rate of 21% (59% at herd) reported in this study was in agreement with the previous finding from Ethiopia (Sahle, 2004) in which seropositivity of 26.5% was reported. The highest district level seroprevalence (26.1%) recorded in Yabello (Fig. 10) as compared to Dire (18.8%) and Moyale (16.1%), probably reflects a better rainfall that favor availability of water and pasture, making this district a dry period refuge for the pastoralists. Moreover, Yabello is a center for cattle market that facilitates contact among cattle from different locations. Among the sampled PAs, the highest seroprevalence was recorded in PAs located in Yabello and Dire (Table 11): Dida Tiyara is a PAs where permanent (artificial) surface water is found; Magado PA boarders Kenya and it is a place where communal crater mineral lick is located. Moreover, this latter PA has a vast pastureland a long Ethiopia-Kenyan border, where animals from both countries frequently mix.

Age specific seroprevalence study revealed an increasing prevalence as the age increases (Fig. 11). This may indicate the cumulative experience of the population with the agent (Murphy *et al.*, 1999). Therefore, those animals aged greater than four years, might have acquired the infection from multiple serotypes, and could produce antibodies against all serotypes of FMD. The relatively low seroprevalence in age group less than two years may be indicative of prevailing passive maternal immunity and low frequency of exposure as the Borana pastoralists keep this age group in *warra* herd, around permanent encampments. The correlation of disease with herd size (Table 12) can be explained by the contagious nature of the disease and mode of transmission, which is enhanced by crowding and frequency of contact. Although sex appeared to have a significant effect on seropositivity in univariate analysis (Table 12), it was found not to have a significant effect ($p>0.05$) in the multivariate analysis (Table. 13).

FMD Serotypes circulating in Borana pastoral areas were O, A, SAT2 and C. This is in agreement with previous findings from Omo National Park and Bale mountain area (Sahle, 2004). Similarly, Dejene (2004) reported serotypes O, A, C, and SAT2 from samples collected during outbreaks in dairy farms in and around Addis Ababa. Three serotypes (O, A, and SAT2) were also isolated from cattle at National Veterinary Institute, Ethiopia, during outbreak investigation from 1982-2000 (Galaye, *et al.*, 2001). The finding of serotype C in this study is however inconsistent with current knowledge that considers serotype C as eliminated from the world. Andersen (1985) however recorded the presence of five serotypes of FMD (O, A, C, SAT1 ands SAT2) in buffalo in support of the present study, and the last outbreak of serotype C occurred in Kenya in 2000 (Kitching, 2002a).

6. CONCLUSION AND RECOMENDATIONS

The observed agreement between informants groups and veterinary literatures regarding most of exhibited signs of important cattle diseases has proven that Borana pastoralists have an enormous wealth of knowledge on diagnosis of cattle diseases. Therefore, Participatory Appraisal methods are useful to complement conventional methods, in the control of complex livestock diseases such as FMD.

The analysis of seasonal calendar demonstrated that FMD is more prevalent during dry seasons: December-February (Bona) and June-July (Hagayya), consistent with seasonal cattle movement, which intensifies during long dry period (December -February). Although cattle movement control is impractical (at least at present) in Borana pastoral system, the level of seropositiviy and loss due to morbidity and mortality, as investigated through participatory appraisal, justify a launching of a control program. This finding suggests that animals should be protected through vaccination before they are subjected to stress of movement and mixing. This study further elucidated that various FMD serotypes are circulating in Borana pastoral area, signifying to periodically assess the efficacy of the vaccinal strains in the field.

Therefore, based on these findings, the following is recommended:

The participatory epidemiological and conventional veterinary methods are complementary; therefore, they should be used side by side during animal health research, especially in pastoral areas, to make use of the rich experience of the pastoral community.

An extensive regular serological survey, virus isolation, and characterizations of FMDV need to be conducted for a possible development of polyvalent vaccine. Based on Borana seasonal calendar, November (end of short rainy season) and May (end of long rainy season), two times per, year can be suggested as the most suitable time for vaccination against FMD in Borana Pastoral system.

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8. ANNEXES

Annex 1. The map of Ethiopia showing Oromia Regional State and Borana pastoral area

Borana pastoral area

Oromia Reginalstate

Reginal state boundary

Figure .2 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	etc.									
E	3	9										
F	4	10										
G	5	11										
H	6	12										

N = negative control sample
P = positive control sample
1, 2, 3.etc = test sample

Annex 3. Plate layout used for liquid phase blocking ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	C^{++}	C^{++}	1	1	9	9	17	17	25	25	33	33
B	C^{++}	C^{++}	2	2								
C	C^{+}	C^{+}	3	3								
D	C^{+}	C^{+}	4	4								
E	C^{-}	C^{-}	5	5								
F	C-	C-	6	6								
G	Ca	Ca	7	7							39	39
H	Ca	Ca	8	8							40	40

C^{++} *strong positive serum control* C^{-} *Negative serum control*

C^{+} *moderate positive serum control* C_a *Antigen control*

1, 2, 3. . Serum samples

Annex. 4. Borana Seasonal calendar, Local and scientific name of season and months of a year

.Season	Status of rain	Month of a year	
		Local name	Scientific name
Gana	Long rain	Gurandhala Bittotesa Camsa	March April May
Adoolessa	Cold dry	Buffa Watabaji Obra Tika	June July August
Hageya	Short rain	Obra Guda Birra Chika	September October November
Bonna	Dry season	Sdassa Abrassa Amaji	December January February

Annex 5 Geographical positioning system data collected in different PAs of Borana pastoral area.

PAs	North (latitude)	East (longitude)	Altitude (M)
DidaTiyara	04^0 56' 52.6"	038^0 13' 22.5"	1530
Dharito	04^0 47' 19.6"	038^0 11' 00.3"	1631
Dida yabello	04^0 54' 52.3"	038^0 10' 24.3"	1596
Garbi Minch	04^0 53' 16.9"	038^0 14' 01.3"	1693
Tedim	04^0 02' 52.4"	038^0 13' 22.5"	1653
Areri	04^0 58' 17.1"	037^0 56' 49.4"	1511
Magado	03^0 53' 41.1"	038^0 13' 22.8"	973
Medhecho	03^0 12' 03.6"	038^0 16' 52.1"	1548
Dembella Bedana	03^0 24' 40.3"	038^0 19' 59.5"	1512
Harallo	03^0 59' 13,7"	038^0 23' 14.0"	1467
Romso	03^0 03' 19.8"	038^0 15' 32.5"	1629
Melbana	03^0 54' 19.9"	038^0 28' 28.2"	1357
Dambi	03^0 37' 48.4"	038^0 58' 09.9"	1209
Tile Mado	03^0 36' 49.8"	039^0 01' 10.1"	1140
Tuka	03^0 37' 23.8"	038^0 51' 50.0"	1232

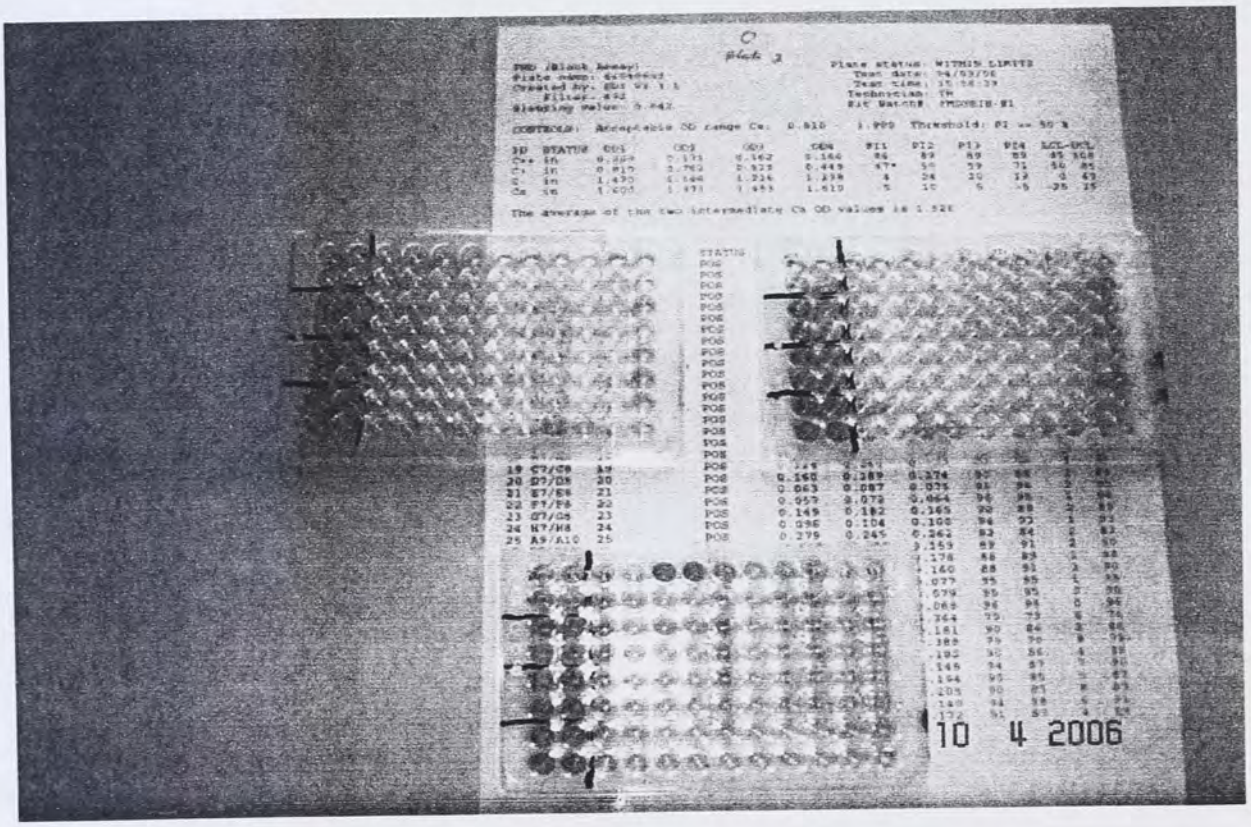
Annex 6 Format used for collection of sample and information on risk factor

Date of collection.....

Region .....Zone.....

District	PAs	Owner Name	No animals	Sample Codes	Age	Sex	Georeferenced data		
							North	East	Altitude
				1					
				2					
				3					
				4					
				5					
				6					
				7					
				.					
				.					
				.					

Annex 7 Microplates lay out indicate laboratory result of liquid phase blocking ELISA



9. CURRICULUM VITAE

PERSONAL DATA

Name:	Tesfaye Rufael Chibssa
Date of Birth:	08 July1968
Place of Birth:	West Shewa, Gendabart
Marital Status:	Married with one child
Languages:	Afan Oromo, Amharic and English
Address:	Asella Regional Veterinary Laboratory
	P.O.Box 212,
	Tel: 0911764972 (Mobile)
	Email rufaelc@yahoo.com

EDUCATIONAL BACK GROUND AND QULIFCATION

- Doctorate Degree in Veterinary Medicine from Addis University Faculty of Veterinary Medicine, Debre Zeit, 1993
- Achieved Senior secondary school Certificate at Ayer Tena High school, Addis Ababa, 1983-1987
- Achieved Junior secondary school Certificate at Kachise Senior secondary School, Gendabart, 1981-1982
- Achieved primary secondary school Certificate at Getare Primary School, Gendabart, 1974-1980

SPECIAL COURSES AND TRAINING

- Certificate, Participatory Disease Search (PDS): A Training organized by CAPE and PACE Ethiopia, 21-26, 2004, Dollo Ado, Ethiopia.
- Certificate, Basic Computer course, PC: Training Sponsored by AsellaRegionalVeterinary Laboratory, Aug. 2002- Jan., 2003, Asella, Ethiopia.

- Certificate, Serological and Bacteriological techniques: Training Sponsored Quality and Sanitary of animal product in Ethiopia and National Animal Health Research Center, Jun. 23- Jul. 4, 2003, Sebeta.
- Certificate, Planning, Monitoring, and Evaluation: Training organized by Ethiopia Agricultural research organization, 24-29 Sep. 2001, Melkassa, Ethiopia.
- Certificate, Epidemiological, and economic investigation as a planning basis for animal health programs: International Training Course organized by GTZ, Jul.02-Aug. 03, 1999, Barnried, and Feldafing. Germany.
- Certificate, Principles and Criteria of Monitoring and Evaluation: Training organized by Borana lowland pastoral Development program /GTZ/, NOV.24-25, 1997, Negelle, Borana
- Certificate, Project Cycle Management (PCM) and Objectives oriented Project Planning: Training organized by Borana pastoral and Development program (BPLDP)/GTZ, May 12-16,1997, Negelle, Borana
- Certificate, Integrated Tsetse and Trypanosomosis control: Training sponsored by Oromia agriculture Bureau, Ethiopian science and Technology commission, and ICIPE, Aug. 21-1996, southern Ethiopia.

WORKSHOPS AND SEMINARS

- National workshop on quality Assurance, Sep, 14-19,2005, at National Veterinary Research center /NAHRC, Sabeta, Ethiopia
- Workshop Pan African Program for the control of epizootics (PACE), Annual report, march 04-05,2004 , Addis Ababa
- Introduction to Participatory Epidemiology, 10-11, Dec.2004, Faculty of Veterinary, Debre Zeit, Ethiopia
- How to use Geographical positions System(GPS), Jan, 5-8, 2003, Debre Zeit, Ethiopia
- Regional strategic planning workshop on animal health problems of Oromia regional state, 23-26 Jan. 2001, Hirna, Ethiopia.
- Cross Border experience, sharing in the field of Community based primary health care (CBPAHC), April. 28-29, 1998, Moyale, Borana.

- Pastoral Oriented Development and extension concept (PODEC) 25-30, May, 1998, Negelle, Borana by GTZ
- Common concept for a community Based Animal Health Care System (CBAHCS), June, 18,1998, Yabello, Borana
- Development of a Curricular for the training of Vet- scouts/ Contact Herders, 30, July 1998,Yabello, Borana

WORK EXPERIENCE

2000 -to date

- Head of Microbiology Department at Asella Regional Veterinary Laboratory
- Sero-surveillance team leader,
- Guest lecturer of Terioginology and animal health at Asella Agricultural Technical College

1995-1999

- Head of animal health and production section Southern Rangeland Development Project (SOURDU), Yabello, and Borana
- Conduct training for capacity building especially CAHW's with NGO's

1994-1995

- Team leader of district veterinary section, North Omo Zone, SRNN

PROFESSIONL AFFILIATIONS

- Member, Ethiopian Veterinary Association (EVA)

RESEARCH WORK

- Review on Peste des Petits ruminants and its current status in Ethiopia, 2005,FVM, Debre Zeit
- Isolation and identification of PPR virus in Arsi Zone of Oromia Region. Preceding of the 17th Annual conference of the EVA, Addis Ababa Ethiopia
- Survey of Chalk brood disease on the golden insects in Arsi of Oromia Regional state. Annual report of Asella Regional Veterinary Laboratory, June 2002, Addis Ababa Ethiopia.
- Adverse Reaction of T_1 44 strain, CBPP Vaccine in Southern Oromia Rangeland Development units (SORDU) Borana, Ethiopia. Veterinary Association Proceeding of 14th Conference, June 2001 Addis Ababa, Ethiopia.
- The importance of mechanically transmitted trypanosomosis in Asella Regional Veterinary laboratory operational areas. Proceeding of the Workshop on animal health problems in Oromia Region, January 2001, Hirna, Ethiopia.
- Report on new Camel, disease Called "Furro" in Southern Rangeland Development project (SORDU), Borena, Ethiopia. Veterinary association proceeding of 10th Conference, June 1996 Addis Ababa, Ethiopia.
- Research on the Epidemiology and Biology of Oestrus ovis in sheep and goats in western Shewa, Ambo. DVM thesis, AAU, FVM, Debere Zeit, Ethiopia. June 1993.
- Seminar on Embryo transfer and its development potential in Ethiopia 1991, FVM, Debere Zeit,

REFERENCES

Dr. Mesifen Sahle (DVM, MSC, PhD)

Center Manager of National Animal Health Research center, Sebeta,

Dr. Berhe G/Egziabher (DVM, MSc)

Director of National Veterinary Institute, Debre Zeit, Ethiopia.

Dr. Asseged Bogale (DVM, MSc)

Assistant Professor, Faculty of Veterinary Medicine, AAU, Debere Zeit Ethiopia

10. SIGNED DECLARATION SHEET

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

Name Tesfaye Rufael Chibssa.

Signature _____

Date of submission _____

This thesis has been submitted for examination with our approval as University advisors.

Dr. Asseged Bogale ----------

Dr. Mesfin Sahle ----------