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EPIDEMIOLOGY AND ECONOMIC IMPACT OF FOOT AND MOUTH DISEASE OF CATTLE IN SELECTED DISTRICTS OF ARSI AND BALE ZONE, OROMIA REGIONAL STATE, ETHIOPIA

MVSc THESIS

BY

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MVSc THESIS

A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Epidemiology

By

Mohammed Aliye Tunfuri

June, 2022

Bishoftu, Ethiopia

Approval Sheet

Addis Ababa University

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STATEMENT OF AUTHOR

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion Test
AHI	Animal Health Institute
BHK	Baby Hamster Kidney
CFT	Complement Fixation Tests
CPE	Cytophatic Effect
CSA	Central Statics Agency
DACA	Drug Administration and Control Authority of Ethiopia
DIVA	Discrimination Infected Vaccination Animal
ELISA	Enzyme-Linked Immunosorbent Assay
ETB	Ethiopian Birr
FAO	Food and Agricultural Organization
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GDP	Gross Domestic Product
ICTV	International Committee on Taxonomy of Viruses
LPBE	Liquid-phase blocking ELISA
MAbs	Monoclonal Antibodies
MOARD	Ministry of Agriculture and Rural Development
MoLF	Minster of Livestock and Fishery
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
NSP	Non Structural Protein
NVI	National Veterinary Institute
OD	Optical Density
OIE	Office International des Epizooties
OP	Oesophageal Pharyngeal
OR	Odd Ratio
ORF	Open Reading Frame
PAs	Peasant Association

PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Information System
RNA	Ribo Nucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAT	South Africa Territories
SP	Structural Protein
SPCE	Solid-phase competition ELISA
SsRNA	Single stranded Ribonucleic Acid
TAD	Transboundary animal diseases
USD	United State Dollar
UTR	Untranslated Region
VN	Virus Neutralization
VNT	Virus Neutralization Test
VPg	Viral Genomic Protein
$\chi 2$	Chi –square

ABSTRACT

Foot and mouth disease (FMD) is a highly contagious viral disease that primarily affects cloven-hoofed animals and causes significant economic losses in Ethiopia. To develop effective control and preventive measures, it is necessary to know the status of FMD through continuous surveillance, outbreak investigation, risk factor assessment and analysis of economic impact. Therefore, a cross-sectional study was conducted from November, 2021 to June, 2022 with the aim of estimate the seroprevalence, identify associated risk factors, identifying circulating serotypes, and analyzing the economic impact of the diseases on cattle in selected districts of the Arsi and Bale zones, Oromia, Ethiopia. A multistage cluster sampling technique for the sero-prevalence study was used, and a total of 779 sera samples and 11 epithelial tissue samples were also collected for serotyping. To estimate the economic loss associated with FMD and assess various factors that affect the occurrence of foot-and-mouth disease, a questionnaire survey was conducted. To detect antibodies against non-structural proteins of foot and mouth disease virus (FMDV), the 3ABC ELISA was employed. In this study, the overall seroprevalence of FMD in cattle was 48.65 % (379/779) (95% CI: 45%- 52%). Multivariable logistic regression analysis showed that districts, breed, age, herd size, and animal composition were significantly associated with FMD seroprevalence ($p< 0.05$). The odds of FMD seropositivity were higher in cattle kept with small ruminants (OR = 1.737, 95% CI: 1.195-2.538, p = 0.0039) than in cattle alone. It also revealed that the odds of seropositivity were 1.756 higher in adults compared with young cattle (OR = 1.756, 95% CI: 1.677-3.528, p=0.0000). Of the eleven (11) tissue samples subjected for serotyping by the antigen detection ELISA, nine (9) samples were found positive and three types of FMDV serotypes were identified. The identified serotypes were: serotype A (77.77%), SAT 1 (11.11%), and SAT 2 (11.11%). During the study period, the total economic loss was estimated to be 374025ETB (7333.82USD). It was found that the disease's impact was extremely severe, resulting in massive economic losses. Therefore, further investigation and characterization of the circulating virus serotype and economic consequences should be studied to design appropriate control options.

Keywords: *Arsi and Bale zone, Cattle, Economic loss, Ethiopia, Foot and mouth disease, Risk factors, Seroprevalence, Serotype*

1. INTRODUCTION

Ethiopia has the largest livestock population in Africa, with 70 million cattle, 42.9 million sheep, 52.5 million goats, 8.1 million camels, 2.15 million horses, 10.8 million donkeys and 57 million chickens (CSA, 2021). The livestock sector plays a crucial role in the livelihoods of the majority of the human population in the country. The agricultural sector constitutes around 45-48% of the gross domestic production (GDP) of the country, and the livestock sector accounts for an estimated 20% of the total GDP without considering other contributions like traction power, fertilizing, and means of transport. Even though the country is gifted with huge livestock population, production and productivity is by far underneath the expectation due to widespread of livestock diseases and other constraints (Tegegne *et al.,* 2020).

Livestock diseases are the major cause of economic losses to the peasant farmers and pastoralists in Ethiopia, amounting to hundreds of millions of birr annually. These diseases are currently widespread in all agro-ecological zones of the country, and annual mortality rates due to these diseases are estimated to be 8–10% of cattle herds, 15% and 12% of sheep and goat flocks, respectively. It is expected that animal diseases reduce the production and productivity of livestock by 50–60% per year (Suleyman *et al.,* 2018). Among the livestock diseases hampering productivity of the sector and restricting Ethiopia's ability to participate in international trade, foot-and-mouth disease (FMD) is perceived as the most economically important trans boundary viral disease of cattle, both at national and household levels (Mada, 2021).

Foot and mouth disease is considered a highly contagious viral transboundary disease affecting cloven hoofed animals, including cattle, pigs, sheep, goats, and more than 70 wildlife species (Calkins and Scasta, 2020). It is caused by the seven immunologically distinct serotypes (A, O, C, Asia 1, and South African Territories 1, 2, and 3) of Foot-and-mouth disease virus (FMDV). All serotypes are clinically indistinguishable but immunologically distinct. The seven serotypes also differ in distribution across the globe. Foot and mouth disease virus is a single-stranded, non-enveloped, positive-sense RNA virus that belongs to the *Aphthovirus* genus and family *Picornaviridae* (Tesfaye, 2021). Currently, in Ethiopia, four of the serotypes; namely O, A, SAT1 and SAT2, are circulated in all regions of the country (Tesfaye *et al.,* 2016).

The disease is one of the major endemic transboundary livestock diseases of socio-economic importance in Ethiopia and in other parts of the globe (Tesfaye *et al.*, 2016). It causes significant economic damage by impeding the export of livestock and livestock products both locally and internationally. It spreads quickly, infecting a large number of animals in a short period of time and causing massive economic loss. Quantifying the economic impact of FMD is an important issue in different regions of the world (Alemayehu *et al.*, 2014). The economic impact of FMD can be direct or indirect, visible or invisible, and it varies between endemic and non-endemic areas of the world (Tadesse *et al.*, 2020).

Foot and mouth disease affects animals' performance directly through reduction of milk yield, loss of drought power, a high number of deaths among young animals and fertility impairment due to increased abortion rate, while the indirect impacts include additional cost of treatment, vaccination, vaccine delivery, movement control, diagnostic tests, culled cattle, and denied access to both local and international markets (Admassu *et al.,* 2015). The annual economic impact of FMD in terms of visible production losses and vaccination costs in endemic regions of the world is estimated between US $6.5-21 billion (Knight-Jones and Rushton, 2013).

The disease is characterized by fever, loss of appetite, salivation, vesicular eruptions in the mouth, on the feet and teats, and sudden death of young stock. The virus can be transmitted either directly, e.g., via contact with an infected host/s, or indirectly, e.g., via contact with a contaminated environment with FMDV-infected secretions and excretions (Tadesse *et al.*, 2021). The disease is diagnosed based on clinical signs. However, the clinical signs can be confused with other diseases, and thus, a laboratory based diagnosis is necessary. For laboratory diagnosis, the samples of choice are epithelium tissue, serum, and esophageal-pharyngeal fluids collected with a Probang cup. Laboratory diagnostic techniques for FMD are achieved by a combination of serological tests, virus isolation, and nucleic acid recognition methods (Tesfaye, 2021).

Foot and mouth disease preventive measures include: control of national borders; prohibition of import of animals and livestock products from endemic countries in accordance with the OIE standards; emergency measures in the event of outbreaks through: stamping-out, followed by cleaning and disinfection to reduce the risk of re-infection; strict movement controls, extending

to movement on and off farms of livestock products; and also, possible emergency vaccination is important (Azeem *et al.*, 2020).

Currently, in Ethiopia, there is no government strategy for FMD control through vaccination and movement control. Lack of vaccination strategies (quality, coverage, and timing) and the presence of free animal movement without certification are thus the main factors that could increase the spread of FMD along the cattle market chain. Moreover, the presence of lack of veterinary infrastructure to handle outbreaks on large scale greatly contributes to the increasing occurrence of the disease (Yalew, 2019).

Foot and mouth disease outbreaks in cattle have been reported on a regular basis in Ethiopia, including the Arsi and Bale zones of the Oromia region. A regular study of the serotypes circulating in the study areas, as well as reporting on the disease's economic crisis, is necessary to motivate the government. For effective control and prevention measures, an appropriate vaccine containing the serotypes circulating in the area must be developed. On top of this, knowing the status of FMD through regular serological surveillance and field-based outbreak monitoring could aid in generating baseline information about foot and mouth disease and institute better mitigation measures in the study area. Also, reports related to the economic impact of foot and mouth diseases in the study area are limited, so it is difficult in decision-making for control and prevention.

Therefore, the objectives of the current study were:

General objective

- ✓ To determine the sero-epidemiology and economic impact of foot and mouth disease in cattle of Arsi and Bale zones, Oromiya Regional State, Ethiopia.

Specific objectives

- ✓ To determine the sero-prevalence of Foot and mouth disease in cattle in selected districts of the Arsi and Bale zone
- ✓ To identify some of the risk factors associated with the disease in the study area
- ✓ To detect the serotypes of FMD virus strains responsible for outbreak cases
- ✓ To assess the economic impact of the disease with emphasis on livestock farms

2. LITERATURE REVIEW ON FOOT AND MOUTH DISEASE

2.1. The Disease Definition

Foot and mouth disease is an extremely contagious, acute viral disease of all cloven-hoofed animals and pigs, characterized by fever, loss of appetite, salivation, vesicular eruptions in the mouth, on the feet and teats, and sudden death of young animals (Quinn *et al.*, 2005). It is one of the most globally important notifiable diseases of livestock due to its highly infectious and trans-boundary distribution nature (Knight and Rushton, 2013).

2.2. Etiology

2.2.1. Taxonomy

The Foot and Mouth Disease virus was defined in 1963 by the International Committee on Taxonomy of Viruses (ICTV) as belonging to the genus *Aphthovirus*, family *Picornaviridae*. The name *picornaviridae* 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. Foot and mouth disease viruses are the causative agents of foot and mouth diseases (Juhar, 2019).

2.2.2. Physicochemical properties

Foot and mouth disease virus is a small RNA virus that is enclosed by a non-enveloped protein shell (capsid). The capsid is made up of polypeptides that lack lipoprotein and are thus resistant to lipid solvents such as ether and chloroform (Rowlands, 2008). The virus is pH sensitive to both acidic and alkaline conditions. It is more stable between pH 7 and 9 at 4°C and -20°C respectively, but all strains are rapidly inactivated when exposed to pH below 6.5 or above 11. In milk and milk products, the virion is protected, and it can survive at 70°C for 15 seconds at pH 4.6. In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes. FMDV-contaminated objects can be effectively disinfected with a 2% solution of caustic

soda (NaOH) or KOH and 4% Na2CO3 in combination with detergent, but the virus is resistant to alcohol and phenolic quaternary ammonium disinfectants (Rueckert, 2006).

At temperatures below freezing, the virus is stable for a long period, while exposure to 56 °C for 30 minutes is sufficient to destroy most strains. On the contrary, sunlight has little or no direct effect on infectivity. The survival of the virus is also influenced by relative humidity, with good survival above 60% relative humidity and speedy inactivation below 60% relative humidity (Geering and Lubroth, 2002).

2.2.3. Antigenic variation

Changes to the genes encoding capsid proteins through mutation can result in antigenic variation and the evolution 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. More frequently, the impacts of this variation derive from the three major surface exposed proteins of the virus (VP1-VP3) (Rweyemamu *et al.,* 2014). Due to antigenic variation over time, the emergence of field variants is increasing. Either the infected or vaccinated species of host may undergo immunologic pressure to generate antigenic variants. The presence of variable antigenic types in different geographical areas and even the concurrence of different antigenic types in the same geographical area means there is always a need to select a vaccine strain based on the antigenic type prior to starting a control and eradication program (Rudresha *et al.,* 2012).

Currently, FMDV isolates are grouped into seven different serotypes, namely, O, A, C, Asia-1, and SAT 1-3, based on the antigenicity of the capsid coating proteins, each with a diversity of topotypes, genetic lineages, and strains. Some lineages have different properties that may contribute to sporadic spread beyond their recognized endemic areas. Due to the change in capsid protein, it led to a lack of cross-protection between variants within serotypes, particularly evident within serotype A (Ding *et al.,* 2013; Chakraborty, 2014).

2.2.4. Genetic variation

The observed genetic variation in the 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 proof reading 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 and Venter, 2004). Genetic variation that arises may be due to the following factors.

Mutation: Foot-and-mouth disease viruses undergo a high rate of mutation during replication. This is mainly due to a lack of replication error checking mechanisms (proof reading). RNA viruses that exhibit such a deficiency mutate at the rate of one nucleotide base change per 103 bases per replication cycle (Holland, 1997). It is also estimated that a mutation rate of up to 10−8 to 10−9 nucleotide substitutions per year during an epizootological cycle of FMD viruses can occur. Therefore, new variants of FMD viruses are continuously arising after each replication cycle, which constitutes an intratypic population of FMD viruses with different degrees of genetic relationships, previously described as the quasi species phenomenon (Domingo *et al.,* 1990). This may result in the generation of viral diversity. Changes in the nucleotide composition of the capsid genes are responsible for the genetic or antigenic variability of the virus. Thus, the generation of new variants is considered one of the major problems in the control of FMD by vaccination (Sangare, 2005).

Immune selection pressure: One of the evolutionary mechanisms employed by RNA viruses is profile mutant production, as detailed above. In addition, the immune system of an infected animal, which presumably provides a powerful selective force, is another driving force in viral evolution for the reason that the viruses are exposed to immune sera. Under this condition, only neutralization escape variants will be able to grow (Domingo *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. However, 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. Therefore, recombination is an important factor in the creation of genetic diversity (Genchwere and Kasanga, 2014).

2.2.5. Genome organization and viral proteins

FMDV has a single-stranded positive sense RNA genome of approximately 8500 nucleotides long enclosed within a protein capsid assembled of 60 copies of each of three major structural polypeptides termed VP1, VP2, and VP3, and a smaller polypeptide termed VP4, encoded by 1D, 1B, 1C, and 1A genomic regions, respectively. Among those, VP4 is internal, whereas others are exposed on the virion surface. The viral genome is generally divided into three sections: a 5' untranslated region (1300 nucleotides), a single Open Reading Frame (coding region), and a 3' untranslated region (24 or 25 nucleotides). The 5'UTR is also made up of five components: an S fragment, a poly C tract, pseudo knot structures, a cis acting replication element, and an internal ribosome entry site (Yalew , 2019).

There is polyadenylated attached on the 3' end and Vpg attached covalently to the 5’ end. A single Open Reading Frame (coding region) of the viral RNA part is used to encode a large polyprotein which is cleaved by viral proteases to form a capsid. The VP1 contains a minimum of two important immunogenic sites: the G-H loop (at amino acid positions 141–160) and the C-terminus with 200 to 213 residues (Tesfaye, 2014). The G-H loop contains an arginine glycine-aspartic acid motif, which is required for virus attachment to the host cell. FMDV's genome contains a large and indeterminate spectrum of subtypes or variants, resulting in significant genetic heterogeneity (León, 2012). The overall genomic organization of the FMDV is illustrated in Figure 1 below.

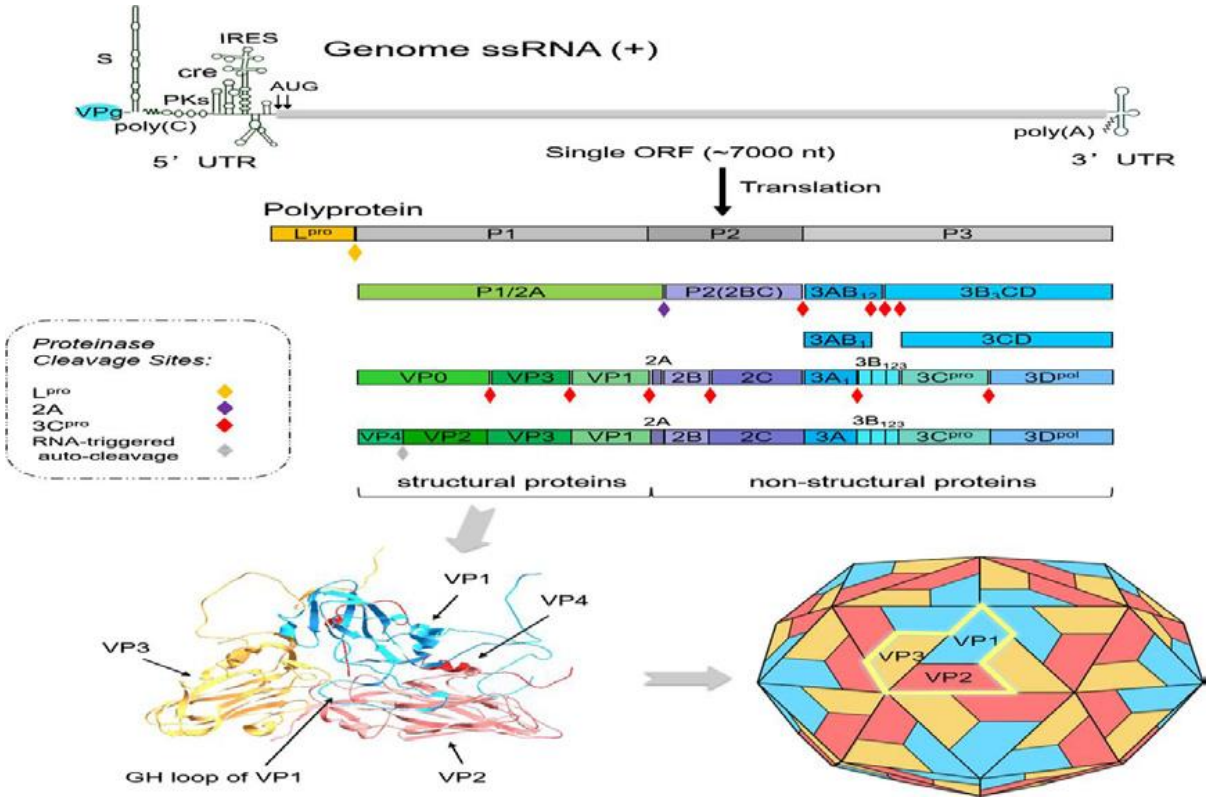

Figure 1: Schematic diagram of FMDV genome organization

Source: (Yalew, 2019)

2.2.6. Serotype and subtype

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, 2007). At present, sequencing of FMD viruses is increasingly being used to establish intratypic variations of FMD viruses and classify viruses into genotypes and lineages (Sahle and Venter, 2004).

2.3. Epidemiology

2.3.1. Global distribution

Foot and mouth disease is one of the most widely distributed diseases worldwide, mainly in South America, Asia, the Middle East and sub-Saharan African countries. Among 178 member states of the World Organization for Animal Health, only 66 countries are FMD free (65 without vaccination, 1 with vaccination); 10 countries have FMD free zones. North America, the majority of South America, Western Europe, Australia, New Zealand, and most island countries in the Pacific are free of the disease (Depa *et al.,* 2012). The global distribution of foot and mouth disease is shown in Figure 2 below.

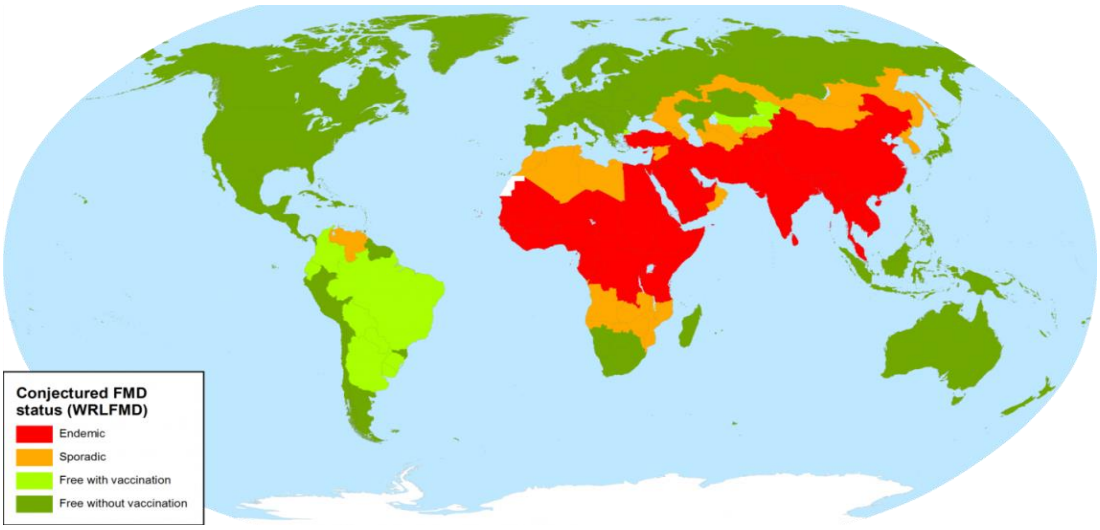

Figure 2: Global distribution of Foot and Mouth Disease
Source: (OIE, 2021)

2.3.2. Distribution of the disease by serotypes

Foot and mouth disease virus is currently classified into seven serotypes, namely A, O, Asia1, C, SAT1, SAT2, and SAT3. The former four are also known as Euro-Asiatic serotypes, circulating mainly in Europe, Asia, and South America, while the latter three are collectively known as South African Territories (SAT) serotypes, found mainly in Africa. This classification scheme

was established based on immunological reactions. According to a study of 919 genomic sequences with serotype records retrieved from the NCBI database, 91.19% of the sequences belonged to the Euro-Asiatic serotypes (O, A, Asia1, and C), and 8.81% belonged to the South African Territories strains (SAT1–3). All SAT strains had an African origin, except for one (SAT 1), of which the origin was unclear. Serotype A, Asia1, C, and O of FMDVs were from the rest of the world, with a few being from the African continent (Aiewsakun *et al.,* 2020).

The seven serotypes of FMDV are not uniformly distributed in the world. Among the seven serotypes of FMD, serotypes O, A, and C have had a worldwide distribution, with serotype O being responsible for the majority of the outbreaks globally. Serotype A has more than thirty subtypes with great antigenic diversity, and there is also often no cross-protection in between. Serotype C was detected in Europe, South America, East Africa, North Africa, Angola and southern Asia. The FMD type C virus was controlled and eradicated by vaccination in Europe and South America, and it has also become extinct in Africa and other Asian countries. The serotypes SAT (South African Territories-1, 2, and 3) are normally confined to sub-Saharan Africa and are prevalent in southern and eastern Africa (Abubakar *et al.,* 2012).

Table 1: Geographical distribution of different FMD serotypes Regions

Country	**Serotype**
Asia	A,O,Asia1
Africa	A,O,C,SAT1,SAT2,SAT3
Europe	A,O,C
South America	A,O,C
Oceania	FMD free
North and Central America	FMD free
Caribbean	FMD free

Source:(Azeem *et al.,* 2020)

2.3.3. Species affected

Foot and Mouth Disease (FMD) is a highly contagious disease and affects various cloven-hoofed domestic and wild mammals, including cattle, sheep, goats, deer, and pigs, which are susceptible to infection and can spread the disease, whereas the African buffalo is known to be the main wildlife reservoir for SAT serotypes in Africa, but depending on the species and virus strain, the development of the disease varies (OIE, 2021). The typical severity of the disease and the level and duration of infectiousness vary widely, with sheep showing less clinical evidence of infection than cattle or pigs. However, horses, pet animals, and birds are resistant to FMD, while camels are moderately susceptible (Chakraborty, 2014).

2.3.4. Carrier state of animals

A carrier, in FMD, is defined as an animal from which FMD virus can be isolated from the esophageal pharyngeal (OP) area more than 28 days after infection. Generally speaking, a carrier is defined in epidemiological terms as an animal that is infected and can disseminate the infection in the absence of symptoms. However, with FMD, carrier animals may or may not be able to transmit infection (Sutmoller and Casas, 2002). The maximum duration of the carrier state that has been reported in cattle is 3.5 years; in sheep, 9 months; in goats, 4 months; in African buffalo, 5 years; and in water buffalo, it is unknown. Pigs, however, clear the infection in 3–4 weeks and so do not become carriers but are considered key amplifiers of the virus since they excrete large amounts of virus in their expiration (Sellers and Gloster, 2008). Consequently, the FMD carrier state is a factor that highly influences national policies directing FMD countermeasures in areas in which the disease is not endemic (Genchwere and Kasanga, 2014).

2.3.5. Incubation period

The length of the incubation period varies depending on the route of inoculation, dose of virus, viral strains, age, and species of animals. In susceptible animals, it can range from two to eight days, but can be up to twenty-one days post infection with the virus. Depending on the infected animal species, the incubation period of FMD is 2 to 14 days in cattle, 2 to 8 days in sheep, and

usually 2 days or more in pigs (with some experiments reporting clinical signs in as little as 18-24 hours)(OIE, 2021).

2.3.6. Source of infection and mode of transmission

Foot-and-mouth disease is highly infectious because a small dose of the virus is infectious and several routes of FMD virus infection and excretion have been reported. The FMDV can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, blood, milk, urine, feces, and semen. The primary mode of transmission is via respiratory aerosols since the virus can replicate mainly in the respiratory tract of animals and a large number of the virus particles are excreted from this area (Juhar, 2019).

Another important means of spread is by direct contact between infected and susceptible animals, which leads to the virus entering the host through damaged epithelium, cuts or abrasions, mucous membranes, or by deposition of droplets in the respiratory tract (Paton *et al.,* 2018). In densely populated areas, the disease may spread extremely rapidly because of the high level of challenge from infected animals. It is widely accepted that the most common mode of FMD transmission is through physical contact between infected and susceptible animals, often as a result of the movement of infected animals. In comparison to aerosol transmission, pigs are infected with the virus through physical contact with infected secretions containing a large amount of virus. The FMD virus also readily spreads indirectly by a variety of inanimate objects, including animal food staff, beddings, farm equipment, livestock holding areas, transport vehicles, and so on, that have been contaminated with acutely infected animals' excretions and secretions such as saliva, milk, faeces, and urine (Rweyemamu *et al.,* 2008).

Outbreaks of FMD can also occur because of viruses escaping from research and vaccine production centers, and the semen of infected bulls can be a source of infection by artificial insemination. Personnel handling infected animals can be contaminated on hands, clothes or in nasal passages with live FMD virus and mechanically carry virus to susceptible animals by direct contact. A person in contact with infected animals can serve as a source of infection for 24 hours post infection (Kitching *et al.,* 2007). The spread of FMD viruses by wind over 250 km across

the sea and 60 km across the land has been reported in temperate climates during an outbreak of the disease. Early recognition of disease, followed by the slaughter of infected livestock and the introduction of movement controls, reduces the risk of mechanical spread. However airborne spread of the virus cannot be controlled by these means (Tesfaye, 2021). The routes of foot and mouth disease virus transmission are described in figure 2 below.

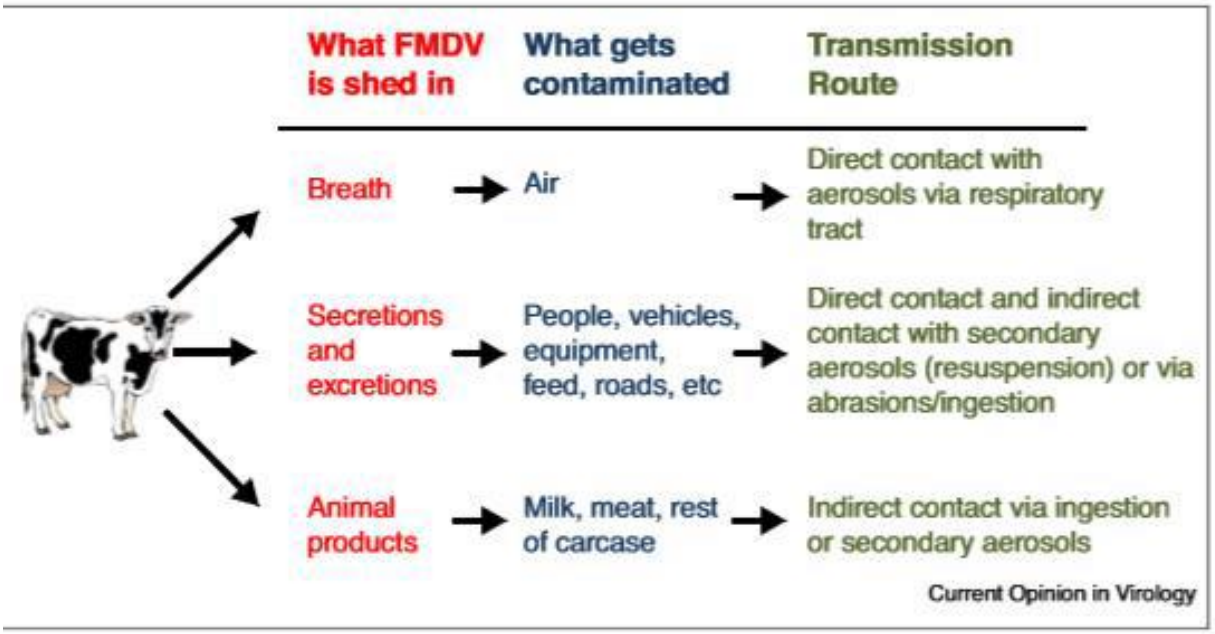

Figure 3: Routes of FMDV transmission

Source: (Paton *et al.*, 2018)

2.3.7. Morbidity and mortality

Foot and mouth disease has a high morbidity rate and a low mortality rate. The type of breed, the production system, age group, absence of restriction of animal movement, animal density, use of communal pasture and watering points, and season are among the major factors associated with the morbidity and mortality rate of the disease (Tesfaye, 2021). The morbidity rate in outbreaks of FMD in susceptible animals can rapidly approach 100%, but some strains are limited in their infectivity to particular species. However, morality is low, about 2% in adults and 20% in young stock. Mortality in adult animals is usually low to negligible; up to 50% of calves may die due to

cardiac involvement and complications such as secondary infection, exposure, or malnutrition. Mortality in suckling pigs and lamps ranges from 20-75% in most extreme cases, and it is highly age-dependent. Infected animals under 4 weeks of age, mortality is high and decreases rapidly as animals get older than 4 weeks (Balemual, 2018).

2.4. Clinical signs

Animals that can be affected include cattle, swine, sheep, goats, buffaloes, and wild ruminants. When susceptible animals are in contact with clinically infected animals, clinical signs usually develop (Kitching, 2002). 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 mild or in apparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2019).

In acutely infected cattle, the initial signs are fever or a rise in temperature (40–40.6oC), dullness, anorexia, and marked drops in milk production. Within 24 hours, there is excessive salivation, with the saliva hanging in long, ropy strings that lead to nasal discharge. These signs are accompanied by vesicle formation on the dorsum of the tongue, soft palate, dental pads, lips, and gums, which results in the opening and closing of the mouth with a characteristic smacking sound. Vesicles may also be found in areas where there is high friction, such as inter-digital space and the coronary band of feet, with consequent lameness, on nostrils, muzzles, and teats. Pregnant cows may abort, presumably as a consequence of fever, and young calves up to 6 months of age may die suddenly before the appearance of any clinical signs of the disease due to virus infection of the developing heart muscle (myocarditis) (Balemual, 2018).

In swine, lameness is often the first sign. Other signs include fever about 40-40.6oC, anorexia, reluctance to move, and squeal when forced to move. These signs are followed by the formation of vesicles on the coronary bands, heels, snout, and in the inter-digital space (foot involvement is usually severe). Mouth lesions are not too common, and when they, occur they are smaller and of shorter duration than in cattle and tend to be a dry-type lesion; there is no drooling; sows may abort; and piglets may die without showing any clinical sign (OIE, 2021).

In sheep and goats the clinical signs of FMD are very mild or in apparent. The disease can easily be overlooked until and unless individual animals are carefully examined for disease lesions. If clinical signs occur in sheep and goats, they may include dullness, fever, and small vesicles (0.5-2 mm) 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 along the coronary band or in the inter-digital space. Infected animals may abort, and nursing lambs may die without showing any clinical signs (Juhar, 2019).

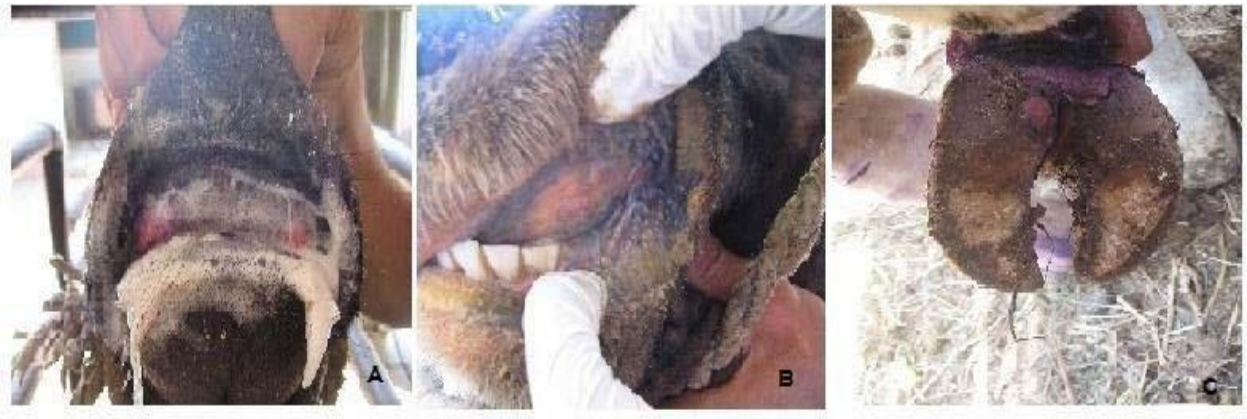

Figure 4: Clinical signs of FMD

(A) Salivation; (B) Erosion of oral mucosa and (C) erosion in interdigital space.

Source: (El-Khabaz and Al-Hosary, 2017)

2.5. Pathogenesis

The respiratory system is the most important route of infection for FMD. Once the virus is inhaled, it can affect the pharynx, which is the primary multiplication site and then transported by lymphatic and blood circulation to the sites of secondary multiplication in the lymphatic glands, epithelial tissues in and around the mouth, feet, and in the mammary glands. Following secondary replication in glandular tissues, the virus appears in different body fluids such as milk, urine, respiratory secretions, and semen, before the appearance of frank clinical signs of FMD. The virus can also persist in the oral cavity of infected animals for long periods after the acute infection (Balemual, 2018).

Gross lesions develop only in areas subjected to mechanical trauma or unusual physiological conditions such as the epithelium of the mouth, feet, or to a lesser extent, the teats. Bacterial complications aggravate the lesions, particularly those of the feet and the teat, leading to severe lameness and mastitis, respectively. In young animals, especially neonates, the virus frequently causes necrotizing myocarditis, and this lesion may also be seen in adults infected with some strains of the virus, particularly type O (Ranjan *et al.*, 2016).

2.6. Diagnosis

The accurate diagnosis of infection with FMD virus is of prime importance for both control and eradication campaigns in FMD endemic areas. The disease is diagnosed based on clinical signs, including high temperature, excessive salivation, and the formation of vesicles on the oral mucosa, on the nose, inter-digital spaces, and coronary bands on the feet. However, the clinical signs can be confused with other diseases. Therefore, confirmed laboratory diagnosis of any suspected FMD case to ascertain the serotype/subtype of the causal virus is a necessity to enable proper control of the disease as a supportive measure to the stamping out policy in FMD-free areas (Longjam *et al.*, 2011).

For laboratory diagnosis, samples include vesicular fluid, epithelium tissue, serum, and esophageal/pharyngeal fluids collected with the Probang cup. 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 signs, samples of esophageal-pharyngeal (OP) fluids are collected by means of a probang and used for virus isolation (Admassu *et al.*, 2015). Laboratory diagnostic techniques for FMD are achieved by a combination of serological tests, virus isolation and nucleic acid recognition methods (Balemual, 2018).

2.6.1. Serological test

Serological tests are essential for supplementary diagnosis of FMD, for certification of animals for import/export, in determining freedom from infection and for demonstrating vaccine efficacy. Virus infection can be diagnosed by the detection of a specific antibody response. Enzyme linked

immunosorbant assay (ELISA), agar gel immunodiffusion test (AGID), virus neutralization test (VNT), and complement fixation test (CFT) are used for serological diagnosis of FMDV. Previous or current infections can be diagnosed by detection of antibodies to viral structural proteins (SP) via virus neutralization test (VNT, Gold standard test), liquid-phase blocking ELISA (LPBE), solid-phase competition ELISA (SPCE), and complement fixation test (CFT), which are serotype specific. Because FMDV vaccines also induce antibodies to structural proteins, these tests can only be used in unvaccinated animals (OIE, 2021).

Antibody levels to NSP can be measured via an agar gel immunodiffusion test (AGID) and a 3ABC NSP ELISA test. One particular application of these serological assays is to identify animals in a vaccinated herd that have been infected with FMDV. This so-called DIVA (differentiating infected from vaccinated animals) principle exploits differences in the antibody (humoral) responses generated in vaccinated animals compared to those animals naturally infected with FMDV (whether or not they have been vaccinated) and can be used in both vaccinated and unvaccinated animals. High quality FMDV vaccines are purified to contain structural protein (SP) viral capsid components from which most of the viral non-structural proteins (NSP) have been removed. In contrast, during natural infection with FMDV, viral NSP are expressed that elicit a corresponding immune response that can be detected using diagnostic approaches (Figure 5) (Yalew, 2019).

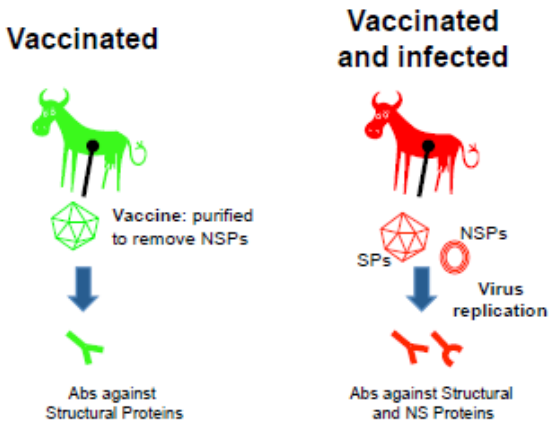

Figure 5: The principle of using NSPs tests to differentiate between vaccinated and infected animals.

Source: (Yalew, 2019)

2.6.2. Virus isolation

Virus isolation in cell culture is considered the "gold standard" technique for FMD diagnosis. Virus isolation is a definitive method of diagnosis for foot and mouth diseases. Virus isolation requires the presence of infectious viruses, but sample quality determines getting a reliable result. The cultures were checked for cytopathic effects (CPE) of the virus. This method is highly sensitive, but it is time-consuming, which takes between 1 and 4 days and requires extraordinary laboratory facilities. The media used for the culture of FMDV are bovine thyroid cells, primary lamb kidney (LK) cells, and baby hamster kidney (BHK-21). Bovine thyroid cells are very sensitive to FMDV, but for preservation within the refrigerator, BHK-21 is better. Cultures with CPE were stored at -70°C until processing for indirect sandwich ELISA (Mahmoud *et al.,* 2019).

2.6.3. Nucleic acid recognition methods

The polymerase chain reaction techniques are the most widely used nucleic acid based diagnostic techniques for rapid identification of FMD viruses and sequence analysis of any PCR positive result. A specific reverse transcriptase polymerase chain reaction was developed and validated for the detection of the polymerase gene (3D) of FMD with an analytical sensitivity equal to 1000 times higher than that of single passage virus isolation (Longjam *et al.,* 2011). RT-PCR is used as a diagnostic tool for FMD viruses, where specific primers are designed to distinguish seven serotypes. In epidemiological studies of FMD viruses, nucleotide sequencing of the VP1 gene has been extensively used to determine the relationships between the field isolates (Porta *et al*., 2013).

2.7. Treatment

Currently, there is no specific curative drug that would be recommended to treat foot and mouth disease. However, treatment of secondary bacterial infection and dressing of lesions with proper animal husbandry practices is recommended in FMD endemic countries in which slaughter policy is hard to apply. Also, sick animals may be treated by applying broad-spectrum antibiotics parentally, tetracycline in particular, in order to control the consequences of secondary bacterial infections (Admassu *et al.,* 2015).

2.8. Prevention and Control

Foot and mouth disease is subject to national and international control and the measures taken depend on whether the country is free from the disease, is subject to sporadic outbreaks or has an endemic infection (Rweyemamu *et al.,* 2008). Control policies and prevention strategies adopted by a country vary depending on their epidemiological condition, FMD status and the risks of incursions of the disease, the importance of the livestock sector in the national economy, and economic capability of the country to invest in control strategies (Paton *et al.,* 2009). Countries free of FMD impose strict import regulations on animals, animal products, and potentially contaminated materials from FMD countries. Quarantine and vaccination programs are also used to control outbreaks and to prevent the spread of disease. In countries where the disease is endemic, efforts are generally directed at protecting high-yielding dairy cattle by a combination of vaccination and control of animal movement (Wubshet *et al.,* 2019).

Preventive measures in the absence of disease should be implemented as follows: control of national borders to regulate or prevent the significant movement of animals and livestock products from non-free neighbors or trade partners. For officially free countries, prohibition of imports of animals and livestock products from non-free countries in accordance with the OIE standards; prohibition of distributing untreated catering waste (human food) to pigs (Getahun, 2021). Emergency measures in the event of outbreaks through: rapid slaughter of infected animals, in contact with animals and herds considered to have received infection by contact, to reduce the quantity of virus released policy of “stamping-out”, followed by cleaning and

disinfection to reduce the risk of reinfection, strict movement controls, extending to movement on and off farms of livestock products (Azeem *et al.*, 2020).

Intensive investigations to determine if infection is likely to have spread to additional locations within or outside of the protection and surveillance zones and containment measures for such herds or villages, depending on the risk identified. And also, possible emergency vaccination is important (Ding *et al.*, 2013). In countries where the disease is endemic, control and prevention of FMD are mainly reliant on repeated vaccination, control of animal movement, and also physical separation of wildlife and livestock. However, the test and slaughter policies shouldn't be implemented due to the economic, social, and regional barriers. For the development of an effective vaccination strategy, it is important to understand the disease dynamics, which indicates the suitable time points to administer vaccine (Chakraborty, 2014).

Vaccination against FMD virus is achieved with inactivated vaccines that should induce protective immunity against each type of antigen incorporated into the vaccine. Therefore, when vaccinating animals, it is important that the vaccine contain the same subtype of virus as that which is in the area. This necessitates frequent checking of the serotype and subtype during an outbreak because FMD viruses frequently change during natural passage through various species (Yalew, 2019).

In the situation of Ethiopia, the control of FMD is practiced by the involvement of quarantine, restriction of animal movement, isolation of infected animals, vaccination programs, and proper disposal of infected carcasses. Currently, there is no country-wide vaccination program aimed to control FMD and a ring vaccination is carried out around infected areas. Considering the wide prevalence of serotypes O, A, and SAT 2, the National Veterinary Institute is producing an inactivated trivalent vaccine (Yalew, 2019).

2.9. Economic Impact of Foot and Mouth Disease

Foot and mouth disease is one of the most economically important contagious diseases of livestock in the world (Garner *et al.*, 2002). The importance of the disease is observed in terms of

its sequelae like contagious nature post outbreak, broad economic impacts on animal wellbeing and productivity, loss of production, cost of veterinary service due to the presence of the disease and the restrictions on the trade of animals and animal's product both locally and internationally (James and Rushton, 2002). The impacts of the disease vary significantly between developed and developing countries and also within many developing countries depending on the species involved, the genotype of animals, the level of productivity, the significance of livestock to livelihoods, and the effectiveness of indigenous coping mechanisms for controlling the effects of FMD (Perry and Rich, 2007).

Economic losses can be attributed to both direct and indirect costs. The direct production effect includes reduced milk production; chronic mastitis due to udder involvement; and reduced draught power due to permanent hoof damage. Clinical disease affects animal performance and causes a reduction of milk yield in dairy cattle by 20%; the growth rate of beef animals by 10–20%; pig meat production by up to 20% per annum; and inflicts fertility impairment due to an increased abortion rate of up to 10% (Knight and Rushton, 2013). Moreover, the death of young animals, the culling of unproductive and chronically infected animals, and the loss of valuable breeding stock and disruption of livestock improvement programs are also attributed to the direct effect of the disease. The impact on livelihoods can be devastating, and similarly, pastoralists severely suffer from the direct impact of the disease since their livelihood depends on livestock production (Rufael *et al.,* 2008).

The indirect cost is associated with the cost of control carried out by the state veterinary services (e.g. vaccination, surveillance, outbreak control, culling and compensation, and movement control). The costs are enormous, with an estimated 2.35 billion doses of FMD vaccine administered in the world every year at a cost of $0.4–3 or occasionally $9 per dose, including delivery and application. In FMD free countries, there are ongoing costs due to efforts to prevent disease introduction, including import controls. In addition, maintaining FMD early detection and control capabilities, including vaccine banks, is costly. Other costs include FMD-related research and permanent restrictions on the livestock sector (such as post-movement standstills and bans on feeding swill) (Knight and Rushton, 2013).

Foot and mouth disease causes the highest economic impact on the poorest countries, like Ethiopia, where the livelihood of most of the people depends directly on livestock. It reduces herd fertility leading to less efficient herd structure, discourages the use of FMD susceptible high productivity breeds, causes trade bans and high control costs. In Ethiopia, FMD is posing a major threat there by causing substantial economic losses through morbidity and mortality (Admassu *et al.*, 2015).

Generally, the overall impact of FMD on the economy is described as direct losses due to reduced production and alteration in herd structure; and indirect losses caused by costs of FMD control, poor access to markets, and limited use of improved production technologies. The annual economic impact of FMD in terms of visible production losses and vaccination costs in endemic regions of the world is estimated at between US $6.5 and 21 billion, while outbreaks in FMD free countries and zones cause losses of more than US $1.5 billion a year (Knight and Rushton, 2013). The economic impacts of FMD are summarized in Figure 5 below.

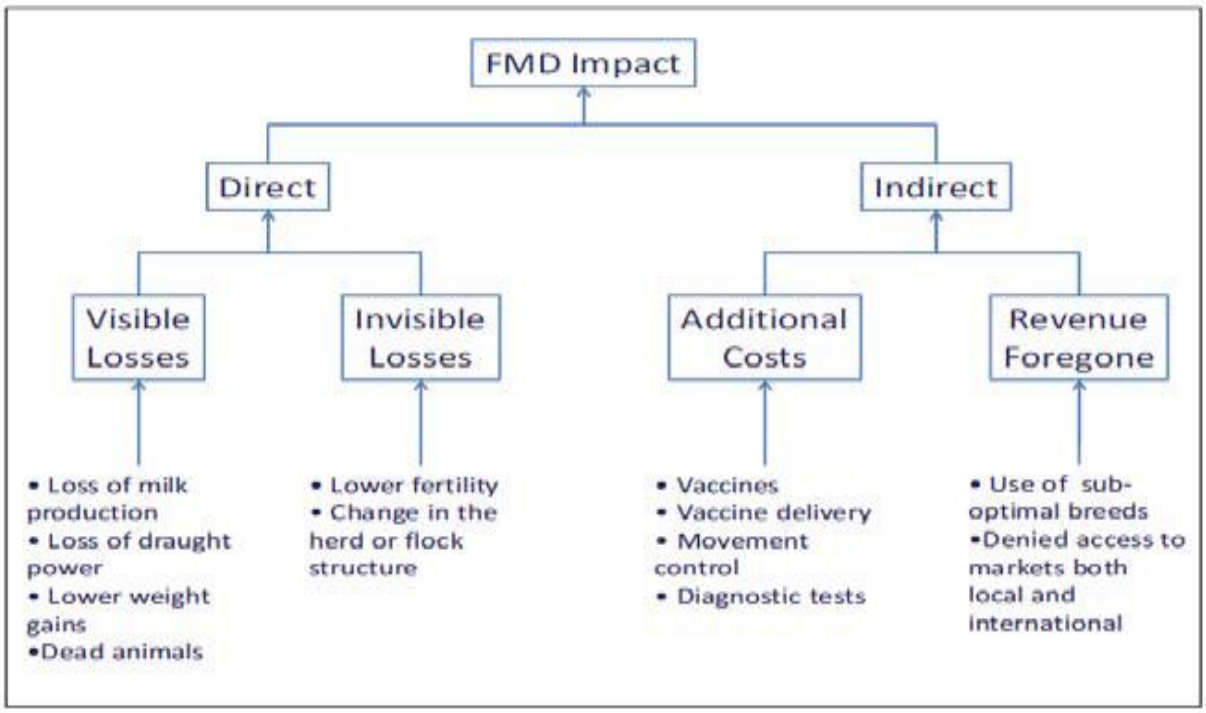

Figure 6: The economic impacts of foot-and-mouth disease

Source: (Knight and Rushton, 2013)

2.10. Foot and Mouth Diseases Situation in Ethiopia

2.10.1. Disease status

In Ethiopia, foot and mouth disease is endemic and a notifiable disease; the national animal health regulatory directorate sends monthly and annual official reports to the OIE (MoLF, 2016). The disease is widely prevalent and has previously been known to occur frequently in the pastoral herds of the marginal lowland areas of the country. However, this trend has changed and the disease is now frequently noted in the highlands of the country (Gudata, 2019).

2.10.2. Spatial distribution

A foot and mouth disease is widely distributed in all areas of Ethiopia, although the level of the disease prevalence may show significant variations across the different farming systems and agro-ecological zones of the country (Wubshet *et al.*, 2019).

2.10.3. Temporal distribution

According to the MoLF, division of epidemiology directorate disease outbreaks report summary, FMD occurs at any time of the year. However, the highest outbreaks of the disease are observed in the extreme dry seasons of the year, which is from January to March. Different researchers reported that this might be associated with factors such as drought. During dry seasons, especially when pastoralists are obliged to move their herds long distances in search of pasture and water, the transmission of highly contagious diseases like FMD is exacerbated at herd gathering sites or communal points (Rufael *et al.*, 2008). On the other hand, in most rural parts of the country, during the rainy seasons of the year, wide areas of farm land are planted with crops. During this time, huge numbers of domestic animals are kept on confined small plots of communal grazing lands that could favor the occurrence and transmission of the disease (Juhar, 2019).

2.10.4. Diseases prevalence

The prevalence of the disease is varies from place to place, and the studies conducted so far did not cover all corners of the country. The lack of well-equipped regional animal laboratories, inaccessibility of certain areas and suboptimal routine surveillance and reporting could hinder to have the overall estimate of the disease magnitude at a national view contrary to its endemicity (Sahle and Venter, 2004).

Table 2: Sero-prevalence of FMD in cattle in different parts of Ethiopia

Study Area	**Study period**	**Seroprevalence %**	**References**
Borana	2006	21.0	Rufael *et al.,* 2008
South Omo	Oct, 2009-May, 2009	8.2	Molla *et al.,* 2010
Somali regional state	Oct,2009-March 2010	14.05	Mohamud *et al.,* 2011
Afar Region	Oct,2007- April, 2008	5.6	Jenbere *et al.,* 2011
Borana	Oct, 2007- Mar, 2008	53.6	Mekonen *et al.,* 2011
Dire dawa	Nov ,2010- Mar, 2011	8.01	Abunna *et al.,* 2013
Kellem-wollega	Nov, 2011- Mar, 2012	21.4	Desissa *et al.,* 2014
Central- Ethiopia	2011	14.5	Alemayhu *et al.,* 2014
Bishoftu	Sep, 2014- July, 2015	10.88	Belina *et al.,* 2016
Centeral-Ethiopia	Sep, 2015- May, 2016	24.22	Sulayeman *et al.,*2018
Oromia and AA	Sep, 2016- May, 2017	38.6	Urge *et al.,* 2017
Tigray	Oct, 2008- Jun, 2009	15.4	Zerabruk *et al.,* 2014
Bale Zone	Nov, 2007-April 2008	21.59	Duguma *et al.,* 2013
Western Ethiopia	Nov, 2011-April2012	9	Beyene *et al.,* 2015
Ahmara,Oromia and SNNs	Oct,2018 -May 2019	24.39	Nato,2019
Central Ethiopia	January, 2019-2021	72.1	Awel *et al.,* 2021
Central Ethiopia	2019	49.2	Urge *et al.,* 2020
Borana Zone	April – Nov,2015	42.7	Melkamsew, 2018
West Shewa Zone	Dec,2017-Nov,2019	40.4	Ahmed *et al.* 2020

Source: (Abdela, 2017)

2.10.5. FMD virus serotypes identified

In Ethiopia, foot and mouth disease was first recorded in 1957 when serotypes O and C were found, while serotype A was identified in 1969 (Martel, 1974; Martel, 1975). The first isolation of SAT2 in Ethiopia was in 1989 in a sample collected from Awassa, Sidamo, and Negelli Borena (Roeder *et al.*, 1994). The presence of FMDV serotype SAT1 in Ethiopia was isolated and reported for the first time in 2008 from three species of animals: cattle, sheep, and goats (Legesse *et al.*, 2013). Currently, FMD is endemic, widely prevalent and distributed in all areas of the country. The presence of foot and mouth disease in the country is a major obstacle for the development of agriculture because of its adverse effects on livestock production and exports (Abdela, 2017).

Endemic distributions of five of seven serotypes of FMDV are maintained in the country and serotypes O, A, C, SAT1 and SAT2 were responsible for FMD outbreaks during 1981–2021 as shown in table (3). From the report, serotype O was the most predominant serotype circulating in the country (Jemberu *et al*., 2016). The serotype C has not been reported in the country since 1983 (Ayelet *et al*., 2009).

Table 3: Serotypes of foot and mouth disease virus isolated in Ethiopia from 1981 to 2021

Study Period	**Serotype**	**Sample origins**	**References**
1988-1991	O and SAT 2	Addis Ababa, Eritrea, Wallaga, Hararge, Dire Dawa, Borena	Roeder *et al.*, 1994
1981-2007	O, A, C, SAT 1 and SAT 2	Addis Ababa, Ahmara, Tigray, Dire Dawa, Beneshangul-Gumuz, SNNPs	Ayelet *et al.*, 2009
2007/08	O and SAT 1	Girar Jarso, Yabello, Surma, Maji, Ankesha Guagusa,	Legesse *et al.*, 2013

2007-2012	O, A, SAT 1 and SAT 2	Koka, Surma, Sheka, Yeki, Sululta Benshangul Gumuz, Debre Zeit, Addis Ababa, Bahir Dar, Harar, Guji Debre Birhan, East Shoa, Arbaminch, Abaya, Borena, Dama, Adama, Mekelle, Jille Timuga, Kombolcha, Wollayta Sodo, Sidama, Mekele	Jemberu *et al.*, 2016
2008/09	O and A	Bahirdar Zuria, South Achefer, Yilmana Densa, and Dangela, East Harereghe (Haremaya University dairy farm), Borena (Yabello District), and Bale (Sinana District)	Negusssie *et al.*, 2011
2009/10	O and SAT 2	Addis Ababa, Debre-Birhan, Debre-Zeit, Sululta	Deribie, 2017
2010/2011	O and SAT 2	Debrezeit (Ada clinic)	Belay and Muktar,2015
2011/12	O	Mekelle University Farm, Aynalem, Shibta, Cholekot,	Kassaw *et al.*, 2013
2011/2012	O	Alage Dairy Farm, Alaba, Adamitulu Jido kombolcha, Debre Zeit, Malga, Adama, Akaki-Kality, Debre Berehan Mekele Universty Farm, Enderta,	Menda *et al.*, 2014
2015/16	A, O and SAT2	Guna, Ludehitosa, Adama, Boset,	Sulayeman *et al.*, 2018
2016/17	O, A, SAT 1 and SAT 2	Wolmera, Adea Berga, Kolfe keranyo, Mulo, Kimbit	Beksisa, 2017
2016/17	O	Addis Ababa, Bishoftu, Adama	Motuma, 2017
2016/17	O and SAT 2	Akaki, Bole, Yeka sub city, Mojo, Koka, Alemtena, Angolela, Birbersa and Godoberet	Wondwossen, 2017
2017/18	O and A	Meki, Bishoftu, Shewarobit, Bole sub city	Metages, 2018

2018/19	A and O	Oromia, SNNPs and Tigray	Shimels, 2019
2018/19	A and O	Guduru, Fitche, Kuyu, Mukaturi, Ale, Gewata and Shashogo	Nato,2019
2020	O and SAT 1	Assela and Woliso	Bayush,2020
2020/2021	O,A,SAT1, and SAT 2	Guzman, Adet and Metema	Betelihem,2021
2021	O,A and SAT2	Addis Ababa, Bishoftu,Modjo, Fitche and Debre Birhan	Mohammed *et al.*,2022

Source :(Yalew, 2019)

2.10.6. Economic losses due to FMD

Foot and mouth disease is the most important livestock disease in terms of economic impact on export earnings; as per estimate in a study about US$ 71026.8 losses are documented by Wagari (2016). An estimated amount of about 3,322,269 USD, equivalent to 56,345,682.24 ETB (1 USD = 16.96 ETB), was an annual economic loss in the year 2011 due to the bulls' rejection from the international market (Alemayhu *et al.,* 2014). Recently, the disease had become the major constraint hampering export of livestock and livestock products to Middle East and African countries; the Egyptian trade ban of 2005/2006, which Ethiopia lost more than US$14 million, being a recent memory (Leforban, 2005). Rufael *et al*. (2008) and Mersie *et al*. (1992) reported mortalities of young animals of 2.8% in Borena and 6% in eastern Ethiopia, respectively during FMD outbreaks. The disease causes about6% of export animals and animal products impediment (Abdela, 2017).

Foot and mouth disease infection in livestock causes a significant drop in milk yield (minimum 25%), reduction in meat and wool production, crippled agricultural draught power, abortion in pregnant animals, poor semen quality in bulls, and increased mortality in calves. Trade barriers for export of FMD infected livestock and their products and massive expenditure spent by governments on FMD control and treatment of ailing animals also cause a great economic loss to the country (Ayelet *et al.,* 2009).

2.10.7. Control and prevention strategies

Foot and mouth disease is considered one of the most important livestock diseases, demanding urgent control intervention to minimize the impact of the disease because of international trade barriers. However, due to the disease's complex nature, wider distribution across the country, short-term immunity with no interserotype cross protection, establishment of carrier status, the presence of multiple host species including wildlife, extreme contagiousness, and the absence of a proper vaccination program with FMD vaccine within Ethiopia, control strategies must be implemented progressively on a short- and medium-to-long-term basis (MoARD, 2006).

In Ethiopia, actions for controlling FMD include involvement of quarantine, isolation of infected animals, restriction of animal movement, proper disposal of infected carcasses, vaccination programs, and other methods that are feasible for the country's economy. Currently, there is no country-wide vaccination program aimed at controlling FMD. However, the vaccination program should give emphasis to the control of outbreaks occurring in the country through ring vaccination and vaccination of all exported cattle before entering the quarantine stations. To protect export animals from contracting the disease while being kept in quarantine sites, cattle found within a 10 km radius of these sites could be vaccinated. All dairy animals should also be vaccinated, and a ring vaccination carried out around an infected area. Considering the wide prevalence of the virus, the National Veterinary Institute is producing an inactivated trivalent vaccine against serotypes O, A, and SAT2 (Admassu *et al.,* 2015).

The recommended dosage of 4ml per head is administered to cattle subcutaneously, preferably in the dewlap region. In order to protect the cattle, two injections at a six month interval are recommended (DACA, 2006). Despite several efforts and attempts to design an FMD control strategy at the national level, an officially endorsed control plan for FMD has not been established. Recently, Ethiopia joined the progressive control pathway (PCP-FMD) network, launched by FAO and OIE in Bangkok in June 2012, and has started implementing this since 2017, with the progress reaching stage one (Wubshet *et al.,* 2019).

3. MATERIALS AND METHODS

3.1. Study Areas

The study was conducted from November, 2021 to June, 2022 to estimate the sero-prevalence of FMD, identify its risk factors and to determine the serotypes of FMD virus strains responsible for outbreak cases in the districts of Arsi (Hetosa, Lemu and Bilbilo, Munesa and Asako), and Bale zones (Sinana and Agarfa), Oromia regional state, Ethiopia.

Arsi zone is located at $6^0$45′N to $8^0$58′N latitude and $38^0$32′ E to $40^0$50′ E longitude. Asella, the capital of the zone, is located at a distance of 175 kilometers southeast of Addis Ababa, at an elevation of 2,430 meters above sea level. The zone is located at an altitude ranging from 1500 to 4245 meters above sea level and is known as the crop belt in Ethiopia due to its optimal agro-ecology and flat terrain. The total number of cattle found in the Arsi zone is 2,904,201 and the area of the zone is approximately 19,825.22km^2. It receives biannual rainfalls and the average temperature ranges from 10 to 25°C (CSA, 2021)

Bale zone is one of the zones of the Oromiya regional state, which is located 430 kilometers southeast of the capital city, Addis Ababa. Bale zone is bounded by four Oromiya zones and Somali Regional state, West Arsi zone in the north, Guji zone in the south, Somali Regional State and West Hararge zone in the east and West Arsi Zone in the west. The study area is located at an altitude range from 300 to 4377 meters and falls between 38°40´-46°3´ E longitude and 4°11´- 8°11´ N latitude. The mean annual rainfall and mean day and night temperatures range from 1100-1300mm and 3.5°C-30°C, respectively. The total number of cattle found in the bale zone is 961,215 (CSA, 2021). The climatic condition is marked by two distinct seasons: a long rainy season which extends from June to early October and a short rainy season between late February to early April. Dry season runs from December to May except for the short rainy season. But the low land (Pastoral) areas get rain only in spring and autumn which covers 9 districts of the zone (NMSA, 2008). The map of study areas is shown in Figures 7 and 8 below.

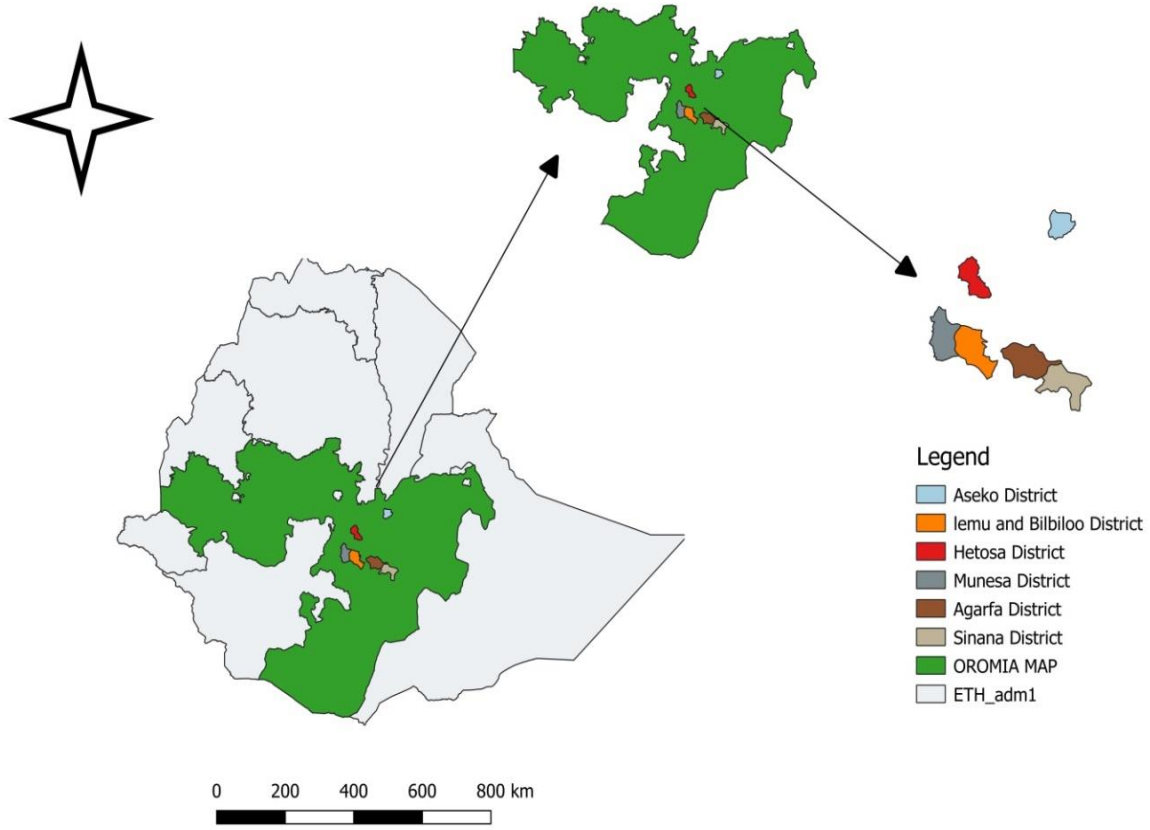

Figure 7: Map of study areas for sero-survey

Source: Done by QGIS version 3.16

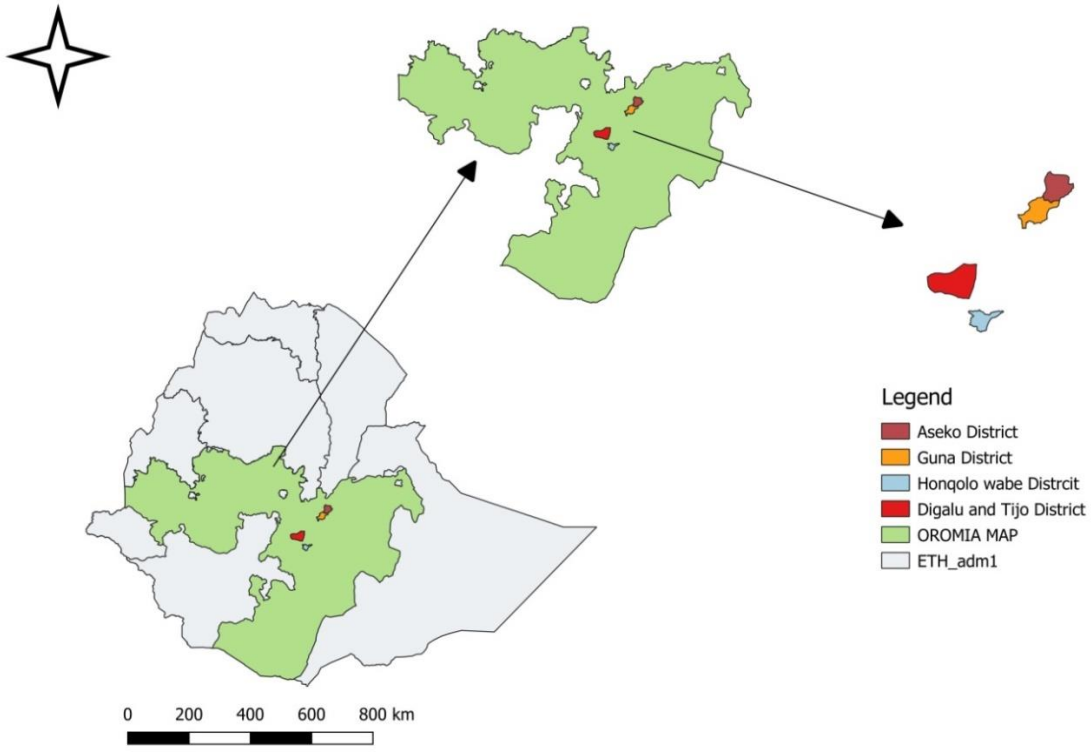

Figure 8: Map of study areas for FMD outbreak investigation
Source: Done by QGIS version 3.16

3.2. Study Population.

The study population was cattle managed under extensive, intensive, and semi-intensive management systems which comprised cross-bred and local breeds with different age groups and sex, selected from six districts of the Arsi and Bale zone. In this study, the age of animals was categorized as an adult (≥ 3 years old) and young (< 3 years old) according to Mesfinie *et al.* (2019). In present study, herd sizes were categorized into three groups: small (1–15), medium (16–30), and large herds (> 30 cattle) (Gelaye *et al.*, 2009b).

3.3. Study Design and Sampling Technique

A cross-sectional study design was implemented to estimate the seroprevalence of FMD in the study areas. Potential risk factors that could be associated with FMD in the study areas were

assessed using semi-structured retrospective questionnaire administered through interviewing comprising of important information which includes geographic information, FMD outbreak information, animal movement information and FMD control strategy in the area.

The study employs a multistage cluster sampling method. The regions, zones, districts, and peasant associations were selected purposively. From the districts, specific peasant associations were again selected based on their accessibility, whereas individual animals were randomly selected. Samples were selected within these areas based on the abundance of livestock population, their accessibility and geographical locations, as well as the owners' willingness. Each individual animal has been selected for sampling regardless of sex, age, breed, herd size, contact with small ruminants, body condition, and production type. During the FMD outbreak, a field investigation was conducted purposively at the specific site of the outbreak within the study areas, and animals with the clear clinical signs of FMDV were sampled.

3.4. Sample Size Determination

The sample size was estimated by using 95% confidence interval, 5% desired absolute precision with the following predetermined parameters via the given formula (Thrusfield, 2007).

$$n = \frac{1.96^2 . Pexp\,(1-Pexp)}{d^2}$$

Where, n = required sample size

Pexp = expected prevalence

d^2= desired absolute precision

The expected prevalence of 22.91% for foot and mouth diseases in the Arsi zone (Sulayeman *et al.,* 2018) was used to calculate the sample size. Accordingly, the total sample size was 272 heads of cattle in the Arsi zone. The expected prevalence of 21.9% for foot and mouth diseases in the Bale zone (Duguma *et al.,* 2013) was used to calculate the sample size. Accordingly, the total sample size was 263 heads of cattle in the Bale zone. Hence, the total sample size 535 cattle. However, to increase the representativeness and improve precision, a total of 779 bovine sera were collected. In addition to the serum samples, 11 tissue samples were collected from the

outbreak, and a questionnaire survey was conducted by involving 100 individuals, comprised of farmers and animal health professionals working in the livestock industry.

3.5. Ethical Clearance

Ethical clearance for this study was obtained from Addis Ababa University College of Veterinary Medicine and Agriculture, Animal Research Ethics and Review Committee. A seven-page request for an explanation of the purpose of carrying out the studies and all possible care planned to reduce animal suffering due to sampling was given to the committee (Appendix 6). After the committee evaluated the significance of this research, approval was given. Before sampling, consent was asked from the owners, and only animals from volunteer owners were sampled.

3.6. Study Methodology

3.6.1. Sample collection

For seroprevalence study, blood samples were aseptically collected using 10 mL plain vacutainer tubes from apparently healthy cattle from the jugular veins of randomly selected animals. The tubes were then labeled. The blood samples were allowed to stand overnight at room temperature to allow serum separation. The serum was then transferred into a sterile cryovial bearing the identification number, species, district, age, and sex. The sera were transported from the collection site to Asella Regional Veterinary Laboratory using an ice-box and were then kept at -20°C until analysis. Finally, at the end of sampling sera were transported in cold chain to AHI, Sebeta, and stored at -20oC. Sampling was done following procedures described in OIE, (2009).

After the identification of the animal as a suspected case, epithelial tissue samples that were un-ruptured or freshly ruptured vesicles were collected from FMD suspect cattle, usually from the tongue, buccal mucosa, or feet by using sterile forceps and scissors, and then placed in a universal bottle with viral transport media (VTM) composed of equal amounts of glycerol and 0.04-M phosphate buffered saline (PBS) solution (pH 7.2–7.6) with antibiotics (OIE, 2004). The

samples were then labeled, kept in an ice box, and transport to the AHI laboratory, Sebeta, Ethiopia. Once the samples arrived at AHI, they were then stored at -20°C until processed and laboratory investigation**.** Sampling was done following procedures described by OIE (2009).

Questionnaire survey was done by participating 100 individuals including farmers and animal health professionals working in the livestock industries. The questioner prepared based on answers to questions such as how many farmers know about FMD; how many diseases rank in the study areas relative to other diseases; the time of incidence of FMD in the area; gathering information on how farmers control diseases; knowing if foot and mouth disease is a challenge for livestock production; and the economic impact of the diseases. The questionnaire survey contained closed and open-ended questions prepared in English and translated to local language and presented in face-to-face interviews to the respondents (farmers and animal health workers).

3.6.2. Serological diagnostic tests

Sera collected from bovine species were tested for the detection of foot and mouth disease non-structural protein (FMD NSP) using competition 3ABC ELISA to identify FMD sero-positive and negative animals. A commercially available test kit (ID Screen ®, FMD NSP Competition, Louis Pasteur, Grables, France) was used. Sera sample were tested with ID Vet competitive ELISA (NSP 3ABC) coated microstate plates was used for this test.

Briefly, the test was carried out stepwise as per the manufacturer's manual. First, all the reagents were kept at room temperature and homogenized by vortexing. The test was carried out in 96-well microplates. A 100µL sample was dispensed into 96 well microplates, which was necessary to avoid incubation differences. Then 50µL of dilution buffer 18 was added to each well. 30 µL of positive control were added to, wells A1 and B1 and the same volume of negative control was also added to wells C1 and D1, while the rest of the wells were filled by 30µL of test sera. Then they were incubated at 37oC for 2 hours. After incubation, the wells were washed five (5) times, adding 300µL of wash solution immediately to avoid drying between washings. After washing, 100µL of the conjugate 1X was added to each well and incubated for 30 min at room temperature. Following incubation, the wells were washed five (5) times with 300 µL of wash

solution, and then 100 µL of substrate solution was added to each well before incubating at room temperature for 15 minutes in the dark. To stop color reaction, 100µL of stop solution was dispensed into each well. Finally, optical density (OD) readings at 450 nm were obtained using a spectrophotometer.

Validation for test

The test result was validated if:

- ✓ The mean value of negative control OD (OD_{NC}) was greater than 0.7

  $OD_{NC} > 0.7$

- ✓ The mean value of the positive control OD(OD_{PC}) is less than 30% of the OD_{NC}

  $OD_{PC}/OD_{NC} < 0.3$

Note: OD, Optical density; NC, Negative Control and PC, Positive Control

Interpretation

For each serum sample, the competition percentage was calculated (S/N %):

$S/N\ \% = OD\ sample/OD_{NC} \times 100$

Sample presented S/N %:

- ✓ Less than or equal to 50% was considered positive
- ✓ Greater than 50% were considered negative

3.6.3. Identification of FMDV serotypes by antigen detection ELISA

An antigen detection sandwich ELISA (IZSLER, Bresica, Italy) was used to determine the serotypes of the FMDV. The kit was created using carefully chosen combinations of anti-FMDV monoclonal antibodies (MAbs), which were used as coated as well as conjugated antibodies. The kit was designed for detection and typing of six FMD serotypes: O, A, C, Asia 1, SAT 1, and SAT 2. A pan-FMD test, detecting any isolates of serotypes O, A, C, Asia 1, and some of the SAT serotypes, was included in the kit to complement the specific typing and to detect FMD viruses which might have escaped binding to selected serotype-specific MAbs. The microplates were supplied with catched MAbs to detect 10 samples at a time, with one positive and one

negative control for each serotype. The controls were already incorporated into the ELISA microplate trapped by the respective catched MAbs.

The test was conducted as per the manufacturer's recommendation. Briefly, first samples were diluted ½ in diluent buffer, and 50µL/well of each sample was distributed to 8 wells of a column (a total of 80 wells of A-H rows). Then, 50µL of diluents per well were added to all wells of 11 and 12 columns (positive and negative control, respectively), and the plates were incubated at room temperature for 1 hour. After incubation, all the fluid in each well was discarded, and the plate was tapped hard to remove all the residual fluid. Then 200µL of washing solution was added and incubated for 3 minutes at room temperature, wells were emptied and the washing repeated twice (three washing cycles in total). Then all residual fluids were removed by tapping on clean absorbent paper and 50µL/well of conjugate A was added from row A to F and the same volume of conjugate B was added into row G and H. Plates were covered and incubated at room temperature for 1 hour. After incubation, discard the conjugate and wash with by fill 200µL/well four times, leaving the last one for five minutes. Then 50µL of substrate per well was added to all wells and the plates were covered and left at room temperature for 20 minutes in the dark. After 20 minutes, the reaction was stopped by adding 50uL/well of stop solution (sulfuric acid (H2SO4)). Immediately after blocking, mix the well content before reading with a spectrophotometer. The optical density (OD) of each well was measured at a 450 nm wavelength using a microplate reader.

Criteria for test validity

The positive inactivated controls were expected to give OD values greater or equal to 1.0 unit while the negative controls for serotypes O, A, C, Asia 1, and Pan-FMDV were expected to give OD values less than 0.1unit, and the negative controls for serotypes SAT1 and SAT2 were expected to give OD values less than or equal to 0.2unit. The interpretation of the result is in Appendix 4.

3.7. Assessment of the Economic Loss Associated with FMD

Foot and mouth disease causes significant economic loss in the livestock industry, but this study focused on direct economic losses such as draft power loss, milk loss on dairy farms, treatment

costs, animal mortality loss, and beef farm costs. Because it was studied by asking farmers or animal owners, using a semi-structured questionnaire format, about the consequences of the diseases and the measures taken during outbreaks. A cost determination model was used to estimate the economic loss due to FMD. Economic loss calculations were done at herd level and the average loss from individual clinically affected animals was also estimated (Jemberu, 2016).

3.7.1. Mortality loss

The mortality loss was calculated using the market value of the died animal. Thus, the economic loss due to mortality per herd was determined by taking into account the various types of animals that died and their respective market prices as follows:

$$\sum_{n}^{1} Hi = (NoDc \times mpc + NoDDc \times mpdc + NoDo \times mpo + Nomc \times mpmc)$$

Where, Hi (individual herd), n (number of herd), NoDc (Number of died calf)*mpc (market price of calf) + NoDDc (number of died dry cow*market price of dry cow) + NoDo (number of died oxen* mpo (market price of oxen corresponding with farm type) + Nomc (number of died milking cow* mpmc (price of milking cow for corresponding farm type) (Tadesse *et al.,* 2020).

3.7.2. Draft power loss

Economic losses due to draught power loss per herd were calculated as:

$$\sum_{n}^{1} Hi = AvSo \times (Adi \times adj\ rate) \times ApDppdpa$$

(Ld) Loss of draft power =AvSo (number of sick oxen in small holder farm) × Adi (number of days of illness) × adjustment factor × ApDppdpa (Price of draft power rent of an ox per day per animals). Draught power for crop production (plowing and threshing) is not required all year due to crop seasonality and cultural beliefs. So an ox can work up to 65 days per year, as an adjustment factor (65/365) (Alemayhu *et al*., 2014; Jemberu, 2016).

3.7.3. Treatment costs

Foot and mouth disease control costs are considered to consist of vaccination, diagnosis, and medication costs, as well as extra labour costs for seeking treatment for sick animals. The cost of control was mainly related to antibiotic treatment of infected animals for bacterial complications. The cost of FMD treatment was calculated based on the number of animals treated and the average price per head. The average numbers of working hours lost by the attendant/owner while seeking treatment for sick animals, as well as the average hourly wage, were calculated. The total cost of FMD treatment was calculated as:

$$\sum_{n}^{1} Hi = (\text{Number of animals treated} \times \text{Price of Treatment}) + (\text{hours Lost} \times \text{payment rate})$$

3.7.4. Economic loss in beef farms

$$\sum_{n}^{1} Hi = (NRf \times NoD \times Dcost/animal)$$

Economic loss in beef farms = NRf (number of re-fatting bulls) and Dcost/animal (daily cost per animal), and NoD (number of refating days), Hi (herd individual) and n (number of herd), with the farmer for feeding of the bull and labor cost per animal as a whole.

3.7.5. Milk loss in dairy farms

Milk loss represents the economic loss caused by milk yield, which was calculated by adding milk losses from all herds.

$$\sum_{n}^{1} (Hi = NoDc \times mL \times MPpL \times NoDo)$$

Hi (individual herd), n (number of herd), NoDc (number of diseased cow in herd), mL (milk loss per litter per day per animal), MPpL (milk price per litter) and NoDo (number of days outbreak occur/ average duration of milk loss or outbreak in the farm (days)) was used to calculate total economic loss within infected herd (Tadesse *et al.*, 2020).

3.7.6. Total economic loss

The overall economic losses per individual herd were aggregated as the sum of all losses arising from draught power loss; beef farm loss, mortality loss, treatment costs, and milk loss in dairy farms (Taddesse *et al.*, 2020).

3.8. Data Management and Analysis

Data generated from laboratory investigations was recorded and coded using a Microsoft Excel spreadsheet and analyzed using R software (version 4.0.3). Descriptive statistics were used to determine the sero-positive animals (seroprevalence) and to analyze the questionnaire data gathered. Morbidity and mortality were diagnosed at farm level and individual animal level, as well as in different districts and different production systems. The animal level morbidity was determined as the number of clinically affected animals divided by the total number of animals at risk. The herd level morbidity was determined as the number of positive herds (herds with one or more clinically affected animals) divided by the total number of herds at risk. Animal level mortality was calculated by dividing the number of animals that died during the outbreak by the total number of animals at risk, and mortality at herd level was calculated as the total number of herds where the animal died divided by the total number of herds at risk.

The seroprevalence was calculated by dividing the number of 3ABC NSP ELISA positive animals by the total number of animals tested. The true prevalence (TP) was derived from the apparent prevalence (AP) based on the sensitivity (Se) and specificity (Sp) of the diagnostic test as described by Thrusfield, (2007).

$$\text{TP} = \frac{AP + SP - 1}{Se + SP - 1}$$

In this case, the test specificity and sensitivity for the ID SCREEN 3ABC FMD NSP Competition ELISA kit were 99.5% and 91.7%, respectively (Donnet *et al.,* 2014).

The degree of association between FMD seroprevalence and categorical independent variables was assessed using an odds ratio (OR) with multivariable logistic regression analyses. Before

regression analysis, the data was checked for fulfillment of assumptions, such as multi-collinearity tests (VIF < 10). And all the tested variables did not show multi-collinearity.

A stepwise logistic regression model was used to analyze and regress those factors having a significant putative effect on the occurrence of disease based on a p-value < 0.05 as the significance threshold for entries and removals. The ROC curve revealed the goodness of fit for the model to analyse this study's finding data. Moreover, goodness of fit for the model was determined (checked) by the Hosmer and Lemeshow goodness of fit (GOF) test. This indicates the model appears fit since the GOF test p-value is greater than 0.05 and the calculated chi-square value is less than the table value. To map the geographic distribution of the disease (outbreak) and serum sampling in the study areas, QGIS version 3.16 GIS software was used. In all the analyses, the confidence level was held at 95% and P<0.05 was set for significance.

4. RESULTS

4.1. Seroprevalence of FMD

The overall seroprevalence of Foot and Mouth Disease, in the study districts of Arsi and Bale zones of Oromia Regional State of Ethiopia, using 3ABC-NSP competitive ELISA was found to be 48.65% (379/779) (95% CI: 45%-52%). The overall true prevalence adjusted for the sensitivity and specificity of the imperfect diagnostic test used was 52.79 %(95% CI: 47%-58%). The higher seroprevalence was recorded in Arsi, 59.02% (95% CI: 54%- 64%) as compared to 41.1% (95% CI: 37%- 46%) in Bale (Table 4).

Table 4: Seroprevalence of FMD in cattle of Arsi and Bale zones

Zones	Number of examined	Number of negative	Number of positive	Seroprevalence (%)	95% CI
Arsi	327	134	193	59.02	54- 64
Bale	452	266	186	41.1	37- 46
Total	**779**	**400**	**379**	**48.65**	**45-52**

The seroprevalence results of FMD at district level are given in Table 5. The highest sero-prevalence (69.33%) was recorded in Asako, followed by Hetosa (65.47%), Munesa (58.33%), Lemu and Bilbilo (44.04%), Agarfa (43.89%) and Sinana (38.52%) districts. The difference among districts was statistically significant (χ^2= 37.70, p = 0.0000).

Table 5: Seroprevalence of FMD in cattle at district level of study zones of Arsi and Bale

Districts	Number of examined	Number of positive	Seroprevalence (%)	95% CI	χ2	p-value
Munesa	84	49	58.33	48- 69	37.70	0.0000
Asako	75	52	69.33	59- 80		
Hetosa	84	55	65.47	55- 76		
Lemu & Bilbilo	84	37	44.04	33- 55		
Sinana	231	89	38.52	32- 45		
Agarfa	221	97	43.89	37- 50		
Total	**779**	**379**	**48.65**	**45-52**		

4.1.1. Animal-related risk factors associated with FMD seropositivity

The seroprevalence of FMD was found to be higher in adult cattle than young cattle with prevalence of 57.51% and 29.38%, respectively and this difference was statistically significant ($\chi2 = 37.382$; $p = 0.000$). The seroprevalence estimate for male and female cattle was 50.00% and 47.24%, respectively (Table 6). The higher seroprevalence was recorded in males than females; however, the difference was not statistically significant ($p > 0.05$). The Seroprevalence of FMD was higher in cross breeds (57.51%) than local breed (41.57%) and the difference was statistically significant ($\chi2 = 18.937$; $p = 0.000$). Regarding the body condition, the seroprevalence in medium, poor, and good body conditioned animals, were found to be 46.28%, 49.42%, and 52.90%, respectively; however, the difference was not statistically significant ($p > 0.05$) (Table 6).

4.1.2. Environment related risk factors for FMD seroprevalence

The seroprevalence of 44.03%, 48.90%, and 59.39% was recorded in small, medium, and large herd size animals, respectively (Table 6). The difference in herd size was statistically significant ($\chi2 = 8.9446$; $p = 0.011245$). Higher seroprevalence was recorded in cattle that were kept together with small ruminants than in herds of cattle without, and the prevalence was significantly varied ($\chi2 = 14.483$; $p = 0.0007$) (Table 6). Higher seroprevalence was recorded in extensive management systems (50.42%) as compared to intensive management systems (31.57%) and semi-intensive management systems (34.14%) and this was found to be statistically significant ($\chi2 = 8.7715$; $p=0.01245$) (Table 6).

Table 6: Overall FMD seroprevalence and associated putative risk factors

Risk factors	No of examined	No of positive	Prevalence (%)	95% CI	$\chi 2$	P-value
Sex						
Female	381	180	47.24	42- 52	0.48662	0.4854
Male	398	199	50.00	45- 55		
Age						
Young	194	57	29.38	23- 36	37.382	0.0000
Adult	585	322	55.04	51- 59		
Breed						
Cross	346	199	57.51	52- 63	18.937	0.0000
Local	433	180	41.57	37- 46		
Herd size						
Small	327	144	44.03	39- 49	8.9446	0.01142
Medium	319	156	48.90	43- 54		
Large	133	79	59.39	51- 68		
Management system						
Extensive	700	353	50.42	47- 54	8.7715	0.01245
Intensive	38	12	31.57	16- 47		
Semi-intensive	41	14	34.14	19- 49		
Animal composition						
With small ruminants	180	108	60.00	53- 67	11.483	0.0007
Only cattle	599	271	45.24	41- 49		
Body conditions						
Good	155	82	52.90	45- 61	2.007	0.3678
Medium	363	168	46.28	41- 51		
Poor	261	129	49.42	43-56		

4.1.3. Univariable logistic regression

The univariable logistic regression analysis showed that cattle in Munesa district had 1.789 times the odds of being infected with FMD than cattle in Agarfa. Similarly, cattle reared in Hetosa and Asako had significantly (P< 0.05), 2.424 and 2.89 odds of being infected compared to those reared in Agarfa, respectively. The effect of breed indicates that cross-bred cattle were 1.902 more likely to be infected by FMD than local breeds (OR = 1.902, 95% CI: 1.4304-2.536), with a significant difference (P = 0.0000). Regarding the management system, cattle that were kept under an extensive management system were 2.204 times more prone to FMD infection than those managed intensively (OR = 2.204, 95% CI: 1.118-4.593, p-value = 0.000) (Table 7).

The risk of FMD occurrence was higher (odds ratio (OR) = 1.815) in herds of cattle and small ruminant than herds of cattle that kept without small ruminants (OR = 1.815, 95% CI: 1.296-2.554, p-value = 0.00056). Large herd size cattle had 1.859 times higher odds of FMD infection than small herd size cattle (OR = 1.859, 95% CI: 1.2378-2.809, p-value = 0.00297). Animals greater than 3 years old (adults) were found to be 2.94 times more FMD sero-positive than young animals (OR = 2.94, 95% CI: 2.085–4.1967, p-value = 0.0000). In this analysis, young, local breed, female, small herd size, Agarfa district, intensive management system, animal composition only cattle and body condition good were used as a reference (Table 7 and 8).

Table 7: Univariable logistic regression by different risk factors

Risk factors	Categories	OR	95% CI	P-value
Districts	Agarfa	Ref	Ref	Ref
	Munesa	1.7896907	1.0794- 2.9923	0.024915
	Lemu and bilbilo	1.00636	0.6046 - 1.667	0.980409
	Hetosa	2.4244579	1.447 - 4.126	0.000891
	Asako	2.8901838	1.67 - 5.123	0.000194
	Sinana	0.8012197	0.55 - 1.165	0.247025
Sex	Female	Ref	Ref	Ref
	Male	1.1166667	0.843 - 1.479	0.442
Age	Young	Ref	Ref	Ref
	Adult	2.9426990	2.085 - 4.1967	0.0000
Breed	Local	Ref	Ref	Ref
	Cross	1.9027589	1.4304 - 2.536	0.0000
Herd size	Small	Ref	Ref	Ref
	Medium	1.2162577	0.8926 - 1.658	0.21522
	Large	1.8591821	1.2378 - 2.809	0.00297
Mgt system	Intensive	Ref	Ref	Ref
	Extensive	2.2041306	1.118 - 4.5935	0.0269
	Semi-intensive	1.1234	0.438 - 2.909	0.8083
Body condition	Good	Ref	Ref	Ref
	Medium	0.7669794	0.525 - 1.117	0.168
	Poor	0.8700111	0.5837 - 1.29	0.493
Animal composition	Only cattle	Ref	Ref	Ref
	With small ruminants	1.8154982	1.3 - 2.554	0.000561

4.1.4. Multivariable logistic regression

The multivariable logistic regression model of risk factors analysis indicated that districts, age, breed, herd size, and animal composition had a significant association with the seroprevalence of FMD and hence are independent predictors ($P < 0.05$)(Table 8).

Table 8: Multivariable logistic regression by different risk factors

Risk factors	**Categories**	**OR**	**95% CI**	**P-value**
Districts	Agarfa	Ref	Ref	Ref
	Munesa	1.6800262	0.9859- 2.885	0.057718
	Lemu and bilbilo	0.9514413	0.555- 1.622	0.855224
	Hetosa	2.5087676	1.441- 4.440	0.001321
	Asako	2.0488437	1.148- 3.733	0.016748
	Sinana	0.7106207	0.475- 1.058	0.093784
Age	Young	Ref	Ref	Ref
	Adult	1.7562568	1.677- 3.528	0.0000
Breed	Local	Ref	Ref	Ref
	Cross	1.9027589	1.296- 2.383	0.000286
Herd size	Small	Ref	Ref	Ref
	Medium	1.2943651	0.9254- 1.8135	0.132457
	Large	1.5770507	1.0106- 2.471	0.045482
Mgt system	Intensive	Ref	Ref	Ref
	Extensive	2.0554642	0.9796- 4.5349	0.063342
	Semi-intensive	1.0148192	0.370- 2.8074	0.8083
Animal composition	Only cattle	Ref	Ref	Ref
	With small ruminants	1.7376921	1.195- 2.538	0.003995

Ref, Reference; OR, Odd ratio; CI, Confidence Interval

4.2. Foot and mouth disease Virus Serotyping by Antigen Detection ELISA

Out of the eleven (11) tissue samples subjected for serotyping by the antigen detection ELISA, nine (9) samples were found positive and three types of FMDV serotypes were detected. Of these nine (9) positive samples, 7 (77.77%) were serotype A, and 2 (22.22%) were SAT 1 and SAT 2. Of the nine positive samples, seven were from Digalu &Tijo and Asako districts, and two samples were from Honqolo Wabe district. The Digalu &Tijo and Asako samples were typed as serotype A and SAT -2, whereas the sample from Honqolo Wabe was serotype A and SAT1, as shown in Table 9 below.

Table 9: Serotypes of FMDV identified in the study areas

Region	**District**	**Peasant association**	**No. of sample collected**	**No. of positive**	**Identified serotypes**
Oromia	Honqolo Wabe	Macitu laman	1	1	SAT1
		Taji walkite	1	1	A
	Asako	Dima Asako	3	3	A
		Chefa Ifa	2	2	A
	Digalu&Tijo	Tulu kite	1	1	SAT2
		Lolee hofi	1	1	A
	Guna	Dima Badosa	1	-	-
		Dima Wocale	1	-	-
Total			**11**	**9(81.81%)**	

4.3. Questionnaire Survey

4.3.1. General information on the demography of interviewers

According to the study, four districts in the Arsi zone participated in interviews. The educational background of most participants was illiterate, and some could read and write, but very few

participants, around 12%, were higher educated. All farmers, regardless of educational level, had nearly identical ideas about the diseases.

4.3.2. Farmer's knowledge about foot and mouth diseases

In the study area, foot and mouth diseases are the fourth (4) ranked diseases causing severe loss in livestock industries. Most respondents said that FMDV is called by its local name "Maasa" in Arsi zones. Among the participants, the majority (83%) replied frequent occurrences of FMD outbreaks in their farms/herds, whereas 17% responded that no outbreak had occurred on their farm/herds.

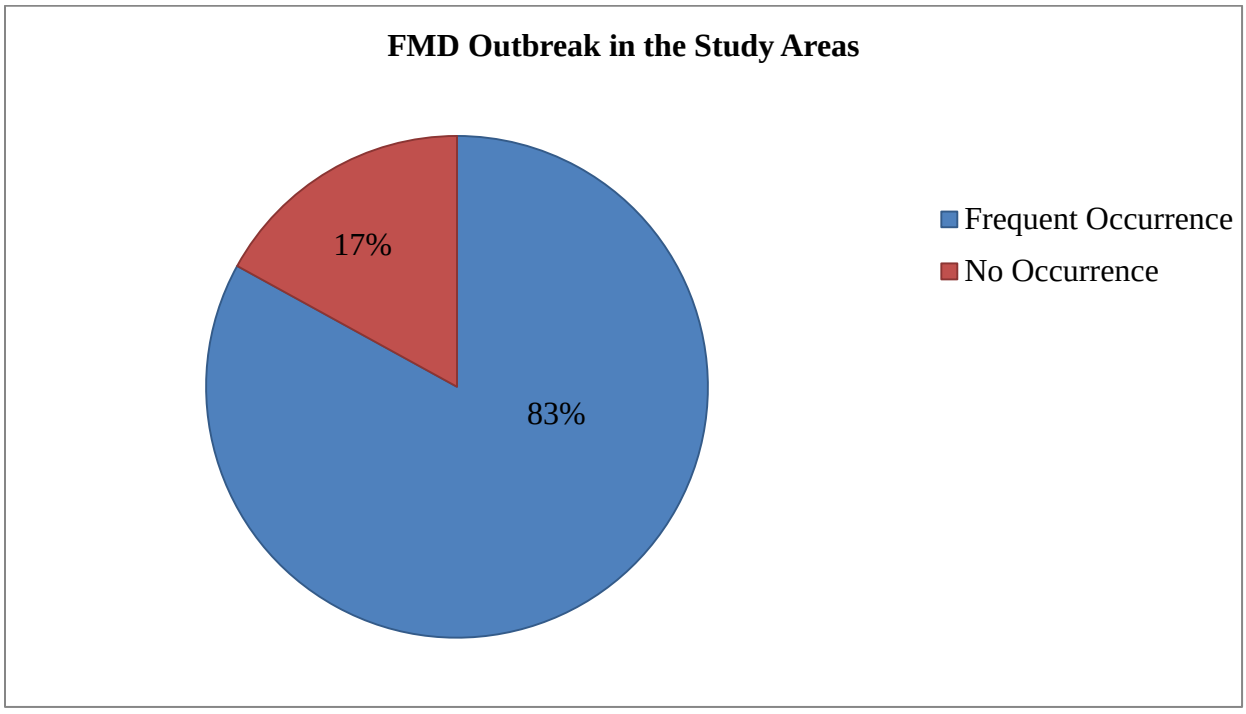

Figure 9: Status of FMD in the study areas according to the respondants

The majority of the respondents gave reference factors for the occurrence of the disease in their farms/herds and surroundings. These include river flow across their area (41.6%); a livestock market nearby (39.4%); communal water points (28%); movement of animals in and out of the farm (53%); and sharing pasture with other neighboring animals (52.8%).

4.3.3. Foot and mouth disease control and prevention strategy applied in the study areas

According to the respondents, 52.9% replied that they follow and treat their animals to manage the spread of the disease in their farms/herds, while the other 19.5%, 18.5%, and 5% of respondents follow vaccination program, movement restriction and all three together approaches, respectively to control and prevent disease incidence and/or spread. On the contrary, 4.1% of respondents use no intervention to control the disease (Figure 10).

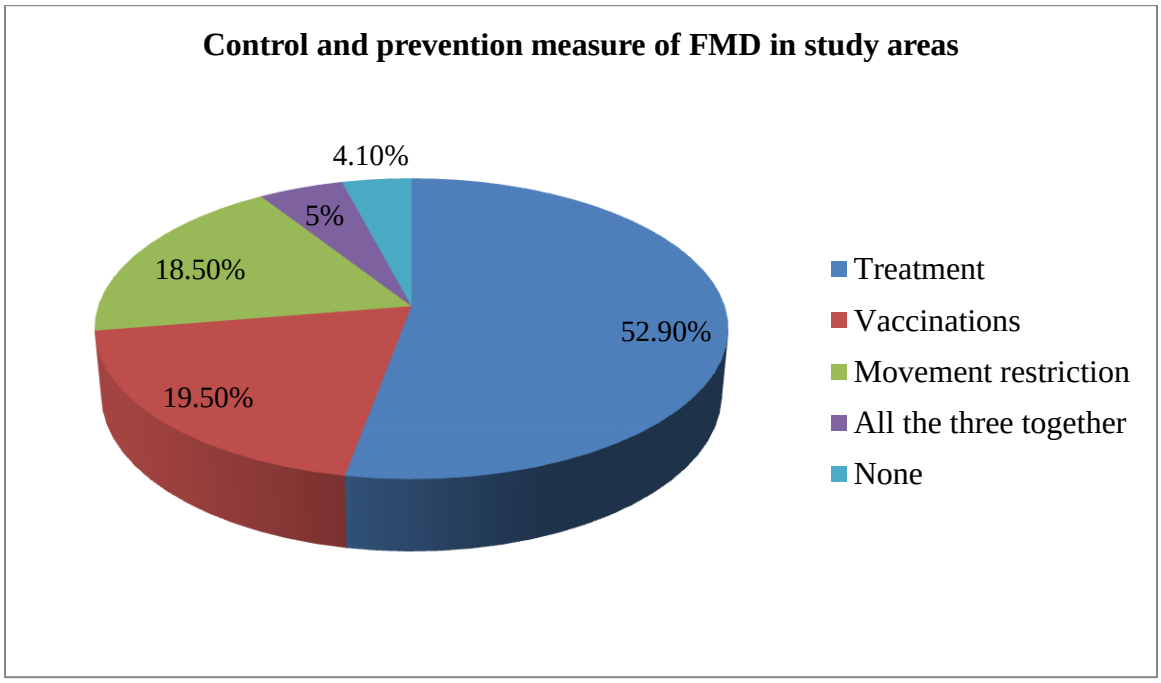

Figure 10: Control and prevention measure of FMD in the study areas

4.3.4. Morbidity and Mortality of FMD

By district level, a higher morbidity was observed in Honqolo Wabe with 36.17% and low morbidity was observed in Guna district with a morbidity rate of 11.86%. In the production system, higher morbidity was observed in beef farms with a morbidity rate of 32.69% and a lower morbidity rate was observed in small holders with a rate of 26.13%. A Mortality rate was also observed both at a production and district level. During the observed period, district higher mortality was observed in Asako with a mortality rate of 7.8%, and lower mortality was

observed in Guna with a mortality rate of 0 %. When comparing the mortality rate with farm type, higher mortality was observed in beef farms with 3.8% and lower mortality was observed in dairy farm with 0%. The total number of diseased animals was 99. Foot and mouth disease mortality and morbidity by animal level were 3% and 27.34%, respectively. The morbidity and mortality rates by herd level were 29% and 27%, respectively.

Table 10: Summary of Questionnaire Survey

Risk factors	**Total animals**	**No of diseased**	**No of herd**	**No of +ve herd**	**Morbidity**	**Mortality**
Districts						
Honqolo Wabe	47	17	16	6	36.17%	2(4.2%)
Asako	64	21	10	3	32.81%	5(7.8%)
Digalu &Tijo	94	24	23	5	25.53%	3(3.19%)
Guna	59	7	13	2	11.86%	-
Farm type						
Small holder	264	69	62	16	26.13%	10(3.7%)
Beef farm	52	17	27	6	32.69%	1(3.8%)
Dairy farm	46	13	11	7	28.26%	-
Total	**362**	**99**	**100**	**29**	**27.34%**	**11(3.03)**

No, number; +ve, positive

4.3.5. Economic losses of foot and mouth disease outbreaks

Mortality loss: The total number of dry cows, milking cows, calves, and oxen that died during the study period was 1, 2, 6, and 2, respectively, and the total economic loss during the examination period was 191000ETB (3745.09USD). In general, it was around 6586.20ETB (129.0USD) per affected herd and around 1910ETB (37.45USD) within any herd. It was 1929.291ETB (37.82USD) for each affected animal and 527.62ETB (10.34USD) for any individual animal.

Draft power loss: The total cost loss in birr due to draft power was 4005 ETB (78.529USD). The cost per affected herd was 138.10ETB (2.70USD), while the cost per affected individual animal was 40.45ETB (0.79USD). The cost loss per herd and loss per individual animal in any herd were approximately 40.05ETB (0.78USD) and 11.06ETB (0.2169USD), respectively.

Treatment cost: There are numerous reasons for loss in the control and prevention of foot and mouth disease, but in this study, the cost of treatment and extra labor costs for seeking treatment for sick animals were considered. In addition to the medical costs, the owner's labor costs are calculated. The cost of treatment for individual animals was 180 ETB (3.529USD). The owner's labor rate was 100ETB (1.96USD) per 12 hours. The total cost during observation was 13420ET B (263.137USD).

Beef farm economic loss: Economic losses estimated as a result of bulls retained from the market were the total number of bulls retained in each beef farm due to FMD time's refatting cost of per day per animal including owner labor. The beef farm had 52 bulls, 17 of which were diseased and required refatting days (to convalesce and again fatten). In the beef farm under consideration, the total economic loss was 107100ETB (2100$USA). The total economic loss per affected herd was calculated by dividing by number of infected herds, 3693.1ETB (72.41$USA) and per any herd 1071ETB (21$USA). The cost per individual animal level with infected animal was 1081.81(21.21$USA) and 295.85ETB (5.82$USA) for any animal.

Economic loss with milk production: Total loss calculated by summation of the whole milk loss during observation was 58500ETB (1147.05USD). In general, it was around 2017.24ETB (39.55USD) milk loss per affected herd and around 585ETB (11.47USD) milk loss within any herd. It was 590.9ETB (11.58USD) milk loss for each affected animal and 161.6ETB (3.16USD) for any individual animal.

Total economic loss: During the study period, the total economic loss was 374025ETB (7333.82USD) as a result of mortality, draft power, beef farm costs, milk loss in dairy farm and treatment costs. The total loss per affected herd was 12897.41 ETB (252.89USD), with a loss of 3778 ETB (74.07USD) per individual affected animal. The total economic loss due to foot and

mouth disease was 3740.25ETB (73.33.86USD) per herd, and the loss per individual animal was 1033.21ETB (20.25USD). For each calculation (1USD=51ETB; data from the Commercial Bank of Ethiopia).

Table 11: Summary of estimated economic loss of foot and mouth disease in study areas

Cost categories	Estimated economic loss
Milk loss	
Milk loss per affected herd	2017.24ETB (39.55USD)
Milk loss per affected individual animal	590.9ETB (11.58USD)
Milk loss any per herd	585ETB (11.47USD)
Milk loss per individual animal with any herd	161.6ETB (3.16USD)
Total milk loss	**58500ETB (1147.05USD)**
Draft power loss	
Draft power loss per affected herd	138.10ETB (2.70USD)
Draft power loss per affected individual animal	40.45ETB (0.79USD)
Draft power loss any per herd	40.05ETB (0.78USD)
Draft power loss per individual animal with any herd	11.06ETB (0.2169USD)
Total draft power loss	**4005 ETB (78.529USD)**
Mortality loss	
Mortality loss per affected herd	6586.20ETB (129.0USD)
Mortality loss per affected individual animal	1929.291ETB (37.82USD)
Mortality loss any per herd	1910ETB (37.45USD)
Mortality loss per individual animal with any herd	527.62ETB (10.34USD)
Total mortality loss	**191000ETB (3745.09USD)**
Beef farm loss	
Beef farm loss per affected herd	3693.1ETB (72.41USD)
Beef farm loss per affected individual animal	1081.81(21.21USD)
Beef farm loss any per herd	1071ETB (21USD)
Beef farm loss per individual animal with any herd	295.85ETB (5.82USD)
Total beef farm loss	**107100ETB (2100USD)**
Treatment cost	

Treatment cost per affected herd	462.75(9.07USD)
Treatment cost per affected individual animal	135.5(2.65USD)
Treatment cost per any per herd	134.2(2.63USD)
Treatment cost per individual animal with any herd	37.07(0.72USD)
Total treatment cost	**13420ETB (263.137USD).**
The overall economic loss	**374025ETB (7333.82USD)**

5. DISCUSSION

Foot and mouth disease is considered to remain endemic with widespread occurrences, causing outbreaks every year in Ethiopia (Ayelet *et al.*, 2012). The situation is complicated by the fact that the virus causes an asymptomatic persistence infection in ruminants and continuously evolves. Despite its occurrence, there is still an inadequate epidemiological understanding and clarification of the disease. Thus, the frequent disease reporting requires monitoring and an update data of the current FMD epidemiological status as well as economic crisis in the country. In this study, an attempt was made to determine the seroprevalence of FMD in apparently healthy cattle, to identify risk factors and circulating serotypes, and to assess the economic impact of the diseases on cattle.

The results of the present study indicated that sero-positivity for FMD in all study areas with an overall sero-prevalence of 48.65% in cattle, indicating the spread of FMD virus throughout the study areas. Similar studies conducted at different times and locations revealed varying prevalences. The current finding was comparable to the earlier reported prevalence of 49.2%, documented by Urge *et al.* (2020). The higher seroprevalence reported in the current study is due to failure to periodically vaccinate animals, improper management practices, and contact of animals with livestock from other neighborhoods where they can get infections from disease affected animals. In addition, in 2021, a year before the current study, there was an outbreak in the Arsi zone that might have increased the number of affected animals. These could be possible reasons for the high prevalence value.

Compared to the present finding, lower prevalences of 19.8% (Dubie and Negash, 2021), 21.57 (Duguma *et al.*, 2013), 24.6% (Mekonen *et al.*, 2011), 24.22% (Sulayeman *et al.*, 2018), 40.5% (Ahmed *et al.*, 2020) and 42.7% (Melkamsew, 2018) were reported from the Afar region, Bale zone, Borana and Guji zones, Central Ethiopia, West Shewa, and Borana zone, respectively. On the other hand, higher prevalence than the present study was reported with a prevalence of 53.6% in Borena, Ethiopia (Mekonen *et al.*, 2011), 72.1% in central Ethiopia (Awel *et al.*, 2021), 52.5% in Kenya (Kibore *et al.*, 2013), 61% in Uganda (Mwiine *et al.*, 2010), 77% in Uganda (Namatovu *et al.*, 2015) and 72.62% in Nigeria (Lazarus *et al.*, 2012) registered much higher

sero-prevalence in cattle. The observed prevalence variation may be due to differences in individual animal breeds, immune status, interaction of cattle with other animals such as small ruminants, unequal distribution of vaccines across the country, the method of sampling based on the existence of a recent outbreak, type of diagnostic tests used, geographic variation and timing of infection, sample size, and variations in the production systems among the study areas.

In the present study, statistically significant variation in FMD seropositivity was observed among age categories, with a higher prevalence in adults (55.04%) than in young animals (29.38%). Thus, adult cattle were 1.75 times more likely to contract FMD than young cattle. This statistically significant higher seroprevalence of FMDV in adult animals than in young cattle in this current study finding was in close agreement with previous study reports by Dubie and Negash (2021), who reported that adult cattle were 2.97 times more likely to contract the disease than young cattle in the Afar region.

The current study is in agreement with the previous reports of Rufael *et al.* (2008), Megersa *et al*. (2009), Molla *et al*. (2010), Negussie *et al.* (2011), Yahya *et al.* (2013), Mohamoud *et al.* (2011), and Kibore *et al.* (2013), which stated that sero-positivity increases with age. According to Murphy *et al.* (1999), as age increases, the chance of exposure to the disease increases. This might be because adults have acquired the infection through repeated exposure to the different serotypes of the virus and could get access to mix with other herds at market places and communal pasture land. These conditions favor disease dissemination and, as a result, disease transmission to adult cattle. The low sero-prevalence in the young may be due to their husbandry system that kept them in separation from adults so that exposure to infection would be minimized.

In this study, a significantly higher FMD seroprevalence was recorded in cross breeds (57.51%) than in local breeds (41.57%). Similarly, higher FMD seroprevalence in cross breed than in local cattle was reported by Sulayeman *et al.* (2018), Urge *et al.* (2020) and Ahmed *et al*. (2020). The relatively higher percentages of FMD seropositivity in cross-bred cattle might be associated with the genetic difference among cattle breeds. These indicated that cross-breeds appeared more susceptible to the FMD viruses endemic to Ethiopia. The direct impact of FMD on livestock in

sub-Saharan Africa depends on the breeds of animals used (Hunter, 1998). Quinn *et al.* (2005) also reported that FMD is more severe in European breeds of cattle than in other breeds. In contrast, Knowles and Samuel (2003) and Sarker *et al.* (2011) reported that breeding was associated with FMD outbreaks, with local breeds being the most affected. This might be due to poor management practices given to the local as compared to other breeds.

In the current study, a significantly higher FMD seroprevalence was recorded in cattle kept with small ruminants (60%) than in those kept alone (45%). Cattle kept with small ruminants had higher odds (1.81 times) of infection than those kept alone. This report corresponds with the studies of Sulayeman *et al*. (2018), Ahmed *et al.* (2020), Beyene *et al.* (2015), Gelaye *et al.* (2009), and Tefera (2010). Similar studies in South America (Barnett and Cox, 1999), where communal farming of cattle, sheep, and goats occurs, identified a high prevalence of antibodies against the virus infection associated with sheep and goats in the absence of clinical signs. This suggests that small ruminants have an important role in the epidemiology of FMD in cattle.

In the present study finding, seroprevalence of FMD was also significantly affected by herd size, which means seroprevalence of antibodies against FMDV increased with increasing herd size. There was a higher seroprevalence on large herd size than small and medium herd size, at 59%, 44.3 %and 44.03%, respectively. In our study results, those animals from large herd size and medium herd size were 1.85 and 1.21 times more likely to develop the disease as compared to those animals from small herd size, respectively. This finding was in agreement with Suleiman *et al.* (2018); Dubie and Negash (2021); Gelaye *et al.* (2009); Bayissa *et al.* (2011), who reported that there is a positive relationship between FMD seroprevalence and herd size. This direct association might be an indication of the contagious nature of the disease and mode of transmission, which is attributed to the crowding of animals caused by a large herd size that can facilitate frequency of direct contact and hence enhance chances of transmission.

The foot and mouth disease seroprevalence was nearly the same in both sex categories, with 47.24% and 50.0% in females and males, respectively. This finding is consistent with previous reports from different parts of Ethiopia, where sex appeared not to have a significant effect on FMD seropositivity (Megersa *et al.,* 2009; Gelaye *et al.,* 2009; Awel *et al.,* 2021). On the

contrary, Mazengia *et al.* (2010) in their study on FMDV occurrence among dairy cattle in the Northwest part of Ethiopia found that female cattle had a higher rate of infection than male cattle. The observed variation in FMD seroprevalence between the sexes of cattle may be related to the unproportionally allocated sample size, in which the sample size of female animals was higher than male animals. The current study confirmed that both sexes have equal chance of getting infection by FMD.

In this study, no significant differences in FMD seroprevalence were observed between the management systems. However, a higher prevalence was recorded in extensive management systems (50.42) as compared to intensive management systems (31.57%) and semi-intensive management systems (34.98%). This finding was consistent with the previous studies by Woldemariam *et al.* (2022), which stated that the management system did not have a significant effect on the seropositivity of foot and mouth diseases. The higher seroprevalence of foot and mouth disease was observed in good body conditions (52.90%) as compared to poor body conditions (49.42%) and medium body condition animals (46.28%). but the difference was not statistically significant.

This study showed the level of prevalence of FMD had significant variations between districts; the highest being from Asako (69.33%), followed by Hetosa (65.47%), Munesa (58.33%), Lemu and Bilbilo (44.04%), Agarfa (43.89%), and Sinana (38.52%). In Asako, there is a high population of small ruminants and there is common practice of grazing land and watering points when compared to Sinana. This difference among districts could be explained by the fact that the variation in a production system, the presence of an active outbreak, and variation in the agroecology of the areas could result in a significant difference in prevalence.

The overall serotyping results of the current findings disclosed that 9 (81.81%) samples were positive for FMD virus and three types of serotypes were identified. Out of 9 positive samples serotyped by sandwich ELISA, 7 (77.77%) were serotype A and 2 (22.22%) were SAT 1 and SAT 2. Serotype A was isolated from the samples collected from Asako, Honqolo wabe, and Digalu &Tijo districts of the Arsi zone. Most likely, the viruses were responsible for the occurrence of the investigated outbreak in these areas. Previously, serotype A was reported from

bovine samples collected from Hadiya and Yabello areas (Ayelet *et al.,* 2013), Sinana and Yabello areas (Negussie *et al.,* 2013), Meki districts of Oromia Regional State (Metages, 2018) and Konso areas (Alemu, 2014); similarly, from bovine and swine samples obtained from different outbreak areas of Ethiopia (Gelaye *et al.,* 2007). In addition, Sulayeman *et al.* (2018) also reported serotype A from Guna district in the Arsi zone.

Serotype SAT 2 FMD viruses were identified in cattle found in the Digalu and Tijo district of the Arsi zone of Oromiya region.This result was corroborated with earlier studies by Sulayeman *et a l.* (2018), Beksisa (2017), Ayelet *et al.* (2009), Tefera (2010) and Rufael *et al.* (2008), who reported serotype SAT 2 virus in the lode hetosa district of the Arsi zone and Adama, Mulo district of North Shoa zones, Benishangul-Gumuz, Gambella, and Borana zone, respectively. Serotype SAT 2 FMDV has also been previously reported from many sub-Saharan African countries (Bastos and Sangare, 2001; Sangare, 2005; Sahle *et al.,* 2007), suggesting the endemicity of the serotype in these countries. On the other hand, the serotype SAT1 FMD virus was isolated in samples collected from Honqolo Wabe district, Arsi zone, Oromiya region. This observation was consistent with previous studies (Beksisa, 2017; Bayush, 2020; and Betelihem, 2021) who reported the presence of serotype SAT 1 in the kimbibit district and Adea berga districts, Woliso, and Guzamn districts of Ahmara regional state, respectively.

In this study, risk factors for FMD were assessed through a questionnaire survey, and it revealed that the disease was endemic in the study areas, causing frequent outbreaks and resulting in the deaths of animals. According to the results of the questionnaire, communal grazing places and watering points, different animals in close contact, herding animals close to rivers and flooded areas, presence of nearby market places, and failure to vaccinate animals could be the possible factors that contributed to the high disease transmission and occurrence. This observation was consistent with previous studies (Woldemariyam *et al.,* 2022; Sahle *et al.,* 2004; Abbas *et al.,* 2014; Admassu, 2005; Dukpa *et al.,* 2011), who stated that factors such as continuous contact with susceptible animals, intermingling of different herds, and sharing of grazing and watering points with the high number of susceptible animals contribute to the occurrence of FMD outbreaks.

The 29% herd level and 27.34% animal level morbidity in the current study are lower than the 85% herd level and 74% animal level morbidity reported by Jemberu *et al.* (2014). The lower herd level morbidity might be due to the limited movement of animals during the current outbreak period. The current outbreak occurred during the cropping season in which mixing of herds from different kebeles is minimal, and this might have reduced the herd level morbidity. The background immunity from previous outbreaks (no vaccination was practiced in both areas) may also be a factor for this difference. On the other hand, the animal level morbidity in the current study is higher than the 19.6% morbidity reported by Negussie *et al.* (2011) in Ethiopia. Similarly, herd level morbidity in beef farms, dairy farms, and small-holder farms was 22.22%, 63.63%, and 25.8%, respectively, in this study.

The current overall economic losses of 374025ETB (7333.82 USD) were lower than the 555321ETB (13221.928 USD) economic losses as a result of milk loss, mortality loss, draft power loss, beef farm cost, and treatment cost reported by Betehilem (2021) in the Ahmara regional state of Ethiopia. In this study, the economic losses per affected herd of 12897.41 ETB (252.89 USD), 3778 ETB (74.07 USD) per individual affected animal, 3740.25ETB (73.33 USD) per herd, and 1033.2ETB (20.25 USD) per individual animal were lower than the economic loss per affected herd of 20567.44ETB (489.69 USD), 5666.54ETB (134.91 USD) per individual animal, 5553.21ETB (132.21 USD) per herd, and 1124.13ETB (26.76 USD) per individual animal reported by Betelihem (2021). This economic loss was only a visible production loss and an FMD treatment cost. In this study, only the cost loss due to FMD in the study area was determined.

6. CONCLUSION AND RECOMMENDATIONS

The seroprevalence of foot and mouth disease was found to be high in the study districts of Arsi and Bale zones of Oromiya Regional State of Ethiopia. There was a significant association of the seroprevalence of the disease with risk factors of districts, age, breed, herd size, and animal composition. Additionally, factors such as the presence of river flow across their area, nearby livestock market, sharing of communal grazing, and water points were found to contribute to the occurrence of FMD outbreaks. In this study, serotypes A, SAT 1, and SAT 2 were identified, with the dominant serotype being serotype A. In the current study, the total economic loss associated with FMD was found to be high, with a huge economic loss. Generally, the current study ascertained and explained the continuity of FMD's introduction and the endemic situation of the disease with widely distributed and identified circulating serotypes in the study areas and very high economic loss.

Therefore, based on the current findings, the following recommendations are forwarded:

- ✓ Great attention should be given to this economically important disease by creating awareness with regard to the clinical and economic consequences of FMD should be given to livestock owners in different parts of the country.
- ✓ An extensive regular surveillance should be conducted to understand the status and trends of the disease, identifying serotypes, and molecular characterization of FMDV for a possible development of polyvalent vaccine.
- ✓ Detailed investigation particularly works on cost-benefit analysis by including all types of economic loss and all infected population groups for a better understanding of the impact of the diseases on livestock production.
- ✓ More attention should be given to controlling FMD through regular vaccination and animal movement restrictions prior to outbreaks.

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8. APPENDICES

Appendix 1: Questionnaire Sheet Format

Instructions: Please Tick ✓ in the appropriate box ☐ and fill-in the blank spaces.

A. General information

1. Age ☐ Young ☐ Adult; Sex ☐ Female ☐ Male

2. Zone_____ District_____ Peasant association _____

3. Educational Status: ☐ No, ☐ primary school, ☐ Secondary school, ☐ High school and ☐ University Bachelor or higher

4. Occupation: ☐ Farmer, ☐ Veterinarian, ☐ student, ☐ herdsmen, ☐ others

B. Geographic location information

5. Please describe the geographic location in your village?

☐ Lowland, ☐ highland, ☐ flood, ☐ wetland, ☐ others

6. Is there any livestock market near or in your village/farm/herd?

☐ Yes, ☐ No, ☐ I don' know

7. List down at least four most common/priority livestock diseases in your area?

a._____

b._____

c._____

d._____

C. Foot-and-mouth disease outbreaks information

8. Do you know FMD? ☐ Yes, ☐ No

9. If your answer is yes in question 8, what clinical signs you observe

☐Salivation, ☐ foot lesions, ☐ lameness, ☐ reduced appetite, ☐others

10. Is there a history of FMD outbreak in your village? ☐ Yes, ☐ No

11. If yes in Question number 10, how could you control spread and transmission in livestock population in your area?

12. Is there other animal disease in your farm before or after FMD outbreak? ☐ Yes, ☐ No

13. If yes, what was the sign and symptom?

14. Please indicate the date of last FMD outbreaks in your village/farm/herd?

☐ ongoing ☐ a month ago, ☐ 2 month ago, ☐ six months ago, ☐ a year ago

15. How many FMD outbreaks per year in your village?

☐ One outbreak, ☐ two outbreak, ☐ three outbreak, ☐ Many more

16. Total number of animal's suffered in foot and mouth diseases outbreak in your Farm/herd?

17. Number of calf suffered with this outbreak?

18. Number of dry cows suffered with this outbreak?

19. Number of fattening bull suffered with this outbreak?

20. Number of oxen suffered with this outbreak?

21. Number of milking cow suffered with this outbreak?

22. How many animals died from this outbreak on your farm/herd?

Calf_____, Milking cow_____, dry cow_____, oxen_____

23. Cost of animals before outbreak?

Calf_____, Milking cow_____, dry cow_____, oxen_____

24. Cost of animals after outbreak?

Calf_____, Milking cow_____, dry cow_____, oxen_____

25. How long did the affected Oxen stay out of work during the illness? _____

26. The daily renting price of an Ox_____

27. What is the daily cost of laborer in your locality? _____

28. What is the cost of milk per litter in your locality/nearby town? _____

29. Average daily milk production before the onset of outbreak ---------- (liter/day)

30. Average daily milk yield production during the FMD illness period ----- (liter/day)

31. How long did the affected milking cow stay out of milking during the illness -------?

32. Average price of milk per litter in your area?

33. Cost for additional feeding and other facilities of refatting bull?

34.Is there retained animal from market? ☐ Yes, ☐ No

35. If yes in Question number 24, how many of them retained?

D. Animal movement information

36. How can you rate the movement of your cattle (or the animals on this farm)?

☐ Free movement, ☐ semi restricted, ☐ restricted

37. Where is your cattle point of watering?

☐At home, ☐communal, ☐Other, identify ……

38. Does your herd have contact with animals/herds of different peasant associations at grazing areas/watering points? ☐ Yes, ☐ No

39. is there animal movement from your farm/herd (in or out) during FMD outbreaks?

☐ Yes, ☐ No

E. FMD control strategy applied in your area

40. What strategy is applied to control FMD in your area (village, farm, and district)?

☐Vaccination, ☐ treatment, ☐movement restriction, ☐ depopulation, ☐ others

41. If your answer of Question number 30 is vaccination, when was the last vaccine administered to the cattle on your farm/herd? ☐ a month ago, ☐ six month ago ,☐ one year ago

42. Did your herd ever experienced out break after the necessary vaccination? ☐ yes ☐ no; if yes, why do you think that happen; ☐ vaccines efficacy ☐ emerging of new virus type ☐ problem of vaccine production

43. What is Cost of vaccination per animal?

44. What total number of vaccinated animal in your farm/herd?

45. What is the cost of treatment per animal?

46. What is the total number of treated animals in your farm/herd?

Appendix 2: Bovine sample collection and associated risk factors recording format

Date of collection ____ /____ /____

Region _____ Zone_____

Code	District	PAs	Sex	Age	Breed	Mgt system	Herd size	Animal composition	Body condition	Sample type
1										
2										
3										
4										
5										

Appendix 3: Plate layout used for 3ABC FMD NSP ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	05	13									
B	PC	06	14									
C	NC	07	15									
D	NC	08	16									
E	01	09	17									
F	02	10	18									
G	03	11	etc									
H	04	12										

PC = Positive control serum; NC = Negative control serum; 01, 02, 03, 04, 05, etc. = Tested serum sample

Appendix 4: Interpretation of OD values as recommended by Sandwich ELISA

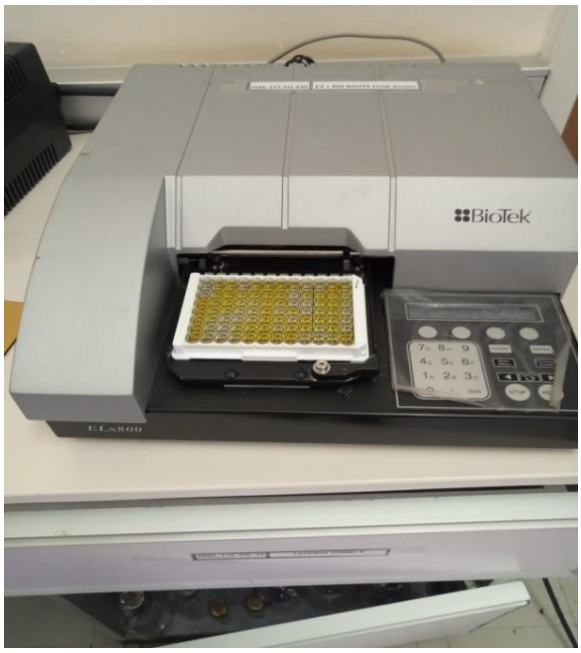
Negative for FMDV	OD <0.1
FMDV Positive for type O	OD ≥ 0.1 with the type O MAb and with the pan-FMDV MAb; Some samples may cross-react with the 1st MAb type A, but OD values with MAb O are higher.
FMDV Positive for type A	OD ≥ 0.1 with at least one of the two type A MAbs and with the pan-FMDV MAb
FMDV Positive for Asia 1	OD ≥ 0.1 with the type Asia 1MAb and with the pan-FMDV MAb
FMDV Positive for type C	OD ≥ 0.1 with the type C MAb and with the pan-FMDV MAb
FMDV Positive for SAT1	OD ≥ 0.1 with the type SAT1 catching MAb; some samples could be positive also with the pan-FMDV MAb
FMDV Positive for SAT2	OD ≥ 0.1 with the type SAT2 catching MAb; some samples could be positive also with the pan-FMDV MAb
FMDV Positive (untyped)	OD ≥ 0.1 with the pan-FMDV catching MAb and < 0.1 with the type specific MAbs

Note: OD values ≥ 0.05 and <0.1 should be considered suspect and retested:

Appendix 5: Miscellaneous photo during study period

1. Photo during sampling

2. Photo during laboratory work



Appendix 6: Ethical clearance certificate

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/10/02/14/2022

Name of Applicant: **Mohammed Aliye Tunfuri (DVM, MSc fellow)**

Address: Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Epidemiology and economic impact of FMD f cattle in selected districts of Arsi and Bale Zone, Oromia, Ethiopia*

Date of application:	**December, 2021**
Nature of the project:	**Mildly invasive**
Target animal species:	**Cattle**
Number of animals involved:	**535**
Study area:	**Arsi and Bale, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/14/022, 01/03/2022**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

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