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EPIDEMIOLOGY AND ECONOMIC IMPACT OF FOOT AND MOUTH DISEASE IN DOMESTIC RUMINANTS IN WESTERN AMHARA REGIONAL STATE, NORTH WESTERN ETHIOPIA

MVSc Thesis

BY

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EPIDEMIOLOGY AND ECONOMIC IMPACT OF FOOT AND MOUTH DISEASE IN DOMESTIC RUMINANTS IN WESTERN AMHARA REGIONAL STATE, NORTH WESTERN ETHIOPIA

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 Medicine in Veterinary Epidemiology

By

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June, 2021

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Title: **EPIDEMIOLOGY AND ECONOMIC IMPACT OF FOOT AND MOUTH DISEASE IN DOMESTIC RUMINANTS IN WESTERN AMHARA REGIONAL STATE, NORTH WESTERN ETHIOPIA**

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DEDICATION

This thesis manuscript is dedicated and memorial to my beloved Mother Ehetinat Esyneh Endalew whom I could not forget her since she has passed away because of diabetes case on March 12/2020!!!

The grief I felt has hurt me so much.

May your soul rest in peace

STATEMENT OF AUTHOR

First, I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an MVSc degree in Veterinary medicine at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under the rules of the Library. I surely declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHY SKETCH

The author was born in Quarit, Gebezemariam on July 10, 1995, from her father Ato Yirdaw Getie and her mother W/ro Ehetnat Esyneh. She completed her secondary and preparatory education at Quarit Secondary and Preparatory School. Then, from October 2012 to June 20, 2018, she joined Gondar University and graduated with a Doctorate degree in Veterinary Medicine. In September 2019, she joined the Ethiopian Institute of Agriculture and served as an Assistant researcher at Asosa center. Subsequently, in September 2020 she joined Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Clinical study for MSc Program in veterinary Epidemiology.

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ABBREVIATIONS

ANOVA	Analysis of variance
BHK	Baby Hamster Kidney
CFT	Complement Fixation Test
CPE	Cytopathic Effect
DIVA	Discrimination Infection Vaccination Animal
ELISA	Enzyme-Linked Immunosorbent Assay
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GDP	Gross Domestic Product
Ig	Immunoglobulin
MAbs	Monoclonal antibodies
MPCR	multiple Polymerase Chain Reaction
NAHDIC	National Animal Health Diagnostic and Investigation Center
NSP	Non Structural Protein
NVI	National Veterinary Institute
OD	Optical density
OIE	Office International Des Epizootics
PCR	Polymerase Chain Reaction
RNA	Ribo Nucleic Acid
RPM	Rotation per Minute
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAT	South Africa Territories
SP	Structural Protein
TAD	Transboundary animal diseases (TAD)
USD	United State Dollar
UTR	Untranslated Region
VNT	Virus Neutralization Test
VP	Virus protein

ABSTRACT

Foot and mouth disease is a highly contagious viral disease that primarily affects cloven-hoofed animals and causes significant economic losses in the livestock industry. A cross-sectional study was conducted in the western Amhara region of Ethiopia during the period from November 2020 to May 2021 with the aim of estimating the seroprevalence and associated risk factors of FMD, to identifying circulating serotypes and determines the economic impact of the diseases on domestic ruminants. A total of 389 sera samples and 14 epithelial tissue samples were collected from ruminants. A questionnaire survey was also conducted to determine economic loss associated with FMD. All the serum samples were subjected to a 3ABC enzyme-linked immune-sorbent assay to detect antibodies against non-structural proteins of foot and mouth disease virus (FMDV). In this study, the overall seroprevalence of FMDV in the domestic ruminants were 4.63% (18/389); (95%; CI: 2.93% - to 7.24%). The seroprevalence of cattle, sheep and goats were 6.38% (12/389), 3.67% (4/389) and 2.17% (2/389) respectively. The multivariable logistic regression analysis showed the occurrence of FMD was higher in Adet than in Banja (OR= 11.48, p=0.02). The occurrence in the semi-intensive production system was significantly greater than the occurrence in the extensive production system (OR=14.2 and p= 0.001). FMDV was detected using real-time reverse transcriptase polymerize chain reaction (RT-PCR) from a total of 14 active tissue samples taken and serotyping was done using antigen detection sandwich ELISA. FMDV was found in all tissue samples, and serotypes O, A, SAT1, and SAT2 were identified. Questionnaire survey data revealed total economic loss was 5553.21ETB (132.21$USA) per herd and 1124.13ETB (26.76$USA) per individual animal. It was determined that the disease's impact is extremely severe, resulting in massive economic losses. Therefore, it was recommended that further investigations on the epidemiology and economic consequences should be studied to design appropriate control options.

Key words; *Amhara region, Domestic ruminants, Economic loss, Ethiopia, Foot and mouth disease, Risk factors, sero-prevalence, serotype*

1. INTRODUCTION

Transboundary animal diseases (TAD) are one of the infectious diseases that have an impact on the livestock sector through decreased productivity, restricted international trade access, the loss of entire herds, biodiversity loss, and the loss of valuable genetic resources (Bayush, 2020; Yirgalem, 2020).

FMD is a highly contagious transboundary viral disease that primarily affects cloven-hoofed ruminants such as cattle, pigs, sheep, goats, buffalo, and artiodactylous wildlife species. The disease is most common in crossbred cattle (Longjam *et al.,* 2011). FMDV of the genus *Aphthovirus* of the family Picornaviridae causes the disease.

The virus has seven different serotypes (A, O, C, Asia1, South African Territories (SAT) 1, SAT 2, and SAT 3) and many subtypes based on the antigenicity of the capsid coating proteins. All serotypes are clinically indistinguishable but immunologically distinct (Tadesse *et al*., 2020). The disease has a clinical signs of vesicular formation, and erosions of the epithelium of the mouth, nose, muzzle, feet, teats and udder, tongue, lips and between the hooves, which leads to salivate profusely or become lame (Tikuye, 2019).

The virus can be spread directly through contact with an infected host or indirectly through contact with a contaminated environment. In addition to contact, viruses can be transmitted orally or through the respiratory tract to a new susceptible animal. Aerosol transmission is the most common method of spreading within a herd (Tadesse *et al*., 2019). FMD is highly contagious and requires diagnosis for prevention and control. Treatment and vaccination are the primary means of control in endemic countries such as Ethiopia (Paixao *et al.,* 2008). FMDV was difficult to control in the country due to its complex epidemiological nature and poor diagnostic facilities (Longjam *et al*., 2011).

FMD epidemiology is the study of the distribution of serotypes, prevalence rates, and risk factors that contribute to the occurrence of FMD. FMD is found all over the world and is classified as a notifiable disease by the World Health Organization. FMDV serotypes are not distributed evenly around the world. Serotype O is the most common serotype in most parts of the world, including Ethiopia (Aman *et al.,* 2020). FMD is endemic in Ethiopia, with several outbreaks reported each year due to the virus's complex epidemiological nature, wider geographical distribution, broad host range, ability to establish carrier status, antigenic diversity leading to poor cross-immunity, the presence of variant type of viral genes, and relatively short duration of immunity. The presence of free animal movement, a high rate of contact among animals at commercial markets, the presence of communal grazing areas and watering, poor diagnostic facilities, poor surveillance, and limited prevention and control strategies by the government are all associated with a high incidence of FMD in Ethiopia (Tikuye, 2019).

FMD is one of the most important diseases in the world because of its socioeconomic impact on the livestock sector. 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. Quantify 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 (Taddess *et al*., 2020).

The epidemiology and economic impact of FMD should be studied on a regular basis in Ethiopia for the purposes of prevention, control, mitigation, and eradication. FMD outbreaks in cattle have been reported on a regular basis in Ethiopia, including the Amhara region. For effective control and prevention, an appropriate vaccine containing the serotypes circulating in the area must be developed. Understanding epidemiological factors for disease occurrence in feedlot farms, dairy farms, and small holder farms is critical for disease mitigation and eradication. A regular study of the serotypes circulating in the region, as well as reporting on the disease's economic crisis, is necessary to motivate the government. Reports related to the economic impact of FMD in Ethiopia are limited so it is difficult in decision making for control and prevention. As a result, more research should be conducted on this disease that poses a threat to animal health.

Therefore, the objectives of this study were:

- To assess the Sero-epidemiology and economic consequences of foot and mouth disease

Specific objectives

- To estimate the seroprevalence of foot and mouth disease in domestic ruminants,
- To assess risk factors associated with the disease,
- To assess the economic impact of the disease with emphasis on livestock farms
- To identify serotypes of foot and mouth disease virus circulating in domestic ruminants in the western Amhara region.

2. LITERATURE REVIEW

2.1. Definitions

Foot-and-Mouth Disease (FMD), also known as aphtous fever, is an acute systemic infectious trans-boundary animal disease that affects both wild and domestic cloven-hoofed animals. Foot and mouth disease is known by various names around the world, including Epizootic aphtae, Infectious aphtous stomatitis, and Aftosa in Italian and Spanish, fever aphtheuse in French, Maul and Klavenseuch in German (Shimels, 2019). Because of the variety of species involved, the disease's rapid spread, and the difficulty in controlling it, it is more difficult to control than other diseases (Tesfaye, 2019).

2.2 Etiology

2.2.1 Taxonomy of the Virus

FMDV was first identified in 1963 by the International Committee on Virus Taxonomy. FMDV is the etiological agent, belonging to the Aphthovirus genus in the Picornaviridae family. Picornaviridae is derived from the Latin words ‘Pico' meaning small and ‘rna' meaning RNA (ribonucleic acid), which refers to the virus's size and genome type. The genus name 'Aphthovirus' refers to the vesicular lesions produced in the animals' feet and mouth. Foot and mouth disease virus is the causative agent of FMD disease. It is a non-enveloped icosahedral, positive-sense single-stranded RNA virus (Issa, Mishamo, 2016). The nature and organization of the genome, mode of replication, and structure of the virus are similar to those of other viruses in the family, but Picornaviridae is divided into four genera: *Enterovirus*, *Rhinovirus*, *Cardiovirus*, and *Aphthovirus*, based on physicochemical properties such as acid inactivation susceptibility, buoyant density, and nucleotide composition (Tesfaye, 2019).

2.2.2 Historical Perspective of FMDV

The idea of Foot-and-mouth disease (FMD) started the date back to 1546 during outbreak near Verona, Italy and 1780 in South Africa. The disease was very threatened to the cattle industry in the last centuries, but it was not known very well until the end of nineteenth century. Detailed information about FMDV was started during the twentieth century, including understanding of its genetic and physical structure, which has three-dimensional structure when observed by X-ray crystallography (Mahy, 2005). The FMDV was first identified as a filterable viral causative agent of an animal disease by two scientists, Loffler and Frosch, in 1897, marking the beginning of the science of virology (Chakraborty *et al.*, 2014).

In Ethiopia foot and mouth disease was first recorded in 1957 when serotypes O and C were found while serotype A was identified in Ethiopia in 1969 (Tikuye, 2019). The history of foot and mouth disease in Ethiopia accounts more than six or seven decades from now. The establishment of three African origin (SAT1, SAT2 and SAT3) FMDV serotypes found in Ethiopia accounts three decades from now (Wubshet *et al*., 2019).

2.2. 3 FMD Virus Morphology and Genomic organization

FMD viral particle or virion composed of an icosahedral protein coat (capsid) and the RNA core (genetic material) (Shimels, 2019; Wubshet *et al*., 2019). The external part of virus (capsid) consists of 60 capsomers each consisting of four structural polypeptides (Wondwossen, 2017). The FMDV genome is with very low molecular weight ranging from 7.2 to 8.4 kb single stranded positive sense RNA and whole virus particles having sedimentation coefficient of 146S (Longjam *et al.*, 2011).

The FMDV RNA genome encodes 12 viral proteins, L, 1A, 1B, 1C, 1D, 2A, 2B, 2C, 3A, 3B, 3C, and 3D. Four viral structural proteins 1A, 1B, 1C, and 1D (VP1 to VP4) make up the protein shell of the virus. 1D is using to traces the nonstructural protein. The other eight viral proteins (L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) play a role in replication and other functions within the host cell (Raiesul-Islam *et al.*, 2009).

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 (Tikuye, 2019).

There is polyadenylated attached on the 3' end and Vpg attached covalently with the 5' end. A single Open Reading Frame (coding region) of viral RNA part used for encodes a large polyprotein which is cleaved by viral proteases to form capsid. The VP1 contains a minimum of two important immunogenic sites, the G-H loop (at amino acid positions 141–160) and 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 (Leon, 2012). Figure 1 shows the virus's structure in greater detail.

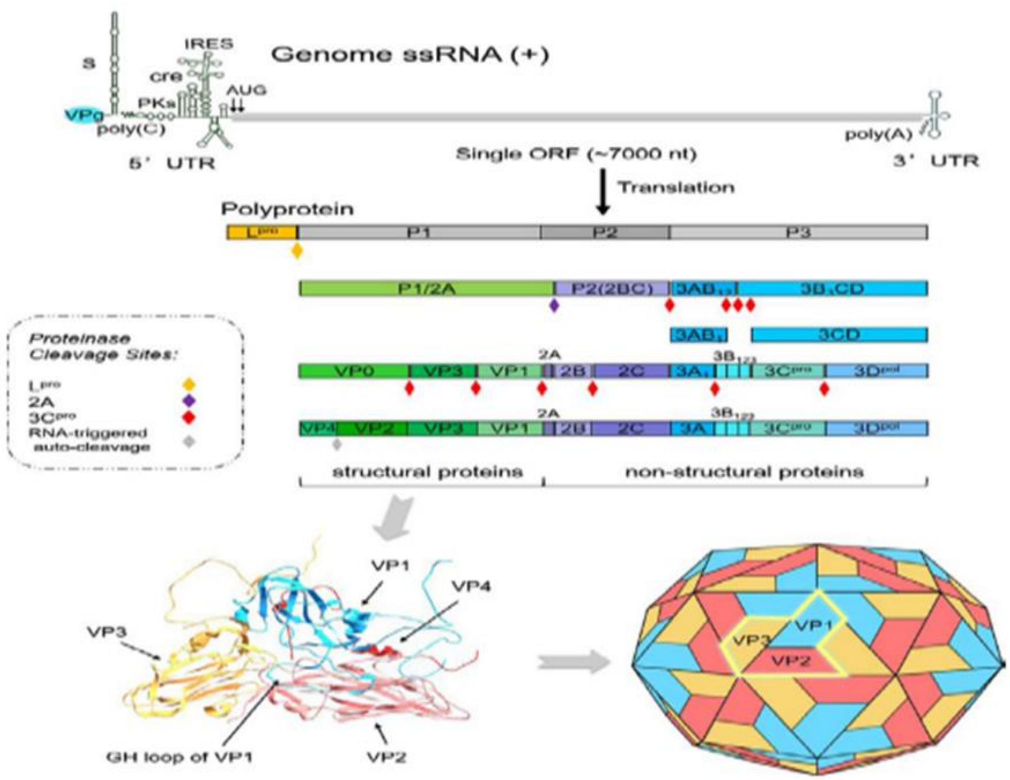


Figure 1: Genomic structure of FMDV

Adopted by (Tikuye, 2019)

2.2.4 Serotype of Foot and mouth diseases virus

There are seven serologically and genetically distinct types of the virus, namely O, A, C, Asia1, SAT1, SAT2, and SAT3, with over 60 subtypes. FMDV has a high mutation rate because the viral RNA dependent RNA polymerase is incapable of proofreading. There is no strain-to-strain cross-immunity (Wernery and Kinne, 2012; Longjam *et al*., 2011). Although clinical signs are indistinguishable between serotypes, the immunological response is distinct because the antigenic structure of each serotype differs (Leon, 2012). In addition to serotype differences, it may differ based on capsid protein sequence analysis. The serotype is further classified as a topotype, which expresses the serotypes' geographic, antigenic, and genetic relationships (Tesfaye, 2020).

2.2.5 Physicochemical properties of FMDV

The virus's physical characteristics indicate that it is a small RNA virus that is inactivated when exposed to pH levels below 6.5 or above 9. However, the virus can survive in milk and milk products with a pH of 4.6. Capsid FMDV is composed of polypeptides but lacks lipoprotein, making it resistant to lipid solvents (Wondwossen, 2017). The virus could be easily inactivated by heat, UV radiation, gamma irradiation, chemicals and disinfectants but at temperature below freezing point, it is stable almost indefinitely. The virus may persist for days or weeks in organic matter under moist and cold conditions. It can survive in frozen bone marrow, lymph nodes and also in cheese during its processing. The virus in milk and meat can be inactivated by heating at the temperature of 70°C for at least 30 min (Tesfaye, 2019).

2.3 Epidemiology of FMDV

FMD epidemiology is complicated due to a variety of factors such as virus properties, a large number of susceptible hosts, ecology, and environment. The distribution and severity of diseases may differ between animals depending on virulence, stability in different microenvironments, and the virus's chances of long-term persistence. FMDV multiplication and spread are also affected by host species, nutritional and immunological status, population density, animal movements, and interactions between domestic and wild host species (Longjam *et al*., 2011).

Identifying FMDV epidemiology was useful in defining strains, understanding transmission events, characterizing biodiversity, performing effective quarantine measures against reintroduction, developing specific diagnostic tests, and producing effective protective vaccines (Menda *et al.*, 2014).

2.3.1 Global distributions

The prevalent of FMD is all over the world with the seven heterogeneous serotypes A, O, C, SAT1, SAT2, SAT3 and Asia1, particularly in Asia, Africa and the Middle East. However, Japan, New Zealand, Australia and some other countries are FMD free countries. Among the seven serotypes of FMDV, serotype O broadly distribution in the world and serotype C low reports with the last report was in 2005 in Kenya (Yan *et al.*, 2017), (Wondwossen, 2017). Generally more than 100 countries are still affected by FMD worldwide and believed that disease still found about two-thirds of the world Figure 2 below (Chakraborty *et al.*, 2014; USDA, 2020).

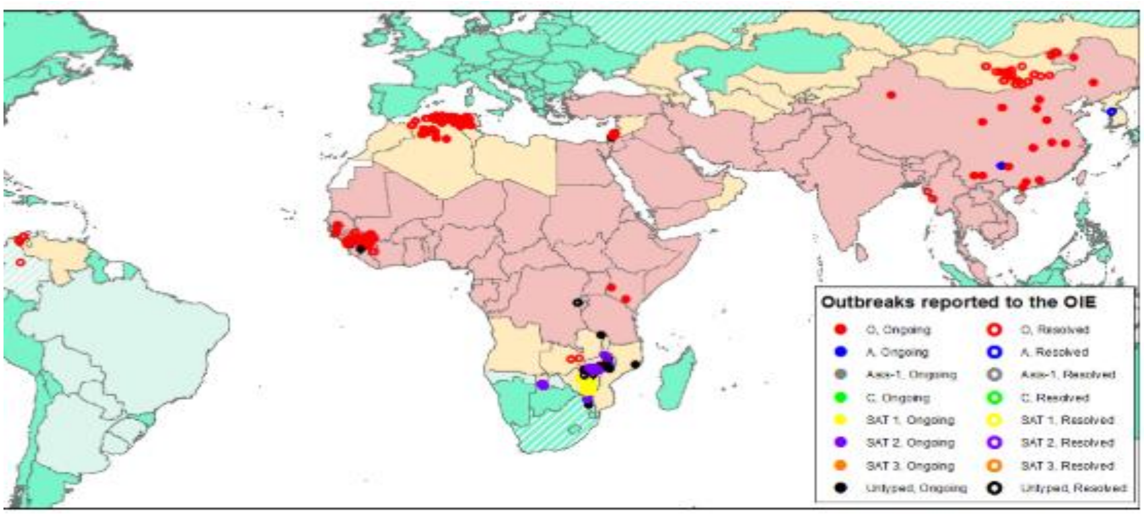


Figure 2: Map indicating the location of significant epidemiological events and disease outbreaks reported to OIE follow-up reports in 2018.

Adopted by (King *et al*., 2016).

2.3.2 Distribution of FMDV in Ethiopia

In Ethiopia, FMD is a common disease. Because it is endemic and notifiable, official reports from both pastoral and highland livestock are sent to the OIE on a monthly and annual basis (Tadesse *et al*., 2019). The temporal and spatial distribution of FMD in Ethiopia is ambiguous so it is difficult to manage as well as prevention, control and eradication. The presence of diverse serotypes, the occurrence of different diseases with clinical signs similar to FMDV, the presence of widely susceptible host species, including wild reservoirs, and the lack of effective control measures are all reasons for the complexity. See Figure 3 for more information (Wubshet *et al*., 2019).

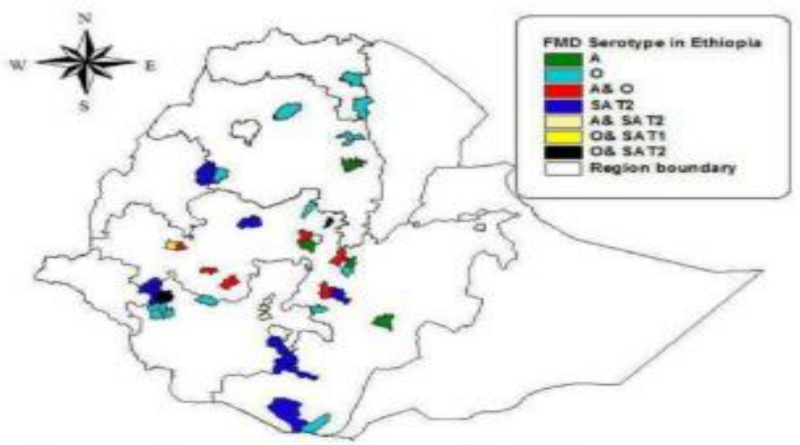


Figure 3: Spatial distribution of FMD serotypes identified from outbreaks (2014–2017)

Adopted by (Wubshet *et al*., 2019)

2.3.3 Current Statuses of the Diseases in Ethiopia

Five of the seven FMDV serotypes have endemic distributions in Ethiopia, and serotypes O, A, C, SAT1 and SAT2 were responsible for FMD outbreaks from 1981 to 2018 (Tikuye, 2019). A cross-sectional serological investigation of FMD was conducted in Amhara region with A total of 1,672 sera samples were collected and the overall prevalence was 12.04% (Mesfine *et al*., 2019).

A total of 2596 bulls were examined for the presence of FMD antibodies using ELISA techniques at the Adama export quarantine station to assess the international live animal trade, with 12.9% (334/2596) found to be positive (Adege and Masebo, 2017).

The research was conducted in 12 areas of northern and central Ethiopia where FMD outbreaks occurred between August and December of 2018. The researchers looked at 43 FMDV-infected clinical samples cultured on BHK-21 cells, 29 of which were isolated using virus serotype identification via RT-PCR and gene sequencing. Serotype O contributed 8 (18.60%) samples, serotype A contributed 20 (46.51%) samples, and mixed infection (O and A) contributed 1 (2.33%) sample (Tesfaye, 2020).

A cross-sectional survey was conducted using serum samples from the North and South Gondar zones of the North Western Amhara Region. In the North and South Gondar zones, the overall seroprevalence of foot and mouth disease in cattle was 14.9% (86/578) of the total sera tested. North Gondar had a higher prevalence rate, 17.8% (66/370), compared to 9.6% (20/208) in South Gondar, and the difference was statistically significant. The districts of Metema (62.5%), Quara (46.7%), and Alefa Takusa (34.9%) in the North Gondar zone, which border Sudan, had the highest prevalence (Tesfaye *et al*., 2016).

The study was based on outbreak cases in the Afar Region's Aba'ala District, and 18 of the 27 samples were inoculated on cultured Baby hamster kidney (BHK-21) monolayer cells. The findings revealed that 72.2 % (n=13) of the cultures exhibited FMDV-induced cytopathic effect (CPE), with the identified serotype being SAT-2 FMD virus (Dubie and Amare, 2020).

The study was conducted in Ethiopia's central region in 2011. A total of 38,187 bulls were tested for foot and mouth disease (FMD) antibody, with 5,536 being positive, indicating a 14.5 % prevalence of FMDV (Alemayehu *et al*., 2014). To determine seroprevalence and risk factors, a study was conducted in the Borana pastoral and agro-pastoral area. A total of 768 sera from 111 herds were collected. Using the ELISA test, the overall individual level seroprevalence was 23.0% and the herd level seroprevalence was 58.6% (Bayissa *et al*., 2011).

2.3.4 Risk factors

Even if the diseases were endemic, there are a variety of factors that contribute to their occurrence. The most common causes of FMDV occurrence are host-related factors, pathogens, and environmental factors. Many factors, which were substituted by common factors that facilitated FMDV distribution in Ethiopia, such as production system, geographic location, age of animals, contact with wildlife, and season of the year, were identified for the disease's spread in Ethiopia (Bayissa *et al*., 2011).

Several host-related risk factors are listed, including breed, age, immunity status, nutritional status, population density, animal species, animal movements, and contacts between different domestic and wild host species, and animals capable of mechanically spreading the virus of the animal (Souley Kouato *et al*., 2018).

FMDV affects animals classified as the suborder ruminant and the family suidae of the order Artiodactyla (Aman *et al.*, 2020). FMD is an infectious epitheliotropic disease that affects up to 70 species of cloven-hoofed mammals including cattle, sheep, goats, pigs and artiodactyl wild ruminants (Al-salihi, 2019).

The FMDV has no effect on humans, horses, pets, or birds. FMD may be able to survive in the human respiratory tract for 24 hours, allowing people who have had very close contact with infected animals to potentially serve as a source of virus exposure for susceptible animals, implying that humans may mechanically transfer the diseases to other animals and thus are not considered a public health threat. Bactrian camels can be infected experimentally, but Dromedary camels can be infected both naturally and experimentally (Chakraborty *et al.*, 2014).

Small ruminants may be naturally infected but do not show clinical signs or persistent infection, whereas cattle do show clinical signs and are considered an indicator host. It occurs less frequently and for a shorter period of time than in cattle and is regarded as a maintenance host in small ruminants. Pigs are highly susceptible to FMDV and are regarded as disease amplifier hosts because they emit high levels of air-borne virus but are relatively resistant to infection via the air-borne route (Upadhayay and Ewam, 2012).

Sheep excrete air-borne virus at comparable levels to cattle, but are thought to be less susceptible to air-borne infection due to their smaller respiration volume. Deer and buffalos can also be infected in sufficient numbers (Leon, 2012).

FMD virus has also been shown to persist in lymph nodes and orophareangial fluid in a non-replicative form. Carriers are animals in which the virus remains in the oropharynx for more than 28 days after infection, but Pigs do not become carriers. In cattle, the carrier state usually lasts no longer than 6 months. Individual African buffalo have been shown to harbor the virus for at least 5 years, but within a herd it can survive for 24 years or longer. Sheep and goats typically do not carry FMD viruses for more than a few months (Mason and Grubman, 2009).

The age of the animal is also a factor in the occurrence of death in animals. FMDV rarely causes death in adult animals, but the virus can cause severe myocardial damage in calves and piglets, resulting in high mortality rates for young animals (Longjam *et al*., 2011).

Physical characteristics of the virus such as variations in virus virulence factor, virus infection dose, antigenic variability, particle stability in different microenvironments, chances of long-term persistence, and host specific property of the virus are examples of pathogenic factors. FMDV is classified as seven immunologically distinct diseases, owing to the seven recognized serotypes that are currently circulating globally; immunity developed by animals to one FMDV serotype does not protect them from other serotypes (Wubshet *et al*., 2019).

Seasonal and environmental factors act as geographical barriers to virus spread and promote spread when suitable atmospheric conditions exist. The agent is also widely distributed in different agro climatic and socioeconomic factors. Mixed animal husbandry practices, unrestricted animal movement and trade, and porous international borders provide a conducive epidemiology niche for the FMDV to flourish, mutate, and persist over time, affecting the susceptible animal population (Longjam *et al*., 2011).

The number of outbreaks increased after the monsoons and remained high throughout the winter. FMD was found to be more prevalent during the dry season than during the cold dry season. During the rainy season, the incidence was lowest. Furthermore, herd contact with wild animals was greater during the dry season than during the rainy season (Molla *et al.*, 2013). Heavy rain, high relative humidity (60%), extremely hot weather, and moist winds during the rainy season may inhibit aerosol transmission of foot and mouth disease. In addition, heavy rains or floods impede the movement and transport of animals from one location to another in some areas (Jamal and Belsham, 2013). FMD diseases are more prevalent in the winter because of favorable climatic conditions such as dry weather and dry winds, low temperatures (weak sunlight), and moderate humidity (Upadhayay and Ewam., 2012).

2.3.5 Sources of infection and transmission

Affected animals shed the virus in all the body secretions and excretions including air, saliva, nasal and lachrymal fluid, milk, urine, feces, semen and blood milk, blood, pharynx, vagina and rectum before onset of clinical manifestations of the diseases. Most of the time, the virus is shed into milk and other body fluids before the animal shows clinical signs of the disease, increasing the possibility of raw milk spreading the virus within the farm and from farm to farm transmission. Infected animals may transmit the virus four days before the onset of symptoms, and some animals can excrete the virus for long periods of time, even years, after re-infection (Chakraborty *et al.*, 2014). A small number of infective particles can infect a susceptible animal. The main route of ruminant infection is inhalation of airborne virus, which virus exhaled into the air by infected animals and can be spread over long distances depending on wind speed and direction. Because the virus's structure is so simple, transmission through the air is facilitated. Ingestion of contaminated food, direct inoculation of susceptible animals, and infection on skin lesions are other possible modes of infection. Virus transmission is generally accomplished through direct contact, airborne and via fomites, or mechanically (contaminated animal products, non-susceptible animals, agricultural tools, people, and vehicles) or indirectly through contaminated organic debris, fomites, or personnel and materials (Souley Kouato *et al.*, 2018).

2.3.6 Molecular epidemiology

Molecular epidemiology is a relatively new science that aids in the taxonomic classification of many viruses, including FMDV. In endemic areas, the distribution of FMDV serotypes and subtypes was studied in detail using molecular epidemiology. Molecular epidemiology is an important tool for understanding FMDV dynamics, as it is used to determine the new topotype and origin of new FMDV lineages (Klein, 2009). It is more concerned with comparing genetic and phenotypic differences in the capsid protein part. The virus's capsid protein, particularly the VP1 region, is the most important part of the virus for studying molecular epidemiology. To comprehend molecular epidemiology the current preferred method for generating the virus is RT-PCR amplification of FMDV RNA followed by nucleotide sequencing (Bayush, 2020).

2.4 Pathogenesis

The virus enters the body via inhalation, skin abrasion, or mucus membrane abrasion. The respiratory tract is the virus's primary replication site. The virus prefers epithelial tissue in adults and heart muscle in young. Following primary multiplicity in the host's pharynx and mucous membrane, the virus affects lymphatic glands, epithelial tissues around the mouth or feet, and mammary glands via the lymphatic system and bloodstream. Following a 3 to 5 day period of febrile viremia, the virus spreads throughout the body, resulting in secondary infection. Before clinical symptoms of FMD appear, the virus appears in various body fluids such as milk, urine, respiratory secretions, and sperm (Tesfaye, 2019).

The virus attacks the pituitary gland, which regulates metabolic functions in the body. Failure of glands causes panting, restlessness, reduced breeding capacity, and weakness in draught animals. FMDV infection of the udders and teats can cause mastitis, which results in permanent teat loss and reduced milk yield (Upadhayay and Ewam., 2012). FMDV RNA replicates within the cytoplasm of infected cells and requires the virus-encoded RNA-dependent RNA polymerase to do so. This enzyme catalyzes the synthesis of a negative strand copy of the viral genome, which is then used as a template for the production of new positive strand RNA molecules (Tikuye, 2019).

The viral structural and nonstructural protein elements of the viral RNA, as well as host proteins or membranes that participate in the viral replication cycle can be considered virulence factors that aid in FMD transmission (Grubman and Baxt, 2004). Poly “C” tract comprising over 90% “C” residues causes virulence. The length of this tract is extremely variable. There are some evidences that length of this tract is associated with virulence and hence persistence (Longjam *et al*., 2011).

2.5 Clinical sign and Differential diagnosis

Disease control and prevention used for reducing farther spread on animals. Clinical diagnosis and observation of sign and symptom lead for laboratory diagnosis. Sometimes clinically may not possible to distinguish FMDV from other vesicular diseases due to similar clinical sign. Clinical sign of diseases used to determine tentative diagnosis of diseases. Studding differential diagnosis is important for differentiate diseases with similar clinical observation. Clinical signs of FMD can vary from mild to severe, and have got species variation (Leon, 2012).

In natural conditions, the incubation period varies depending on the virus strain, the susceptibility of the individual host, the exposure dose, and the route of entry. It can last anywhere from 2 days to 14 days in most cases. Signs are mostly seen in cattle because the bovine species is an indicator host. Vesicular lesion on teats or mammary gland in female, inter digital space of feet and other hairless parts of skin, pyrexia, shivering, drooling the main sign of FMDV (Chakraborty *et al*., 2014). The virus remains in the esophageal-pharyngeal region for more than 28 days after infection, but the animal exhibits no clinical signs (Wernery and Kinne, 2012). More information about FMD clinical signs can be found in Figure 4 below.

Figure 4: Clinical sign shown due to FMD infection

Adopted by (Dubie and Amare, 2020)

Differential diagnosis is necessary to differentiate diseases having similar clinical signs. Diseases like bovine mucosal disease, rinder pest, peste des petits ruminants, malignant catarrhal fever, blue tongue and epizootic hemorrhagic disease, Vesicular Stomatitis and Swine vesicular disease are among the diseases have similar clinical sign with foot and mouth diseases. To differentiate from thus diseases laboratory diagnosis is very important (Chakraborty *et al.*, 2014).

2.6 Diagnostic methods

Timely and rapid diagnosis of Foot and Mouth Disease (FMD) is very essential in controlling the disease by treating patients and reduce farther spread of the diseases. Diagnosis of FMD in susceptible animals is based on clinical signs and laboratory methods. Due to the presence of diseases having similar clinical sign we can not sure whether the diseases is 100% FMD positive or not by physical examination. Laboratory diagnosis is important for conformation by collecting clinical samples (Sharma *et al.*, 2015).

2.6.1 Clinical and epidemiological diagnosis

Clinical examination is important type of diagnosis used for either tentative diagnosis or lead to laboratory diagnosis. The disease is diagnosed based on clinical signs of the diseases. FMD examination is performed by measuring body temperature, auscultation, vision, palpation whether there is vesicles on the oral mucosa and non-haired part of the body or not, by looking inter-digital spaces (laminas) and presence of salivation or not. Postmortem findings also other method of diagnosis which include as erosions of the mucous membranes rumen and lesions on the oropharyngeal part (Mahmoud *et al*., 2019).

The other diagnosis method is epidemiological diagnosis, which is expressed by risks of introducing live animals and animal products legally or illegally from potentially infected areas, the contact history of wild animals, farming system, movement of animals across the national and international boundaries, geographical area, season of the year and the herd size are valuable to diagnosis of FMD (Tikuye, 2019). However, the clinical signs can be confused with other diseases having similar clinical findings and epidemiological observation may not always give correct perception, so other method of diagnosis is important for screening of diseases (EL-Shehawy *et al*., 2011).

2.6.2. Laboratory Diagnosis

The majority of FMD diagnoses are made in the clinic based on clinical signs, but laboratory testing is also done on occasion. For laboratory diagnosis, vesicular fluids, epithelial samples (the most commonly used tissue for diagnosis), oropharyngeal fluid, throat swab, blood samples, sperm samples, serum samples, milk, and environmental samples (air samples) can be used. Samples of epithelial tissue should be collected (Paton and King, 2012).

There are different methods of diagnosis for FMDV like Complement Fixation Test (CFT), Virus Isolation (Primary cell culture), Enzyme-Linked Immunosorbent Assay (ELISA), Reverse Transcription-Polymerase Chain Reaction (RT- PCR, Real-Time Polymerase Chain Reaction, Multiplex Polymerase Chain Reaction (mPCR) (Longjam *et* *al.,* 2011). Generally diagnostic methods can be classified virus isolation (culture), immunological (serological) and antigen detection methods (Paixão *et al*., 2008).

Virus isolation and characterization of the virus are the “golden standard” for the diagnosis of viral diseases. Virus Isolation is definitive method of diagnosis for foot and mouth diseases. Virus isolation requires the presence of infectious virus, but sample quality determines getting reliable result (Tikuye, 2019). The cultures were checked for cytopathic effects (CPE) of the virus (Paixão *et al.,* 2008). This method is highly sensitive, but it is time-consuming, which takes between 1 and 4 days and requires extraordinary laboratory facilities. The medium used for culture FMDV are bovine thyroid cell, primary lamb kidney (LK) cells and baby hamster kidney (BHK-21). Bovine thyroid cell very sensitive to FMDV but for preservation within refrigerator BHK-21 is better. Cultures with CPE were stored at -70°C until processing for indirect sandwich ELISA (Mahmoud *et al*., 2019).

In Genome based methods, PCR is one of the recent diagnostic techniques and the most widely used nucleic-acid-based diagnostic techniques by amplify and detect the genome fragments of FMDV. The diagnostic tools also used for detect serotypes by using specific primers. It is an investigating whether there the presence of Viral RNA in the samples. RT-PCR developed to amplify RNA targets for many workers have great usefulness more reliable tool for FMD diagnosis (Tesfaye, 2014). Serotypes can be identified by conventional RT-PCR techniques depending on the magnification of the VP1 gene 43. Real-time PCR has engendered wider acceptance of PCR due to its improved rapidity and sensitivity (Longjam *et al.,* 2011).

Immunological methods are the most common immunological diagnostic methods include ELISA, complete fixation test and viral neutralization test. ELISA came into use as diagnostic methods for many infectious diseases including FMD around the year 1975 till now (Fosgate, 2009). ELISA and its various modifications were applied for detection, typing, quantification and strain differentiation of FMDV with a high percent of specificity and sensitivity. Enzyme-linked immunosorbent assay is preferred procedure for the detection of FMD viral antigen and identification of viral serotype obtained within

3-4 hours after sample is received by the laboratory. ELISA has better sensitivity than CFT, which is criticized by lack of sensitivity and specificity (Longjam *et al*., 2011).

There are many type of ELISA as direct, indirect, competitive ELISA, Sandwiched ELISA, liquid phase blocking ELISA and DIVA are the most common applicable diagnostic method. DIVA (discrimination vaccination and infection of animal test) is tests for antibodies to non-structural proteins of FMDV (NSP tests), which is detect antibodies to NSPs of FMDV. This is best option for discrimination natural infection from infection due to vaccination. Test of antibodies against the 3ABC non-structural polyproteins of FMD virus in serum is important for differentiate sera positive is whether due to vaccine or due to infection. This assay has the ability to detect antibodies 3ABC from 10-900 days post-infection in experimentally infected cattle (Adege and Masebo, 2017). Virus neutralization tests are a gold standard for antibody detection. It used as one of the most accepted serological techniques. It is time consuming with variable sensitivity used in certification trade of animals and animal products (Mahmoud *et al.,* 2019). Serological tests (virus neutralization and liquid phase enzyme-linked immunosorbent assay) are not time-consuming, but they are indirect tests and do not always allow for differentiation between infected and vaccinated animals, so it is not the technique of first choice to detect an acute infection (Mason and Grubman, 2009; Paixão *et al*., 2008). Table 1 below shows major diagnostic method for FMD and their diagnostic test capability in detail.

Table 1: Common laboratory diagnostic methods, diagnostic test value, advantage and disadvantage

FMD diagnostic assay	**Sensitivity**	**Specificity**	**Advantage**	**Disadvantage**
Sandwiched ELISA	80%	100%	Easy to perform, suitable for handling large number of samples	Less sensitive and not suitable for a certain clinical samples
Multiplex PCR	Minimum detection limit of 1×10^{-1} TCID 50 mL^{-1}	100% specific for cross serotype detection	Rapid and sensitive, suitable for samples like semen and milk	High risk of generating false positives
TaqMan Real Time PCR	Minimum detection limit of 10^{-1} TCID50 mL^{-1}	100% specific for cross serotype detection	More sensitive and specific than gel based assay	High risk of generating false positives
Virus isolation and neutralization			Gold standard assay for FMD for Diagnosis	Slow takes 1-4 days for confirmatory results Require
3AB3 I ELISA	95.8%	97.45%	Sensitive and specific respectively	Only for Bovine specious
3AB–C ELISA	91.7%	(96-98)%	Specific assay Universal for all species	Less sensitive than I ELISA
LAMP RNA	LAMP RNA Minimum detection limit up to 1.1×10^{-4} TCID 50 mL^{-1}		Requires spcicialized instrument, can be used as a point of care Diagnosis	High risk of generating false positives

Adopted by (Mahmoud *et al*., 2019; Hosamani *et al*., 2015; Roche *et al.,* 2015).

2.7. Control, Prevention and Treatment

In veterinary science, control, prevention, and treatment should always be rationale and logical. Controlling strategies may differ between countries depending on the disease's status, as well as the financial and technical capabilities of different countries (Tadesse *et al*., 2017). Controlling virus transmission entails lowering an animal's risk of virus exposure as well as lowering susceptibility through vaccination or limiting animal movement. Because socioeconomic factors, that are cost effective management, are always conceders in veterinary science, a fixed cost investment is mostly used for prevention of foot and mouth diseases (Knight and Rushton, 2013; Akalu, 2017).

FMD is difficult to control due to its highly contagious nature, multiple hosts, viral stability, multiple antigenic types or subtypes, and short term immunity, as well as the extraordinary genetic and antigenic complexity of the FMD virus. There are no effective vaccination control methods available because one serotype of FMDV does not cross-protect against other serotypes even within a serotype, vaccination against one strain may not cross-protect against other strains, and due to antigenic differences between the strains. In general, there has never been an official FMD control plan in Ethiopia, with the exception of vaccination in some market-oriented dairy farms and ring vaccination in urban and peri-urban areas during disease outbreaks (Jemberu, 2016).

Controlling a disease is commonly accomplished through the use of an equipped laboratory, rapid and accurate diagnostics, rapid response measures, continuous monitoring or surveillance, vaccination, quarantine, restriction of animal movement, isolation of infected animals, and a stamping out policy in both epidemically affected countries and disease-free zones. However, test and slaughter policy shouldn't be implemented in our country, Ethiopia due to the economic, social and regional barriers but applied in developed countries (Leon, 2012). Furthermore, good infrastructure, trained veterinary staff, and good governance are required to effectively control the disease. In general, there has never been an official FMD control plan in Ethiopia, with the exception of vaccination in some market-oriented dairy farms and ring vaccination in urban and peri-urban areas during disease outbreaks (Jemberu, 2016).

Control and prevention of FMD in endemic countries rely primarily on repeated vaccination, good infrastructure, trained veterinary staff, and good governance, as well as control of animal movement and physical separation of wildlife and livestock. In FMD-free areas or countries, most control methods such as quarantine, animal movement restrictions, vaccination (mass and compulsory vaccination) programs, and disease awareness may be used to supplement other preventive measures (Leon, 2012).

Vaccination is the most common method of prevention in both endemic and diseases free area of the world. There are different types of vaccination based on preparation such as Conventional vaccine, Protein vaccine, protein fragments and viral subunits vaccine, peptide vaccine, genetically engineered attenuated strain vaccine and DNA vaccine (Sobrino, 2001).

In Ethiopia available vaccine type includes trivalent inactivated vaccine manufactured from locally isolated FMDV serotypes trivalent vaccine (O, A and SAT2) and bivalent vaccine (Serotype O and A) which are produced by the National Veterinary Institute (NVI). The virus is propagated from cell culture and absorbed into aluminum hydroxide gel and inactivated with 0.3% formaldehyde and adjuvant with saponin (Tikuye, 2019).

There are different vaccination type based on application, emergency FMD vaccine and protective vaccination. Emergency FMD vaccines used to provide protective immunity as rapidly as, possible to susceptible stock and to reduce the amount of virus release or limit the spread of disease. The protective vaccination is used effectively in animals not already exposed to FMD virus. It would therefore be employed outside the 3 km protection zone and a form of ring vaccination (Mahy, 2005).

Most viral diseases, including FMD, have no treatment. Symptomatic treatment, rather than specific treatment, can be used depending on the clinical presentation of the diseases. Some treatments include potassium permanganate mixed antiseptic mouth wash, sodium carbonate, boric acid, and glycerin applied over the lesion. The affected animals' feet are washed with a solution of washing soda and 2 percent copper sulphate, and a topical application of honey or finger millet has traditionally been found to be effective in foot lesions.

Proper animal husbandry practices, treatment of secondary bacterial infection, and dressing of inflamed areas to prevent secondary infection are all recommended, particularly in endemic countries where slaughter policy is not followed (Yirgalem, 2020).

2.8. Economic challenge of Foot and mouth diseases on Livestock Production

The livestock production in Ethiopia can be broadly classified into three systems according to husbandry and ways of life. These are crop-livestock mixed, pastoral and market oriented production system. Each production system mostly performed in central highland parts of the country, arid and semiarid peripheral parts and urban and peri-urban parts of the country respectively. According to management, production systems can be divided into three types: intensive production systems, extensive production systems, and semi-intensive production systems. In the Amhara region, particularly in western Amhara, a mixed livestock production system with intensive, extensive, and semi-intensive production systems was practiced, with the extensive production system accounting for more than 85% of the total (Jemberu, 2016).

In terms of economic impact, FMD is regarded as the most important livestock disease in the world. It currently affects the majority of the world's countries, including Africa, Asia, South America, and parts of Europe, but the consequences are not uniform. The impacts vary between endemic and non-endemic countries or developed and developing countries, even within developing countries. In most studies, Ethiopia ranked FMD as the most economically important viral disease, and it was ranked among the top five most important diseases. The disease has both direct and indirect economic consequences, primarily due to restrictions on international trade in animals and animal products, as well as the costs associated with disease outbreak control (Souley Kouato *et al*., 2018).

Despite the fact that FMD has a low mortality rate of less than 5%, it is considered one of the most economically important livestock diseases in the world because it reduces livestock productivity. Severe economic losses occurred as a result of high morbidity associated with outbreak frequency, large numbers of animals affected in each outbreak, and export trade restrictions imposed on affected countries (Knigh and Rushton, 2013).

Direct effects of FMD on livestock productivity include reduced feed intake, changes in digestion and metabolism, increased morbidity and mortality, decreased rates of weight gain, loss of milk production, retarded growth, and loss of draught power, abortion in pregnant animals and deaths in calves, kids and lambs. Indirect losses are those related to the significant costs of FMD control, management, limited use of improved production technologies and poor access to international markets, which is often less visible than the obvious effects of clinical disease but may be equal or more important in their overall economic impact (Alemayehu *et al*., 2014; Tadesse *et al.*, 2020). Additional costs include application of control measures such as quarantines, slaughter, compensation, vaccination as well as conducting scientific surveillance after an outbreak in order to confirm the disease are other economic impact of foot and mouth diseases virus (Tikuye., 2019; Menda *et al*., 2014). In general, FMD has the following consequences, as shown in the chart 5 below, which shows the impact of the diseases on production and productivity in both developed and developing countries, including Ethiopia.

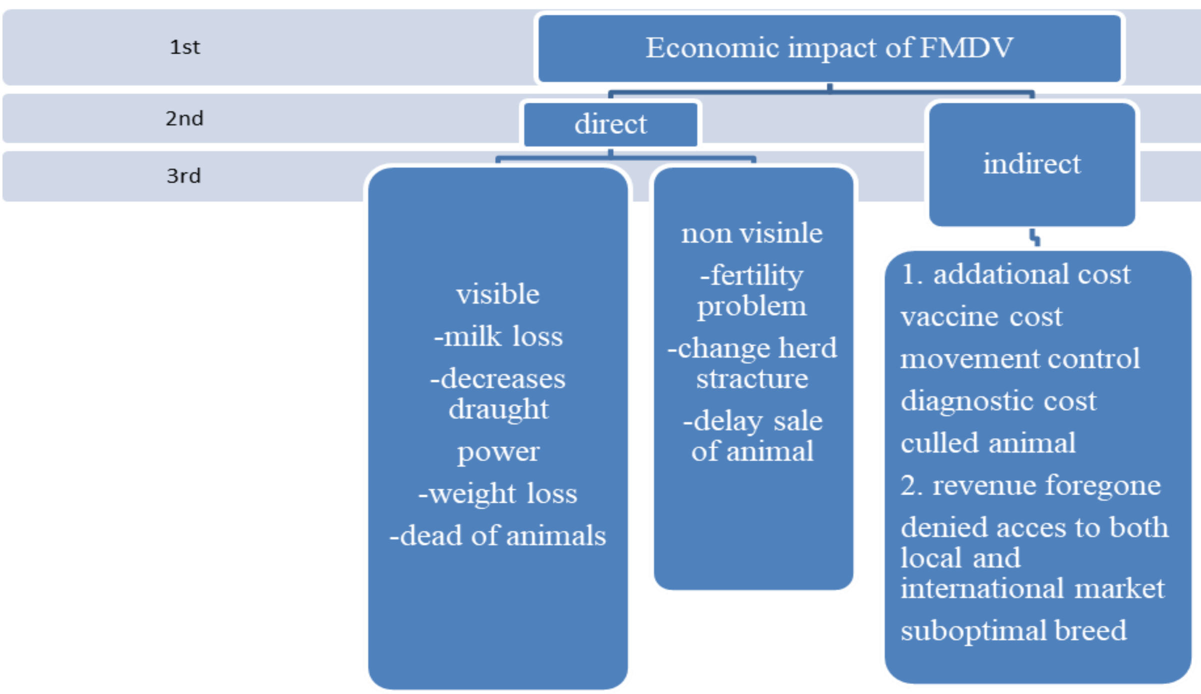


Figure 5: Generalized economic consequences of FMD

Adopted by (Tesfaye, 2019)

3. MATERIALS AND METHODS

3.1. Study Area

The research was carried out in the northwestern part of Ethiopia's Amhara regional state from November 2020 to May 2021. It is located between the latitudes of 8° 45′–13° 45′N and the longitudes of 35° 15′–40° 20′E, and it covers an area of 157,127 km2 (Figure 6). The average annual rainfall in the region ranges between 598.3 mm and -1692 mm. It is bordered by four national regional states of Ethiopia; Oromiya in the south, Afar in the east, Tigray in the north, and Benshangule-Gumuz and Sudan in the west. Rural areas are home to 87.3% of the population, while urban areas are home to 12.7%. The region's main farming system is a mixed crop livestock production system (Sewnet and Kameswara, 2011). The regional state is divided into 11 administrative zones, but only six of them (North Gondar, South Gondar, East Gojjam, West Gojjam, Awi, and Bahir Dar special zone) were included in my research. A questionnaire survey was conducted in each of the six zones, with one district selected from each, but blood samples were collected from four districts (Adet, Banja, Simada and Ebenat).

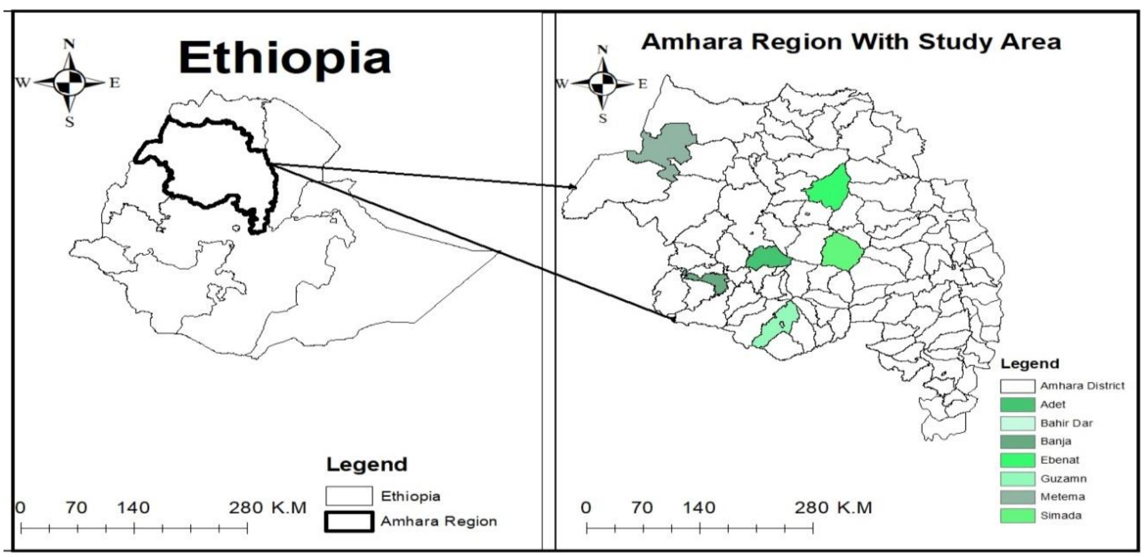


Figure 6: Map showing the study area

Source; Adopted by Arc GIS 10.8

Banja, the first study district, is bordered on the south by Ankesha, on the west by Guangua, on the north by Fageta Lekoma, and on the east by the West Gojam zone. Injibara town is the capital of Banja district. It is 442 kilometers north-west of Addis Abeba and 116 kilometers south of Bahir Dar, with a latitude of 43° 37' 38.10" N and a longitude of 20° 53' 46.82" E, and elevations ranging from 1870 to 2570 m.a.s.l. (Fentie *et al.,* 2020). Adet, located in Amhara Region, has the coordinates 11°16'N latitude and 37°29'E longitude and an elevation of 2216 m above mean sea level. The annual precipitation average is 1250 mm (Abera *et al*., 2019). Guzman is the third study district in east Gojjam, with latitude and longitude of 10°20′N and 37°43′E, and an elevation of 2,446 meters. Simada, located in the Debub Gondar Zone at 11° 29' 59.99" N latitude and 38° 14' 60.00" E longitude, was the fourth study district. The district is topographically classified as 10% highland, 30% mid-highland, and 60% lowland (Aragaw and Tilay, 2012). The fifth district was Metema, which is located in the north Gondar zone with a geographical reference of latitude and longitude of 12°58′N 36°12′E and an elevation of 685 meters above sea level in the Amhara region. Metema is bounded by Qwara on the south, Sudan on the west, Mirab Armachiho on the north, Tach Armachiho on the northeast, and Chilga on the east, and on the southeast by Takusa. The sixth district was Ebenat, which was located in the Debub Gondar Zone and was bounded on the south by Farta, the southwest by Fogera, the west by Libo Kemekem, the north by the Semien Gondar Zone, the northeast by the Wag Hemra Zone, the east by Semine (North) Wollo Zone, and the southeast by Lay Gayint. Ebnate district in south Gondar is 122 kilometers from Bahirdar and has latitude and longitude coordinates of 12°21′ 44.608″ N and 38°18′06.460″E, respectively, and an elevation of 2213 meters above sea level (Melkegnaw, 2017). In general, 11 peasant associations from four districts were chosen from the study area for serosurvey, and eight peasant associations from six districts were chosen for questioner survey. For the serosurvey study, three peasant associations were chosen from each district and classified as lowland (kola), highland (dega), and temperate (wyna dega), with the exception of Adet, which had only two peasant associations classified by availability of different farming type. Based on outbreak information, three districts were chosen for the questionnaire survey, with only one peasant association chosen in each. In contrast, three districts were chosen in parallel to the survey, each with one peasant association, with the exception of Banja and Adet, which each had two peasant associations.

3.2 Study Population

The study population consists of cattle, sheep, and goats of various ages, sexes, herd sizes, and reared under various production systems and farming types. **Appendices 1** and **Appendix table 1** contain the age determination of animals for each species. For the purpose of estimating the economic impact of FMDV, only cattle from any type of farm were included, based on their accessibility in the study area. Except for urban, this region has a nearly similar population density with a mixed crop livestock production system and similar animal management, with the exception of beef or dairy farms with intensive or semi-intensive production systems.

3.3 Study Designs

Serum sample collection: A cross-sectional study was carried out in the western Amhara region to determine FMDV seropositivity and associated risk factors in domestic ruminants. The study employs a multistage sampling method from region to individual animal level but within each stage purposive, convenient and random sampling method was applied. The first region chosen was the western Amhara region due to its accessibility and relatively good security. Four districts were selected conveniently by their accessibility and secured area but peasant association in the district selected by considering agro-ecology. Herds and individual animals were chosen based on their presentation in the clinic, which means that animals found in the clinic for treatment or vaccination were used as sampling units with the owner's permission. A maximum of a quarter of the animals in one herd were used as sampling units, but if the owner had a small number of animals, all of them could have been included.

Questionnaire survey**:** A semi-structured retrospective questionnaire survey was conducted in parallel with the serosurvey, primarily in smallholders, but some questions were asked of dairy and beef farms based on convenience for sampling and outbreak information within the study area. Some owners in the peasant association were selected randomly but some selected based on information of outbreak in their farm.

The questioner prepared based on answers to questions such as how many farmers know about FMDV, how many diseases rank in the region relative to other diseases, the time of incidence of FMDV 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. Face-to-face interviews with respondents (farmers and animal health workers) in the local language (Amharic) were conducted by the researcher in each peasant association. Questionnaire survey format in **Appendix 7**

3.4 Sample Size Determination

For seroprevalence data: The sample size was determined using a sample size calculation (Thrusfield, 2005) based on the desired precision and previous prevalence in the study area. According to the previous report (Mesfine *et al.*, 2019), the seroprevalence of FMDV in cattle was 14.3%, 7.10% in goats, and 7.07% in sheep.

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

Where Z a/2 value of the critical region =1.96, N = sample size, Pexp =expected prevalence, d = absolute precision used as a significance level and 95% confidence interval. Based on the formula 188 cattle, 101 sheep and 100 goats were used in this study. But there is no required number of goat found so 92 goats and 109 sheep was used. This has not much difference from the required sample size. The sample size in each district was calculated using the disproportionate stratified sampling method, which divides the total sample size by the number of strata or districts, as well as a similar flow at the peasant association level.

Sample size determination for questionnaire data: The number of farmers who are entitled to respond determines the appropriate sample size estimation of the survey based on the standard error of 5%. The number of respondents was calculated using the formula 0.25/SE2 and assuming maximum variation between farming types (Stevenson, 2020). A pilot test was conducted with ten farmers in order to eliminate unanswered questions and determine the level at which farmers can respond during the interview.

The data collected by the pilot survey is not part of the study, and one animal health worker was present in each peasant association to support farmer ideas, particularly about vaccination, disease type, and treatment.

3.5 Ethical Considerations

Animal research ethical review Committee of Addis Ababa University, College of Veterinary Medicine and Agriculture would check on ethical issues for sample collection and preservation and Agriculture the thesis is part of Addis Ababa University thematic project part with (Reference No: VM/ERC/07/04/13/2021) **Appendix 9**. Animals would be treated with kindness and care, with the goal of minimizing discomfort, distress, or pain during sample collection. Professionals with experience and qualifications collected the samples.

3.6 Study Methodology

3.6.1 Sample collection

In blood sample collection**,** the blood was drawn from the jugular vein of each animal using a 10 ml plain vacationer tube. Each vacationer tube was carefully inspected. It was kept at room temperature overnight before being separated into sera. When overnight stored blood did not yield serum, a centrifuge was used to separate it. During collection, the serum was carefully harvested and labeled for each species of individual's animal id before being placed in cryovials. It was then transported to the Bahirdar animal diseases diagnostic laboratory using cold chine. The sera samples were kept at -19oC until the laboratory investigation was completed. Detail procedures found in **Appendix 2**.

For epithelial tissue sample collection, there were clinically infected animals present while collecting serum in the field. Freshly ruptured oral and foot lesions from suspected FMD-infected cattle were collected aseptically. The collected samples were immediately placed in a sampling tube containing virus transport medium made up of 50% glycerol and 0.04M phosphate buffer salphate (PBS) with antibiotics at pH 7.2- 7.4. Detail procedures found in **Appendix 3**.

Species, identification number, sex, age, peasant association, and sample type (swab or tissue) were all labeled, and samples were immediately placed in an ice box with ice packs. It was kept at -19°C until it was processed and tested in the lab. The research was carried out in the laboratory at the National Animal Health Diagnosis and Investigation Center (NADIC) in Sebeta, Ethiopia.

3.6.2 Serological Examination

Serum samples were subjected to foot and mouth disease non-structural protein (FMD NSP) Competition 3ABC ELISA (ID Screen ®, ID.Vet, and Montpellier France). A total of 389 sera were tested with ID Vet competitive ELISA (NSP 3ABC) coated microstate plates was used for this test. A test was performed as per the manufacturer's instructions with series of procedures with 96 well micro plates. Detailed procedure in **Appendix 4**

Validation for test

The test result was validated when the mean value of the negative control O.D. (ODNC) was greater than 0.7 (ODNC > 0.7) and the mean value of the positive control O.D. (ODPC) was less than 30% (OD = Optic density, NC = negative control, PC = Positive control).

Interpretation

For each serum sample, the competition percentage was calculated S/N % = ODS/ODNC X 100, ODS (Optical density for sample) and ODNC (optical density for negative control) and less than or equal to 50% were considered positive but greater than 50% were considered negative but between 48% and 51% was border line.

3.6.3 RNA Extraction and Molecular Detection

In tissue sample preparation, samples are ground with a tissue-grinding device (mortal and pistil) to disrupt viral cells. The sample is ground with silica sand and an effective phosphate buffer. For each 14 sample, the procedure is repeated by cleaning the material for the next procedure and transferring the suspension of the grinded part to the sampling tube.

RNA extraction: Total RNA was extracted from FMDV-infected clinical samples tissue suspension using the QIAamp® Viral RNA Mini extraction Kit, cat.no. 52906 done with manufacturer's instructions and more detail in **Appendix 5**

Master Mix preparation: A real-time assay was carried out by Superscript Taq one-step rRT-PCR kit. The composition of the 20µl Master Mix for the one-step rRT-PCR included the following: 10µl 2x- reaction buffer, 2.0µl of each of the forward and reverse primer, 1.5µl of the probe, 3.0µl extracted RNA, 1.5µl of Rnase free water.

3.6.4 Real-time reverse transcription polymerase chain reaction (rRT-PCR)

Detection of 3D (pol) region of FMDV was performed using the Qiamp viral RNA mini kit as per instructions and selected catalog number of 52906. A 3D pol region forward primer 5'ACT GGG TTT TAC AAA CCT GTGA-3', reverse primer of 5'GCG AGT CCT GCC ACG GA-3' and TaqMan probe 5' TCC TTT GCA CGC CGT GGG AC TAMRA-3'was used to screen the samples (Menda *et al*., 2014). Positive and negative control was added to each and keeps in to rRT PCR machine for amplification. The real-time RT-PCR reactions were carried out in a 20 µl reaction mixture containing 3 µl templates RNA and 17µl other reaction mixtures in a 96 micro plate well. Real-time RT-PCR amplification started with programmed as the following temperature, 50°C for 30 minutes, 95°C for 10 minutes, 95°C for 15 seconds, 60°C for 1 minute with a total of 50 cycles (Tesfaye, 2020; EL-Shehawy *et al*., 2011). A threshold cycle (CT) values used to assign samples as either FMDV positive or negative. According to OIE Reference Laboratory negative test samples and negative controls should have a CT value >50. Positive test samples and positive control samples should have a CT value <40. Samples with CT values 40–50 are designated "borderline" and strong positive FMD samples have a CT value below 20.

3.6.5 Identification of serotypes by antigen detection

FMDV serotypes were identified and detected using FMDV antigen detection and Serotyping sandwich ELISA (IZSLER, Bresica, Italy). The kit was created using carefully chosen combinations of anti-FMDV monoclonal antibodies (MAbs), which were used as coated as well as conjugated antibodies.

It was created to detect and type six FMD virus serotypes: O, A, C, Asia 1, SAT 1, and SAT 2. A pan-FMD test can detect isolates of serotypes O, A, C, Asia 1, and some of the SAT serotypes. The kit also includes FMD viruses that may have escaped binding to selected serotype-specific monoclonal antibodies.

The cached MAbs were used to detect 10 samples at a time, with one positive and one negative control for each serotype. The test was carried out in accordance with the manufacturer's instructions and detail in **Appendix 6**

Criteria for validity of antigen detection ELISA

The positive inactivated controls were expected to give OD values greater or equal to 1.0 unit while the negative control for serotype O, A, C, Asia 1 and Pan-FMDV are expected to give OD values less than 0.1unit and the negative control for serotype SAT1 and SAT2 are expected to give OD value less than or equals to 0.2unit. **Table 2:** below detail about interpretation

Table 2 : Interpretation of antigen detection ELISA result

Negative for FMDV	OD<0.1
FMDV Positive type O	OD>=0.1 with the type O MAbs; some samples may cross react with the first MAb type A, but OD values with MAb O are higher
FMDV Positive type A	OD>=0.1 with at least one of the two type A MAbs and with the pan-FMDV MAbs
FMDV Positive type Asia1	OD>=0.1with the type Asia1MAb and with the pan-FMDV MAbs
FMDV Positive type C	OD>=0.1with the type C MAbs and with the pan-FMDV MAbs
FMDV Positive type SAT1	OD>=0.1with the type SAT1 catching MAbs
FMDV Positive type SAT2	OD>=0.1with the type SAT2 catching MAbs; some samples could be positive also with the Pan-FMDV MAbs.
FMDV Positive (untyped)	OD>=0.1with the pan-FMDV catching MAb and <0.1 with the type specific MAbs

Note: OD value >=0.05 and <0.1should be considered suspected and retested.

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 milk production loss, draft power loss, treatment costs, animal mortality loss, and beef farm reffating costs. To demonstrate the economic impact of the FMD burden, estimate the approximate impact of the diseases in endemic countries, taking into account. It was studied by asking farmers or animal owners, using a semi-structured questionnaire format, about the consequences of the diseases, particularly milk loss, and the measures taken during outbreaks. A cost determination model was used to estimate economic loss due to FMD (Jumberu, 2016).

3.7.1 Milk loss in dairy cows

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

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

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) was used to calculate total economic loss within infected herd (Tadesse *et al.*, 2020).

3.7.2 Loss due to Mortality

The mortality loss was estimated by market value of died animal. Thus, the economic loss due to mortality per herd was calculated by considering the different age categories of animals that died and their corresponding market price as:

Loss due to death=

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

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). To calculate the loss in per affected herd divided by number of infected herd and to calculate any herd divide by total herd (Jumberu, 2016; Tadesse *et al*., 2020).

3.7.3 Economic loss in beef farms

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

Economic losses estimated in beef farm was done cost used for re fatting was calculated. Economic loss =NRf (Number of re fatting bull 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 bull and labor cost per animal as a whole.

3.7.4 Draft power loss

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

(Ld) Loss of draft power =AvSo (number of sick oxen in small holder farm) × Adi (number of days of illness) × adj rate × ApDppdpa (Price of draft power per day per animal). The economic losses caused by draft power loss are calculated as the number of oxen affected multiplied by the length of illness in days of an affected ox in the herd multiplied by the adjustment factor. An adjustment factor was calculated by multiplying the price of an ox's draft power rent per day by the price of an ox's draft power rent per day per animal. Draught power for crop production (plowing and threshing) is not required all year due to crop seasonality and cultural beliefs. So ox can work up to 65 days per year so as adjustment factor (65/365) (Alemayehu *et al*., 2014; Jumberu, 2016).

3.7.5 Treatment loss

The costs of controlling and preventing foot and mouth disease include vaccination, diagnosis, treatment, and additional labor costs for seeking treatment for sick animals. Vaccination is only used in some dairy production, but not in small-scale or beef farms. Antibiotic treatment of infected animals for bacterial complications was used as a control measure in the study areas. The cost of FMD treatment was calculated based on the number of animals treated and the average price per head. Also, the average number of working hours lost by the attendant/owner while seeking treatment for sick animals, as well as the average hourly wage was calculated.

$$\sum_{n}^{1} Hi = (NTrAni \times \text{Price of Treatment}) + (\text{hours Lost} \times \text{payment rate}),$$ (Jumberu, 2016).

3.7.6 Total economic loss

Total economic loss per infected herd = (Milk loss + mortality loss + economic loss in beef farm + draft power loss + treatment cost (Alemayehu *et al*., 2014; Jumberu, 2016). **Limitations:** The impact estimate does not account for most invisible costs, vaccination cost and surveillance costs. In addition it does not include the losses national and international level cost loss by FMD and not considers cost loss due to small ruminants. The cost calculated only from outbreaks from the last one year**.**

3.8. Data Management and Analysis

The serum data was entering into Microsoft (Ms) excel spread sheet program and coded for analysis. The overall seroprevalence and prevalence over each factor was analyzed by Stata software Version 13 with chi-square statistical tools. Risk factors were analyzed by univariable and multivariable logistic regression analysis using R software (version 3.6.1). A stepwise analysis performed by R and the last model determine and multivariable logistic regression was applied then only the significant variables written here. The model diagnosis was performed and there was no significance difference between the two models with $p>0.05$; $p=0.61$. The questioner survey data entered in to SPSS version 20 software and analyzed using descriptive statistics but data indicate mortality and morbidity entered into R software and analysis by ANOVA statistical tools for comparison between production and district. In all the analysis, confidence level was set as 95% and 5% set for significance level.

4. RESULTS

4.1 Seroprevalence of FMD

A total of 389 serum samples from domestic ruminants from four districts and three zones in Northwestern Amhara regional state were tested using the 3ABC non-structural competitive ELISA test. The overall apparent seroprevalence of FMDV in domestic ruminants was 4.63% (18/389) in this study (95%; CI: 2.93% to - 7.24%). A significant (P = 0.039) higher seroprevalence was recorded in Adet district 10% (n = 9) as compared to other districts as shown in Table 3. The districts in the south Gondar zone had relatively similar seroprevalence records, with 3.39 % in Ebenat and 3.85 % in Simada. In general, the prevalence of FMDV varied by district was higher in Adet and lower in Banja, Figure 7, which depicts the prevalence of FMDV in study districts and their proportion by percentage in the study area.

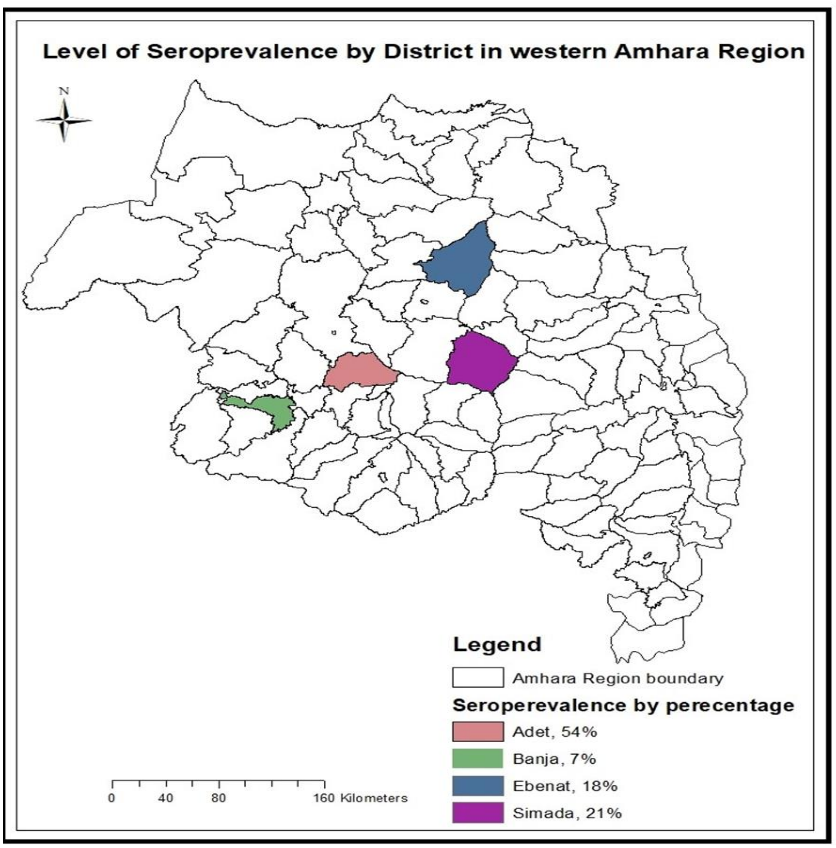


Figure 7: The Distribution of FMD in four districts by prevalence level

Significantly (P =0.015) higher seroprevalence was found in the W/Gojjam zone (n = 9) as compared to the South Gondar Zone (n = 8) and the Awi zones (11.3 % (n = 1). The proportion also varies by peasant association level within the same district, with zero ande having a prevalence of 17.39 % and zero hult having a prevalence of (2.27 %). There was a statistically significant difference among peasant associations with (P=0.001), but the prevalence of FMD was zero in some peasant associations, including Brkorch, Meslcyti, Kesa, Shasho, and Sergawit.

Table 3: The prevalence of FMDV among Zones, Districts and Peasant association level

Variable		**No test**	**No positive test**	X^2	**P value**
Zone	Awi zone	77	1(1.3%)	8.34	0.015
	W/Gojjam	90	9(10%)		
	South Gondar	222	8(3.6%)		
District	Simada	104	4(3.85%)	8.37	0.039
	Ebenat	118	4(3.39%)		
	Adet	90	9(10%)		
	Banja	77	1(1.3%)		
Peasant association	Amstya	40	2(5%)	29.7	0.001
	Selamya	43	2(4.65%		
	Brkorch	35	--		
	Meslcyti	33	--		
	Askun abo	12	1(8.33%)		
	Kesa	32	--		
	Zero ande	46	8(17.39%)		
	Zero hult	44	1(2.27%)		
	Dubaduba	35	4(11.43%)		
	Shasho	32	--		
	Sergawit	37	--		
Total		389	18(4.63%)		

No test, number of animals tested and No positive, number of positive sample for FMD

FMD prevalence between risk factors**: -** Seroprevalence was compared in various risk factors such as age group, gender, species, and number of animals, production systems, and animal rearing practices. Although not statistically significant (P = 0.25), higher seroprevalence was found in Bovine 6.38% (n = 12) as compared to ovine 3.67% (n = 4) and Caprine species 2.17% (n = 2). Higher seroprevalence was recorded in female 4.69% (n =12) as compared to male 4.51 (n = 6) but there was no statistically significant difference. Adults had a lower seroprevalence of 4.27 % (n = 12) than young, who had relatively higher seroprevalence of 5.56% (n = 6), but the difference was not statistically significant. Although not statistically significant (P = 0.05), higher seroprevalence was found in herds larger than five (5.22%) compared to herds smaller than five (3.77%). Seroprevalence was found to be significantly (P =0.013) higher in dairy farms, with a prevalence of 16.67%, than in small holder farms, with a prevalence of 3.91%, and in beef farms, with no prevalence. Seroprevalence was found to be significantly (P=0.00), higher seroprevalence was recorded in semi intensive production system 25% as compared to intensive production system 6.25% and extensive production system 3.4%. Seroprevalence was found to be significantly (P=0.032), higher seroprevalence was recorded in cross breed 12.12% as compared to local breed 3.93%. Table 4 below showed more description including X^2 value.

Table 4: Seroprevalence of FMD across corresponding risk factor

Factors	Categories	No test	No positive	CI	X^2	P value
Species	Bovine	188	12(6.38%)	(3.64-10.93)%	2.79	0.25
	Ovine	109	4(3.67%)	(1.37-9.43)%		
	Caprine	92	2(2.17%)	(0.53-8.37)%		
Sex	Female	256	12(4.69%)	(2.67-8.09)%	0.006	0.937
	Male	133	6(4.51%)	(2.02-9.72)%		
Age	Adult	281	12(4.27%)	(2.43-7.38)%	0.29	0.59
	Young	108	6(5.56%)	(2.50-11.88)%		
Breed	Local	356	14(3.93%)	(2.34-6.54%)	4.59	0.032
	Cross	33	4(12.12%)	(4.53-28.57)%		
Herd size	≤5	159	6(3.77%)	(1.69-8.18)%	0.44	0.505
	> 5	230	12(5.22%)	(2.98-8.99)%		
Farm type	Small holder	358	14(3.91%)	(2.32-6.50)%	8.34	0.013
	Dairy farm	24	4(16.67%)	(6.24-37.53)%		
	Beef farm	7	0(0%)	---		
Production system	Intensive	16	1(6.25%)	(0.81-35.1)%	20.11	0.000
	Extensive	353	12(3.40)	(1.93-5.90)%		
	Semi intensive	20	5(25%)	(10.52-48.57)%		

No test –number of test, No positive- number of positive and X^2 - chi square, CI-confidence interval

FMD prevalence between risk factors**:** The occurrence of FMD seroprevalence was compared among zones, districts, and peasant associations using a univariable logistic regression analysis. The diseases occurred 8.44 times more frequently in west Gojjam than in Awi zone and 2.97 times more frequently in south Gondar, with a significance difference of P=0.045 and p=0.03, respectively. With a significant difference (P=0.045), the diseases occurred 8.44 times more frequently in Adet than in Banja. The effect of breed indicates that FMD occurs 3.36 times more frequently in cross breed cattle than in local breed cattle, with a significant difference (p=0.043). FMD occurred 9.47 times more frequently in semi intensive production systems than in extensive production systems, with a significant difference (P=0.000). The occurrence of FMD in dairy farms was 4.9 times higher than in small holder farms, with a P=0.009 significance difference. FMD was found to be more prevalent in the Zero ande and Dubaduba peasant associations when compared to Amestya, with a significance difference of p=0.006 and 0.022, respectively. There was no statistically significant difference between species, but FMD occurred 3.06 times more frequently in bovine than in Caprine. Table 5 provides a more detailed explanation.

Table 5: Univariable logistic regression result

Factor	Categories	OR	P value	CI
Zone	W/Gojjam vs Awi zone	8.44	0.045	1-68.2
	W/Gojjam vs S/Gondar	2.97	0.03	1.1-7.9
District	Adet vs Banja	8.44	0.045	1-68.2
	Adet vs Ebenat	3.16	0.06	0.9-10.6
Peasant association	Zero ande vs Amestya	4	0.006	0.8-20.1
	Dubaduba vs Amestya	2.45	0.022	0.4-14.28
Species	Bovine vs Caprine	3.06	0.14	0.67-14.3
Breed	Cross vs Local	3.36	0.043	1.04-10.1
Production system	Semi intensive vs Extensive	9.47	0.000	2.95-30.3
Farm type	Dairy farm vs Small holder	4.9	0.009	1.5-16.3

OR, odds ratio; vs. versus; CI, confidence**;**

Only the district and production system show a significant difference in the occurrence of FMD in multiple logistic regression analysis. By district, the disease prevalence in Adet is 11.48 times higher than in Banja (P=0.028). FMD was 8.42 and 7.93 times more common in Simada and Ebinat, respectively, than in Banja, but there was no significant difference between them. FMD is 14.2 times more common in semi-intensive production systems than in extensive production systems, with a significant difference (p=0.001). For a more in-depth understanding, see Table 6.

Table 6: With multi variable analysis on risk factors

	Factors	OR	P value	CI
District	Adet vs Banja	11.48	0.028	1.3-100.8
	Ebenat vs Banja	7.39	0.11	0.6-86.6
	Simada vs Banja	8.42	0.09	0.8-98.8
Production system	Semi intensive vs extensive	14.2	0.001	3-65

OR, odds ratio; vs, versus; CI, confidence

4.2 Molecular Results

Clinical observations during blood sampling or collection revealed some animals with salivation, lameness (foot lesion), fever, depression, and lesion on the tongue, dental pad, and gum as shown in Figure 8

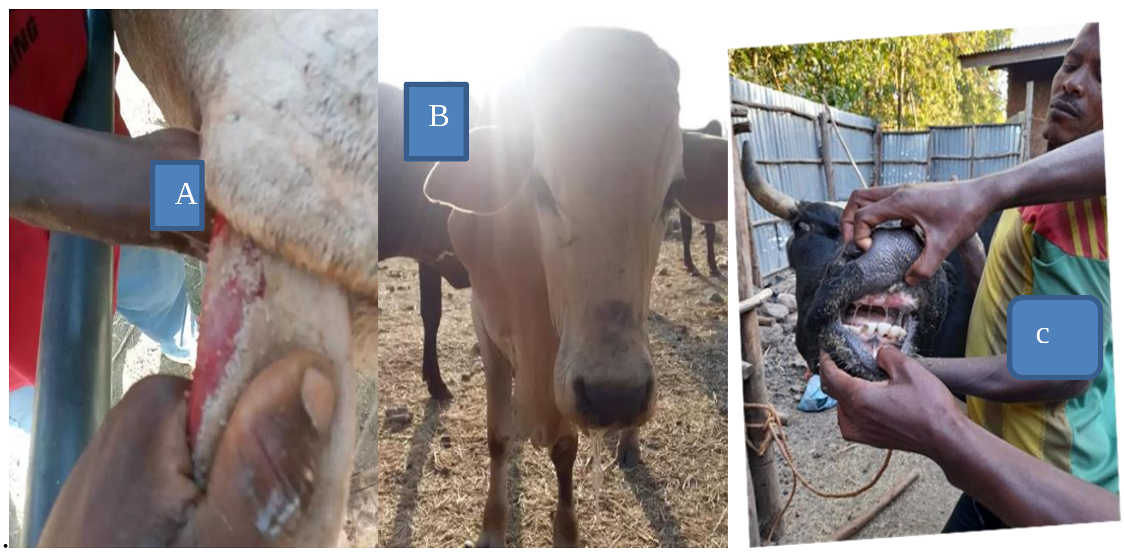


Figure 8: Images show FMDV taken from during sample collection.

(a) Erosion of the tongue (b) Mouth discharge (saliva from mouth) (c) Erosion of gum

The Figure 9 below, Figure A showed that all 14 epithelial tissue samples were detected by rRT-PCR with detection of FMDV 3D (pol). FMDV was found in all 14 samples, with a minimum CT value of 15.07 and a maximum CT value of 30.08.

Six samples had a lower CT value than the positive control. Following RT PCR detection, all 14 samples were tested by sandwich ELISA and serotypically identified. ELISA detection identified four serotypes from Guzamn, Adet, and Metema: O, SAT2, A, and SAT1. Guzman provided half of the sample, and serotypes SAT1, SAT2, and O were found with single and mixed infection. Serotypes O and A were found in the Adet district, as well as one animal with a mixed infection. Only SAT2 was discovered in Metema. Out of all serotypes, 42 % were O, and 35.7% were SAT2 from total samples, both in single and mixed infection. Generally the distribution of serotypes in western Amhara region is varying from one district other district.

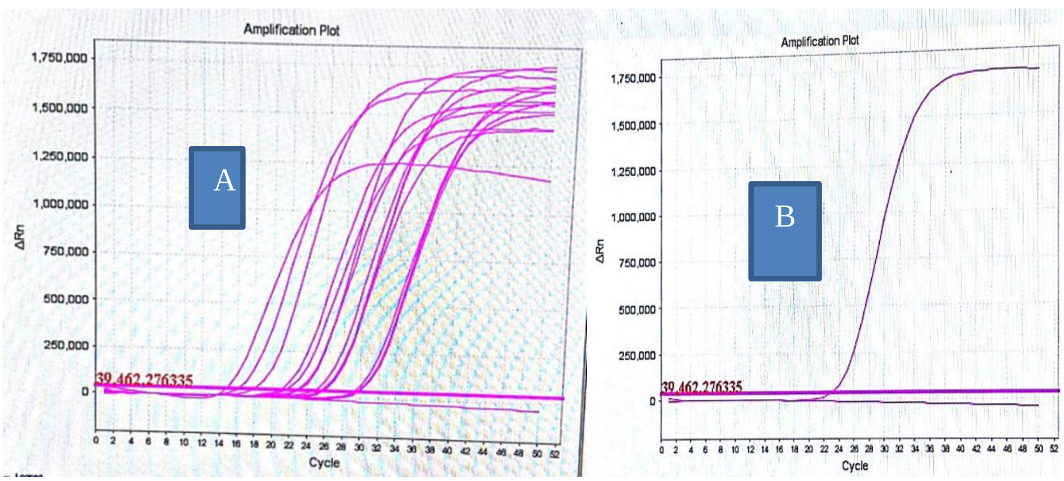


Figure 9: RT PCR Result shows the CT value and Fluorescent produced

In Amplification plot A (14 samples), B(postive and negative control)

Table 7: CT value and serootype of epithelala tissue sample results

Sample No	District	Date of collection	Spacious	Sex	Breed	CT value result from test with RT PCR	Serotype result from antigen detection
1	Guzman	20/01/220	Bovine	Female	Cross	30.09	SAT2, SAT1
2	Adet	14/10/2020	Bovine	Male	Local	18.34	A,O
3	Metema	27/02/2020	Bovine	Male	Local	16.52	SAT2
4	Adet	14/10/2020	Bovine	Male	Local	29.57	A
5	Metema	27/02/2020	Bovine	Female	Local	30.08	SAT2
6	Adet	20/11/2020	Bovine	Male	Local	26.20	O
7	Adet	20/11/2020	Bovine	Male	Cross	25.20	O
8	Guzamn	20/01/2020	Bovine	Female	Cross	21.35	O
9	Guzamn	27/02/2020	Bovine	Female	Local	15.07	SAT2, O
10	Guzamn	20/01/2020	Bovine	Female	Cross	23.21	O
11	Guzamn	20/01/2020	Bovine	Male	Cross	25.93	O
12	Metema	27/02/2020	Bovine	Male	Local	22.04	SAT2
13	Guzamn	20/01/2020	Bovine	Female	Cross	26.14	SAT1
14	Guzamn	20/01/2020	Bovine	Female	Cross	30.05	SAT1
Positive control						23.44	

4.3 Questionnaire Survey Result

4.3.1 General demography of farmers and herd structure

According to the study, 100 farmers from six districts in the Amhara region participated in interviews. Three districts were chosen based on their similarity to seroseruvey, and three more were chosen based on the presence of an active case in the study area. FMD was confirmed using the RT PCR diagnostic method in three districts: Metema, Guzman, and Adet.

Questionnaire includes all type of production systems (intensive and extensive) but about two third of the respondent was in traditional way of life that was 66 herds were small holders, 18 were beef farm and 16 dairy farms. Primarily most farmers participate in day to day activities with mixed crop and livestock production but for some farmer's only livestock were their livelihood. By their educational background most participants were illiterate and some were read and write but very few participants, around 6% were higher educator. All farmers of any educational level have had almost similar concept about the diseases. Morbidity and mortality was diagnosed by farm level and individual animal level as well as in different districts and different production system. By district level a higher morbidity was observed in Simada with 37.14% and low morbidity was observed in Banja district with morbidity rate of 2.23%. In production system higher morbidity was observed in dairy farm with morbidity rate of 30.33% and lower morbidity rate was observed in small holders with a rate of 15.99%. Mortality rate also observed both in production and district level. During observed with district higher mortality was observed in Guzamn with a mortality rate of 5.38% and lower mortality was observed in Metema with a mortality rate of 1.69%. When comparing mortality rate with farm type higher mortality was observed in small holders with 1.57% and higher mortality was observed in beef and dairy farms with 3.37% and 3.49% respectively. The general animal level morbidity was 19.83% and the general animal level mortality was 2.23% during study period. The number of animals in diseased herd with beef farm, small holder farm and dairy farm was 30, 76 and 45 respectively. There were 151 animals in diseased herd. Total number of diseased animals in diseased herd in beef farm, small holder farm and dairy farm was 19, 46 and 33. The total number of diseased animals was 98 so the morbidity in individual animal level from affected herd was 64.9%. The morbidity in beef farm, small holder farm and dairy farm was 63.3%, 60.5% and 73.3% respectively. For more detail information Table 8 below.

Table 8 : Summerize the questionery survey results

Risk factor	Categories	No+ herd	No herd	Total No	No of diseased	Ave Herd size	Morbidity	mortality
District	Adet	6	17	76	22	5	28.947 %	1(1.3%)
	Banja	1	19	89	2	5	2.247 %	---
	Simada	7	15	70	26	5	37.14 %	2(2.85%)
	Guzamn	7	23	130	30	6	23.076 %	7(5.38%)
	Metema	4	11	59	10	5	16.95 %	1(1.69%)
	Tanahyk	2	15	70	8	5	11.43 %	----
Farm type	Small holder	13	66	319	51	5	15.99 %	5(1.57%)
	Dairy farm	7	16	89	27	6	30.33 %	3(3.37%)
	Beef farm	7	18	86	20	5	23.25 %	3(3.49%)
Total		27	100	494	98	5	19.83%	2.23%)

No+ herds means number of positive herd, Ave Herd size means Average herd size and No means number

4.3.2 Farmer's knowledge about foot and mouth diseases

In the study area foot and mouth diseases as the second diseases causing sever loss in livestock industries. As most respondents said that FMDV called by its local name as maize in Gondar and Bahirdar zuria and kortem in and around Gojjam. The majority of respondents ranked foot and mouth diseases from one to three, and secondly from four to six. The majority of respondents provide information on the time of occurrence for foot and mouth diseases, which is mostly from January to February and then from September to December, but some respondents stated that there was an occurrence from June to August as well. In this study area, treatment was the primary control method, with traditional methods coming in second.

Initially, treatment and vaccination were carried out in intensive dairy farms. Approximately 70% of farm owners are aware about foot and mouth disease. There were 27 out of 100 respondents who had a history of disease outbreaks within the previous year (April 2020 to March 2021). There were 13 small holder herds, 7 beef farms, and 7 dairy farms among the 27 herds with a history of outbreak.

4.3.3 Morbidity and Mortality of FMD

The number of animals infected during the outbreak divided by the total number of animals at risk was used to calculate animal level morbidity, and animal level mortality was calculated by dividing the number of animals died during the outbreak by the total number of animals at risk. Morbidity and mortality at the herd level were calculated as the number of positive herds divided by the total number of herds at risk, and the total number of herds where the animal died divided by the total number of herds at risk. FMD mortality, morbidity, and case fatality rates by animal level were 2.23 percent, 19.8 percent, and 12.23 percent, respectively. Morbidity and mortality rates by herd level were 27% and 9%, respectively. There were a significant difference of morbidity between district Guzamn $p<0.05$; p= 0.0384, Simada with $p<0.05$; p= 0.0154 and Adet with a significance level of $p<0.05$, p= 0.0281. By production there is significant difference between beef farm with small holder farm with a significance level of $p<0.05$; p=0.0019. Only Guzamn has significance difference in mortality from other districts with $p<0.05$; 0.0478. With a p value of 0.0247, there was a significant difference in district interaction with the production system.

4.4 Economic impact of FMD on livestock Production

4.4.1 Economic loss due to milk loss

Total loss calculated by summation of the whole milk loss during observation and gives 22322ETB (531.47$USA). The cost lost due to FMD in dairy animals indicated in Table 9 below.

Table 9: Milk loss due to FMD

Variables	Estimated milk loss
Milk loss per individual animal in affected herd	227.78ETB(5.42$USA)
Milk loss per individual animal with any herd	45.19ETB(1.1$USA)
Milk loss per affected herd	826.7ETB(19$USA)
Milk loss per any herd	223.22 ETB(18$USA)

The milk loss per individual animal in the affected herd was calculated by dividing the total cost loss due to milk by the number of diseased animals during the study period. Milk loss was calculated for each individual animal in any herd by dividing the total cost loss due to milk by the total number of animals at risk. The milk loss per affected herd was determined by dividing the total cost loss due to milk by the total number of diseased herds. Milk loss was calculated for each herd by dividing the total cost loss due to milk by the total number of observed herds, as shown in Table 9.

4.4.2 Mortality loss

The total number of milking cows, calves, oxen, and dry cows that died during the study period was 2, 5, 3, and 1, respectively, and the total economic loss during the examination period was 368000ETB (8761.9$USA). In general, it was around 13629.63ETB (324.5$USA) per affected herd and around 3680ETB (87.6$USA) within any herd. It was 3755.1ETB (89.4$USA) for each affected animal and 744.94ETB (17.74$USA) for any individual animal.

4.4.3 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 64 bulls, 20 of which were diseased and required refatting days (convalesce and again fatten).

In the beef farm under consideration, the total economic loss was 154000ETB (3666.67$USA). The total economic loss per affected herd was calculated by dividing by number of infected herd and 5703.7ETB (135.8$USA) and per any herd 1540ETB (36.67$USA). The cost per individual animal level with infected animal was 1571.43ETB (37.41$USA) and 311.74ETB (7.42$USA) for any animal.

4.4.4 Draft power loss

The total cost loss in birr due to draft power was 4277.32 ETB (101.846ETB). The cost per affected herd was 158.41.36 ETB (3.77$USA), while the cost per affected individual animal was 43.65ETB (1.04$USA). The cost loss per herd and loss per individual animal in any herd were approximately 42.77ETB (1.02$USA) and 8.66ETB (0.206$USA), respectively.

4.4.5 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 owner's labor rate was 50ETB per 12 hour, with a two-day payment of 50ETB (1.19$USA). In some farms, an animal health worker visits the farm and treats the animals; in this case, the payment was 50ETB birr per day. Generally 21 farm owners was treating their animals, from them 6, 8 and 7 from dairy, small holder and beef farm respectively. Total cost during observation was 6722ETB (160.045$USA). Total loss per affected herd was 248.96ETB (5.93$USA) and within individual affected animal was 68.59ETB (1.633$USA). The total loss per any herd was 67.22ETB (1.6$USA) and total loss for any animal was 13.61ETB (0.324$USA).

4.4.6 Total cost determination

During the study period, the total economic loss was 555321ETB (13221.928$USA) as a result of milk loss, mortality, draft power, refatting cost, and treatment. The total loss per affected herd was 20567.44ETB (489.69$USA), with a loss of 5666.54ETB (134.91$USA) per individual animal.

The total economic loss due to foot and mouth disease was 5553.21ETB (132.21$USA) per herd, and the loss per individual animal was 1124.13ETB (26.76$USA). For each calculation (1$USA=42ETB; data from the Commercial Bank of Ethiopia)

5. DISCUSSION

5.1 Seroprevalence of FMD

The overall seroprevalence of FMDV in the western Amhara region was 4.63% in this study, but in another study (mesfine *et al*., 2019), the overall prevalence in the entire Amhara region was 11.04 %. Even though FMDV was endemic in our country and found in all regions of Ethiopia, the prevalence and distribution varied across districts (Mohamoud *et al.,* 2011; Gelaye *et al.,* 2009), which could be attributed to differences in land scape, agro ecology, and animal movement within Ethiopia (Mesfine *et al.,* 2019). Most of the districts chosen for this study had low prevalence in previous studies, with Awi zone having a relatively low proportion of FMD prevalence of 4.2% (Ayelet *et al.*, 2012) and in similar Awi zone having a prevalence of 1.3 % in this study. This could be due to the zone being in a relatively central location, or it being a non-boundary area with less movement of animals, and there being exported rather than imported from other areas. The second zone in this study was the south Gondar Zone, which had a prevalence of 9.6 percent in the previous study (Tesfaye *et al.,* 2016). However, the prevalence was 3.6 % in the current study. The overall prevalence as (Abunna *et al*., 2013) was 8.01 %. It was relatively approach to present study. Prevalence varies from place to place with seropostivity that ranges from 5.6% to 42.7% in cattle and from 4% to 11% in small ruminant. By means of a peasant association organization Dubaduba was highland than the other study peasant associations, and the prevalence of FMD was higher in this peasant association. On the other hand, zero ande were relatively mid land (temperate), whereas Banja Askun abo was relatively low land but had a higher prevalence. FMD was present in all ecosystems. In this study, cross-bred cattle had higher percentages of FMD seropositivity than local breed cattle, and the occurrence of FMDV in cross breed cattle was 3.4 times higher than in local breed cattle. In a previous study, crossbred cattle were 2.79 times more likely than local cattle (Sulayeman *et al,* 2018), and crossbred cattle were 6 times more likely than local cattle (Ahmed *et al*., 2020). Similar to this study in the previous study, there was no statistically significant difference between the six groups in this study (Mohamoud *et al*., 2011; Mazengia *et al*., 2010; Tesfaye *et al.,* 2016).

There was no statistically significant difference between age groups in this study similar to (Misgana *et al.,* 2013). In previous studies (Mohamoud *et al*., 2011; Gelaye *et al.*, 2009; Tesfaye *et al.,* 2016), there was a significant difference between age groups. The difference could be attributed to age delegation among researchers. There was a difference with herd size in this study, but it was only marginally significant. A herd size of more than five animals has a prevalence of 5.22%, while a herd size of less than five has a prevalence of 3.77%, but there is no statistically significant difference between them. In this study with questioner survey works, there was a significant difference in morbidity among production systems with p=0.0019, as there was in the serosurvey study with p=0.000. The prevalence of FMD in the semi-intensive production system is 25%, while the prevalence in the extensive production system is 3.4%. This difference may be due to animal density variation, as the semi-intensive production system had a higher density of animals in a fixed area than the extensive production system. FMD prevalence had increased in densely populated than scattered one, so ventilation and animal density may cause for variation in occurrence and distribution of the diseases. This is again proof in dairy farm and small holder farm which is the occurrence of FMD in dairy farm 4.9 times occurrence in small holder farms.

5.2 Molecular and Antigenic Detection

In the current study, out of 14 samples tested by real time PCR to detect the presence of genetic material (targeting of the 3D pol regions) of FMDV in the sample all 14 were found positive. Previously, (Paixo *et al.,* 2008; Longjam *et al*., 2011; Kasanga *et al.*, 2014) suggested that real-time RT-PCR targeting the 3D region of the viral genome was a powerful technique for FMDV detection, which is now a recent and reliable diagnostic test used to confirm the diseases. Previous research (Negussie *et al*, 2013) found serotype O to be the most prevalent and dominant serotype in Adet, causing the majority of outbreaks in the Amhara region. Similar to previous study Serotype O was also identified in Adet and Guzamn in our study. Serotype O considered as the most widely studied and common FMD serotype in the world as (Wubshet *et al.*, 2019) similar to that in this study 42% from the serotypes was O.

In other study (Tesfaye, 2020; Ayelet *et al.*, 2013) it was the most prevalent in central Ethiopia. In this study the next most prevalent serotype was SAT2 which was 35.7%. In previous study (Dubie and Amare, 2020) SAT2 was the cause for outbreak in Afar Region. In a previous study (Tesfaye, 2014), serotypes O, A, and SAT2 were found in Debre Birhan, Debrezyiet, and Addis Abeba, whereas in this study, serotypes O, A, SAT1, and SAT2 were found in Adet, Metema, and Guzman. The results show that the most common serotype in the Amhara region was serotype O, but all serotypes SAT1, SAT2, and A were found in this study district at different study times. In another study (Urge *et al*., 2020), the three serotypes O, A, and SAT2 were found to be circulating in the Adea berga district of the western Shewa zone.

5.3 Questionnaire Survey

In this study, approximately 70% of farmers are aware of foot and mouth diseases, whereas in previous studies in other districts in the Amahra region, approximately 85% of farmers are aware of the diseases, indicating that disease experience in the study area varies (Mesfine *et al*, 2019). According to this study, FMD was ranked one through three in terms of occurrence and impact. FMD was found to be more prevalent during the dry season than during the cold dry season. Another study (Molla *et al*., 2013) found that the fourth most important disease from other cattle health and highest outbreaks of the disease was observed during extreme long dry seasons of the years which were December to May. In this study also more outbreaks occur in dry season of the year when most animal movement occurs. This could be due to factors such as animal movement, as most of Ethiopia's crops are harvested during the dry seasons of the year and animals move freely from place to place. Smallholder and semi-intensive farms were more likely to have FMD. According to another study (Rufael *et al.*, 2008), the majority of cattle and herds are moved during the dry season to access grazing and water, and more transmission occurs across animals during this time. Individual animal morbidity from the affected herd was 64.9 % in this study. Another study (Jemberu *et al*., 2014) found that morbidity rates for FMD at the animal level were 74.3 % in the affected herds in a crop–livestock mixed system.

Similarly, morbidity in beef farms, small holder farms, and dairy farms was 63.3 % in beef farms, 60.5 % in small holder farms, and 73.3 % in dairy farms in this study. Almost similar result was found by serosurvey study that was the herd level seroprevalence was 58.6% (Bayissa *et al*., 2011).

The total annual costs of FMD under the current status of production and control cost estimated 1,354 million birr ETB and the major cost was due to production losses (Dabasa and Abunna, 2021). In this study the total economic loss due to FMD per any herd was 5553.21ETB (132.21$USA) and per any individual animal total loss was 1124.13ETB (26.76$USA). This economic loss was only with visible production loss and FMD treatment cost. In this study only cost loss due to FMD in study area was determined.

6. CONCLUSION AND RECOMMENDATION

FMD is more prevalent and endemic diseases in Ethiopia including Amhara region on rural communities as well as dairy and beef farms because of free movement of livestocks in different regions and ineffective control measure. Due to the frequent occurrence of FMDV in Ethiopia most animal owner know about foot and mouth diseases and use different control option by their selves. In this study the seroprevalence of foot and mouth diseases was 4.63% and it varies among study districts with a minimum prevalence of 1.3% in Banja to a maximum prevalence of 10% in Adet. The diseases mostly not influenced by agro ecology as it was found in every ecosystem. There was frequent outbreak in Ethiopia and during the study period serotype O, A, SAT 1, and SAT2 was identified from the collected tissue samples from Adet, Metema and Guzamn. The outbreak leads for huge economic crises. In the study area total economic loss was estimated as 555321ETB (13221.928$USA) during study period. Total loss per affected herd was 20 567.44ETB (489.69$USA) and 5666.54ETB (134.91$USA) per affected individual animal. The total economic loss due to foot and mouth diseases per any herd was 5553.21ETB (132.21$USA) and per any individual animal total loss was 1124.13ETB (26.76$USA). The impact of these diseases is very high with a huge economic loss so I strongly recommended that

- Awareness creation with regard to the clinical and economic consequences of FMD should be done to livestock owners
- Work more on serotypes, molcular epidemiology and vaccine trial for each serotypes circulating in the different part of Ethiopia.
- Detailed epidemiological investigations should be conducted
- Work on more about cost benefit analysis by including all type of economic loss and all infected population groups.

7. REFERENCES

Abera, E.A., Getnet, M. and Nigatu, L. (2019): Impacts of Climate Change on Bread Wheat (Triticum aestivum L) Yield in Adet, North Western Ethiopia. *J Pet Environ Biotechnol*, **10:** 396.

Abunna, F., Fikru, S. and Rufael, T. (2013): Seroprevalence of Foot and Mouth Disease (FMD) at Dire Dawa and Its Surroundings, Eastern Ethiopia. *Global Veterinaria*, **11(5):** 575-578.

Adege, H. and Masebo M.N. (2017): Assessment of the Major Animal Health Problems and its Economic Impact on Beef Cattle Export Industry at Adama Quarantine Station, Central Ethiopia. *Int. J. Vet. Heal. Sci. Res*. **5:** 165–170.

Ahmed, B., Megersa, L., Mulatu, G., Siraj, M. and Boneya, G. (2020): Seroprevalence and Associated Risk Factors of Foot and Mouth Disease in Cattle in West Shewa Zone, Ethiopia. *Veterinary medicine international*, 2020.

Akalu, B., (2017): Review on Common Impact and Management of Transboundary Animal Diseases. *Juniper Online J. Immuno Virol***, 2:** 1–7.

Alemayehu, G., Zewde, G. and Admassu, B. (2014): Seroprevalence of foot and mouth disease (FMD) and associated economic impact on Central Ethiopian cattle feedlots. *Journal of Veterinary Medicine and Animal Health*, **6(5):** 154-158.

Al-Salihi, K.A. (2019): The epidemiology of foot-and-mouth disease outbreaks and its history in Iraq. *Veterinary world*, **12(5):** 706

Aman, E., Molla, W., Gebreegizabher, Z. and Jemberu, W.T. (2020): Spatial and temporal distribution of foot and mouth disease outbreaks in Amhara region of Ethiopia in the period 1999 to 2016. *BMC Veterinary Research*, **16(1):** 1-8.

Aragaw, M. and Tilay, T. (2012): Measles outbreak in Simada District, South Gondar Zone, Amhara Region, May-June 2009: immediate need for strengthened routine and supplemental immunization activities (SIAs). *Ethiopian Journal of Health Development*, **26(2):** 115-118.

Ayelet, G., Gelaye, E., Negussie, H. and Asmare, K. (2012): Study on the epidemiology of foot and mouth disease in Ethiopia. *Rev Sci Tech,* **31(3):** 789-98.

Ayelet, G., Soressa, M., Sisay, T., Belay, A., Gelaye, E., Jembere, S., Skjerve, E. and Asmare, K. (2013): FMD virus isolates: the candidate strains for polyvalent vaccine development in Ethiopia. *Acta tropica*, **126(3):** 244-248.

Bayissa, B., Ayelet, G., Kyule, M., Jibril, Y. and Gelaye, E. (2011): Study on seroprevalence, risk factors, and economic impact of foot-and-mouth disease in Borena pastoral and agro-pastoral system, southern Ethiopia. *Tropical animal health and production,* **43(4):** 759-766.

Bayush, W. (2020): Isolation and molecular characterization of foot and mouth disease viruses in cattle from outbreaks occurred in different parts of Ethiopia. Addise Abeba University

Chakraborty, S., Kumar, N., Dhama, K., Verma, A.K., Tiwari, R., Kumar, A., Kapoor, S. and Singh, S.V. (2014): Foot-and-mouth disease, an economically important disease of animals. *Adv. Anim. Vet. Sci*, **2(2):** 1-18.

Clyde D. and Lane, Jr. (1970): Age determination in beef cattle Beef, Sheep and Horse, The Interstate printers and Publishers, Inc., Danville, IL adapted from the Stockman's Handbook, 4th edition.

Dabasa, G. and Abunna, F. (2021): Review on Epidemiology of Foot and Mouth Disease (FMD) in Ethiopia. *J Trop Dis*, **9:** 269.

Dubie, T. and Amare, T. (2020): Isolation, Serotyping, and Molecular Detection of Bovine FMD Virus from Outbreak Cases in Aba'ala District of Afar Region, Ethiopia. *Veterinary Medicine International*, 2020.

EL-Shehawy, L., Abu-Elnaga, H., Abdel Atty, Ma, Fawzy, H., A.-W. (2011): Laboratory diagnosis of FMD using real-time RT-PCR in Egypt. *Life Sci. J.* ***8:*** 384–387.

ESGPIP, 2009: Estimation of weight and age of sheep and goats. *Technical bulletin no,* p 23.

Fentie, M.B., Goshu, D. and Tegegne, B. (2020): Potato Value Chain Analysis in Banja District, Ethiopia.

Fosgate, G.T. (2009): Practical sample size calculations for surveillance and diagnostic investigations. *Journal of veterinary diagnostic investigation*, **21(1):**3-14.

Gelaye, E., Ayelet, G., Abera, T. and Asmare, K. (2009): Seroprevalence of foot and mouth disease in Bench Maji zone, Southwestern Ethiopia. *Journal of Veterinary Medicine and Animal Health,* **1(1):** 005-010.

Grubman, M.J. and Baxt, B. (2004): Foot-and-mouth disease. *Clinical microbiology reviews,* **17(2):** 465-493.

Hosamani, M., Basagoudanavar, S.H., Selvan, R.T., Das, V., Ngangom, P., Sreenivasa, B.P., Hegde, R. and Venkataramanan, R. (2015): A multi-species indirect ELISA for detection of non-structural protein 3ABC specific antibodies to foot-and-mouth disease virus. *Archives of virology,* **160(4):** 937-944.

Issa, Mishamo, S. (2016): Isolation, Molecular Characterization and Sero-prevalence study of foot-and-mouth disease virus circulating in central Ethiopia. Addis Ababa University college of Veterinary Medicine and Agriculture.

Jamal, S.M. and Belsham, G.J. (2013): Foot-and-mouth disease: past, present and future. *Veterinary research,* **44(1):** 1-14.

Jemberu, W.T. (2016): Bioeconomic modelling of foot and mouth disease and its control in Ethiopia (Doctoral dissertation, Wageningen University).

Jemberu, W.T., Mourits, M.C., Woldehanna, T. and Hogeveen, H. (2014): Economic impact of foot and mouth disease outbreaks on smallholder farmers in Ethiopia. *Preventive veterinary medicine,* **116(2):** 26-36.

Kasanga, C.J., Wambura, P.N., Rweyemamu, M.M., Yamazaki, W., Mioulet, V., King, D.P., Mulumba, M., Ranga, E., Deve, J., Mundia, C. and Chikungwa, P. (2014): Rapid, sensitive and effective diagnostic tools for foot-and-mouth disease virus in Africa: proceedings. *Onderstepoort Journal of Veterinary Research,* **81(2):**1-5.

King, D., Di-Nardo, A. and Henstock, M. (2016): OIE/FAO Foot-and-Mouth Disease Reference Laboratory Network: Annual Report 2017. Disease FWRLfF-aM.

Klein, J. (2009): Understanding the molecular epidemiology of foot-and-mouth-disease virus. *Infection, genetics and evolution,* **9(2):** 153-161.

Knight-Jones, T.J.D. and Rushton, J. (2013): The economic impacts of foot and mouth disease–What are they, how big are they and where do they occur? *Preventive veterinary medicine,* **112(3-4):** 161-173.

León, E.A. (2012): Foot-and-Mouth Disease in pigs: current epidemiological situation and control methods. *Transboundary and emerging diseases*, **59:** 36-49.

Longjam, N., Deb, R., Sarmah, A.K., Tayo, T., Awachat, V.B. and Saxena, V.K. (2011): A brief review on diagnosis of foot-and-mouth disease of livestock: conventional to molecular tools. *Veterinary medicine international*, 2011.Ltd. **(2005):** 232

Mahmoud, M.A.E., Ghazy, A.A., Shaapan, R.M. (2019): International Journal of Dairy Science Review Article Diagnosis and Control of Foot and Mouth Disease (FMD) in Dairy Small Ruminants ; Sheep and Goats. *J. Dairy Sci*. **14**: 45–52.

Mahy, B.W. (2005): Introduction and history of foot-and-mouth disease virus. *Foot-and-Mouth Disease Virus,* **1:** 8.

Mason, P. and Grubman, M. (2009): Vaccines to Control Foot-and-Mouth Disease. Vaccines for Biodefense, *Emerging and Neglected Diseases*, **361**:377.

Mazengia, H., Taye, M., Negussie, H., Alemu, S. and Tassew, A. (2010): Incidence of foot and mouth disease and its effect on milk yield in dairy cattle at Andassa dairy farm, Northwest Ethiopia. *Agriculture and Biology Journal of North America*, **1(5):** 969-973.

Melkegnaw, E. (2017): Beneficiaries'attitude towards productive safety net program in selected rural kebeles of Ebinat woreda, northwest Ethiopia. *Ethiopian Renaissance Journal of Social Sciences and the Humanities*, **4(2):** 13-13.

Menda, S., Jenberie, S., Negussie, H., Ayelet, G. and Amasalu, K. (2014): Molecular epidemiology of foot and mouth disease Virus Outbreaks in Ethiopia in 2011/2012. *AJAD*, **3:** 8-16.

Mesfine, M., Nigatu, S., Belayneh, N. and Jemberu, W.T. (2019): Sero-epidemiology of foot and mouth disease in domestic ruminants in Amhara Region, Ethiopia. *Frontiers in veterinary science,* **6**:130.

Misgana, D., Yasmin, J., Ahmed, I. and Addisalem, H. (2013): Sero-prevalence of foot and mouth disease of cattle in Bale Zone, Oromiya regional state, Ethiopia. *Global Veterinaria*, **11(1):** 59-64.

Mohamoud, A., Tessema, E. and Degefu, H. (2011): Seroprevalence of bovine foot and mouth disease (FMD) in Awbere and Babille districts of Jijiga zone, Somalia Regional State, Eastern Ethiopia. *African Journal of Microbiology Research,* **5(21):** 3559-3563.

Molla, B., Ayelet, G., Asfaw, Y., Jibril, Y. and Gelaye, E. (2013): Participatory epidemiology and associated risk factors of foot-and-mouth disease in cattle in South Omo zone, South-Western Ethiopia. *Journal of Veterinary Medicine and Animal Health,* **5(11):** 322-328.

Negussie, H., Ayelet, G., Jenberie, S., Minda, S. and Tesfaw, L. (2013): Molecular epidemiology and vaccine matching study on foot-and-mouth disease virus circulating in Ethiopia. *African Journal of Microbiology Research*, **7(44):** .5101-5106.

Paixão, T.A., Neta, A.V.C., Paiva, N., Reis, J.R., Barbosa, M.S., Serra, C.V., Silva, R.R., Beckham, T.R., Martin, B.M., Clarke, N.P. and Adams, L.G. (2008): Diagnosis of foot-and mouth disease by real time reverse transcription polymerase chain reaction under field conditions in Brazil. *BMC veterinary research*, **4(1):**1-6.

Paton, D., King, D. (2012): FMD Diagnostics Institute for Animal Health, Research and surveillance to prevent virus diseases of livestock and their transmission to humans.

Raies-ul-Islam, M., Kumar, V.A., Yadav, S.K., Pal, B.C. and Udit, J. (2009): Genetic and antigenic relationship between foot and mouth disease virus serotype Asia-1 isolate and vaccine strain. *Online Journal of Veterinary Research*, **13(2):** 114-119.

Roche, S.E., Garner, M.G., Sanson, R.L., Cook, C., Birch, C., Backer, J.A., Dube, C., Patyk, K.A., Stevenson, M.A., Yu, Z.D. and Rawdon, T.G. (2015): Evaluating vaccination strategies to control foot-and-mouth disease: a model comparison study. *Epidemiology & Infection,* **143(6):** 1256-1275.

Rufael, T., Catley, A., Bogale, A., Sahle, M. and Shiferaw, Y. (2008): Foot and mouth disease in the Borana pastoral system, southern Ethiopia and implications for livelihoods and international trade. *Tropical animal health and production*, **40(1):** 29-38.

Sewnet, A. and Kameswara, K.R. (2011): Hydrological dynamics and human impact on ecosystems of Lake Tana. Northwestern, Ethiopia.

Sharma, G.K., Mahajan, S., Matura, R., Subramaniam, S., Ranjan, R., Biswal, J., Rout, M., Mohapatra, J.K., Dash, B.B., Sanyal, A. and Pattnaik, B. (2015): Diagnostic assays developed for the control of foot-and-mouth disease in India. *World journal of virology*, **4(3):** 295.

Shimels, T.Y. (2019): Antigen detection and molecular characterization of foot and mouth disease virus from outbreak cases in Ethiopia. Adise Abeba University.

Sobrino, F., Sáiz, M., Jiménez-Clavero, M.A., Núñez, J.I., Rosas, M.F., Baranowski, E. and Ley, V. (2001): Foot-and-mouth disease virus: a long known virus, but a current threat. *Veterinary research*, **32(1):** 1-30.

Souley Kouato, B., De Clercq, K., Abatih, E., Dal Pozzo, F., King, D.P., Thys, E., Marichatou, H. and Saegerman, C. (2018): Review of epidemiological risk models for foot-and-mouth disease: implications for prevention strategies with a focus on Africa. *PloS one*, **13(12):** 296.

Stevenson, M.A. (2020): Sample size estimation in veterinary epidemiologic research. *Frontiers in veterinary science*, 7.

Sulayeman, M., Dawo, F., Mammo, B., Gizaw, D. and Shegu, D. (2018): Isolation, molecular characterization and sero-prevalence study of foot-and-mouth disease virus circulating in central Ethiopia. *BMC veterinary research*, **14(1):** 1-10.

Tadesse, B., Alamir, K. and Demssie, E. (2017): Review on the Control of Foot and Mouth Disease by Vaccination

Tadesse, B., Molla, W., Mengsitu, A. and Jemberu, W.T. (2019): Transmission dynamics of foot and mouth disease in selected outbreak areas of northwest Ethiopia. *Epidemiology & Infection*, 147.

Tadesse, B., Tesfahun, A., Molla, W., Demisse, E. and Jemberu, W.T. (2020): Foot and mouth disease outbreak investigation and estimation of its economic impact in selected districts in northwest Ethiopia. *Veterinary medicine and science*, **6(1):** 122-132.

Tesfaye, A., Mengistu, A., Rufael, and T. (2016): Sero-prevalence status of foot and mouth disease in the North Western Amhara Regional State, Ethiopia. *Ethiop. Vet. J*. **20:** 43–53.

Tesfaye, J. (2019): Review on the Epidemiology and Economic Impact of Foot and Mouth Disease in Ethiopia. *Agricultural Journal,* **14(5):** 79-93.

Tesfaye, Y. (2014): Isolation, Molecular characterization and vaccine matching of foot and mouth disease virus circulating in Ethiopia (Doctoral dissertation, MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Bishofu, Ethiopia).

Tesfaye, Y. (2020): Molecular characterization of foot-and-mouth disease viruses collected from Northern and Central Ethiopia during the 2018 outbreak. *Vet. World* **13:** 542–548.

Thrusfield M. Veterinary Epidemiology. 2nd ed. Oxford, UK: Blackwell Science Ltd (2005) p.232.

Tikuye yalew shimels. (2019): Antigen detection and molecular characterization of foot and mouth disease virus from outbreak cases in Ethiopia (Doctoral dissertation, Addis Ababa University).

Upadhayay, U.P.P.D.D. and Ewam, P.C.V.V. (2012): Epidemiology and diagnosis of foot-and-mouth disease: A review. *Indian Journal of Animal Sciences***, 82(6):** 543-551.

Urge, Beksisa, Fufa Dawo, Helen Aklilu, Neima Arebu, Markos Tadele, Tamirat Siyoum, Melese Eshete, Temesgen Kassa, Endale Yadesa, and Brehanu Abera. "Detection and Molecular Characterization of Foot and Mouth Disease Virus Serotypes from Outbreaks in Adea Berga District." *Livestock Research Results*, (2020), pp 40**.**

USDA (2020): The Foreign Animal Disease Preparedness and Response Plan, in: 4700, U.S.D. of A. (Ed.), Foot-and-Mouth Disease (FMD) Response Plan. Animal and Plant Health Inspection Service, United States, p3–46.

Wernery, U. and Kinne, J. (2012): Foot and mouth disease and similar virus infections in camelids: a review. *Rev Sci Tech*, **31(3):** 907-918.

Wondwossen T. (2017): Isolation, molecular characterization and vaccine matching of foot-and-mouth disease virus circulating in central Ethiopia. MSc thesis Submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University, 61p.

Wubshet, A.K., Dai, J., Li, Q. and Zhang, J. (2019): Review on outbreak dynamics, the endemic serotypes, and diversified topotypic profiles of foot and mouth disease virus isolates in Ethiopia from 2008 to 2018. *Viruses*, **11(11):** 1076.

Yan, H., Wang, J., Zhu, M., Miao, H., Peng, Z., Li, H. and Xin, A. (2017): Complete Genome Sequence of Foot-and-Mouth Disease Virus Serotype O Strain YNTBa from Yunnan Province of China. *Genome announcements*, **5(22).**

Yirgalem, M., Dawo, F., Gizaw, D., Mamo, B., Bilata, T. and Shegu, D. (2020): Phylogenetic and Sequence Variability Analyses of Vp1 Protein of Foot and Mouth Disease Viruses in Cattle in Amhara Region of Ethiopia.

8. APPENDICES

Appendix 1: Age determination for cattle

All animals categorized by age as adult and young, in this study the categories was suspection of their age by observe their teeth (dentation system). For bovine under 3 year (Six broad incisors showing wear) age considered as young and for sheep and goat lower than 2 year (that is on pair teeth wear of sheep and 2 pair of teeth wear of for goat).

12 months - All the calf teeth are in place.

15 months - Centre permanent incisors appear.

18 months - Centre permanent incisors showing some wear.

24 months - First intermediates up.

30 months – Six broad incisors up.

36 months – Six broad incisors showing wear.

39 months – Corner teeth up

42 months – Eight broad incisors showing wear

Adapted by (Clyde and Lane, 1970)

Appendix Table 1: Age estimation in sheep and goat by dentation

Number of permanent incisors	Estimated age range	
	Sheep	Goat
0 pair	Under one year	Under one year
1 pair	1-1½ years	1-2 year
2 pair	1½-2years	2-3 year
3 pair	2½-3years	3-4 year
4 pair	More than three years	More than four years
Broken mouth	Aged	Aged

Adopted by (ESGPIP, 2009)

Appendix Table 2: Sample collecting format for epithelial tissue

No	Id	Owner name	District	Peasant association	Breed	species	Sex	Age	Sample type	Date	Lat	long

Appendix Table 3: Sample collecting format for blood sample

Owner name	Animal Id	Zone	District	PA	Species	Sex	Age	Breed	Herd size	Farm type	Production system	Long	Lat	Date

PA: Peasant association

Appendix 2: Detailed Procedure during sampling of blood sample

1. Restrain cattle's within crash, and for small ruminants manually, hold with head elevated and jugular vein exposed
2. Antiseptic with alcoholic gauze to remove superficial dirt or debris and more visualizing raised vein.
3. Occlude jugular vein by applying pressure at the base of the jugular groove and insert needle firmly into vein at 20° angle then push the plain vacutainer tube into hub
4. 6ml to 8ml blood was collected and keep overnight at room temperature
5. Carefully decant the serum into cryovials, if the serum was not separated within overnight, centrifuge with manual centrifuge.
6. Keep under -20◦C until laboratory diagnosis

Appendix 3: sampling procedure for tissue and swab sample

1. Prepare viral transport media and dispense with Plain tube
2. Restrain the animal and open mouth and hold the tongue
3. Then mop up with a cotton swab and immediately inserted in to tube
4. Live tissue which was abrade from the tongue collected with forceps

5. The tube put into ice box containing icepack (ice)

6. Then keep with -20°C until processed

Appendix 4

A 100µl sample was dispensed into 96 well microplates, which was necessary to avoid incubation differences. Reagents were kept at room temperature and homogenized using a vortex machine. Each well was first filled with 50µl of dilution buffer 18. 30µl of positive control was added to wells A1 and B1, and the same amount of negative control was added to wells C1 and D1. The remaining micro well filled with 30µl sample. Then incubated at $37^{o}C$ for 2hours, after incubation the wells were washed 5 times with adding 300µl of wash solution immediately to avoid drying between washing. After washing 100µl of the conjugate 1X were added in to each wells and incubated for 30min at room temperature. Following incubation, the wells were washed 5 times with 300µl of wash solution, and 100µl of substrate solution was added to each well before incubating at room temperature for 15 minutes in the dark. 100µl of stop solution was dispensed into each well to stop the color reaction. Finally, optical density (OD) readings at 450nm were obtained using a spectrophotometer.

Appendix 5: In general, the extraction of a tissue suspension sample consists of four stages. First, total RNA was extracted by combining 140µl of the original tissue suspension sample with 560µl of viral lysis buffer and incubating it at room temperature for 10 minutes. The second stage was called binding, and it consisted of adding absolute alcohol and mixing it with a vortex for 15 seconds. All mixtures were transferred to a minspin column and centrifuged for one minute at 8000 revolutions per minute. Transfer the minspin column to another micro centrifuge tube and centrifuge at 8000 RPM for one minute. The third stage, washing, involved the addition of AW1 and AW2. Transfer the minspin column to another micro centrifuge tube and add 500µl AW1, then centrifuge at 8000 RPM for one minute. Wash once more with 500µl AW2 and centrifuge at 14000 rotations per minute for three minutes. Then, for another minute of drying, centrifuge at 14000 RPM for one minute. The fourth step is elution which was performed by adding the 60µl elution buffer (AVE) to each minspin column contain tube and incubate at room temperature for five minutes.

In the last centrifuge 8000 rotation per minute for one minute and was removed the minspin column. Extracted RNA with nuclease-free H_2O was stored in aliquots of 10 µl at -80 °C until required (Kasanga *et al*., 2014).

Appendix 6: To be more specific, first samples were diluted 12 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 in all wells including column 11 and 12 (positive and negative control, respectively) and plates were incubated at a room temperature for 1hour. After incubation, all the fluid in each wells were discarded and the plate were tap hard to remove all the residual fluid. Then 200µl of washing solution were added and incubated for 3min at room temperature, subsequently 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 1hour. After incubation discard the conjugate and wash with by fill 200 µl/well four times and leave for the last one for five minutes. Then 50µl of substrate per well was added to all wells and plates were covered and left at room temperature for 20minutes in the dark. The reaction was stopped by adding 50µl/well of stop solution (sulfuric acid (H_2SO4)). Immediately after blocking, mix the well content before reading with a spectrophotometer. The optical density (OD) of each well was done at 450 nm wavelength using micro plate reader

Appendix 7: Questionnaire format for survey data

Identification of each farmer

Zone

Districts

Village (PA)

Educational level of head of the household

Marital status

Total family size

Religion of the house hold

Do you have own farm land?

If yes its total area in hectar

If no source for farm land (specify)

Do you have own livestock?

Total number of livestock

If yes what is the number of livestock you own currently?

Number of oxen in the farm?

Number of milking cow?

Number of Cow?

Number of sheep?

Number of goat?

Number of calf

What type of cattle production system do you use?

Cattle grazing practice in the area?

Where is your cattle point of watering?

How do you house cattle?

Do you have a farm?

If yes for question number 8 what type of farm do you have?

Target specious?

Among your entrie income sorce, which item you use first for market to full fill your need?

What is your challenge to you to keep your livestock or cattle?

List down the most common diseases in your area?

Rank of foot and mouth diseases when comparing from other diseases?

Time of incidence of foot and mouth diseases?

Control method the owner use?

If an animal died of acute diseases and you got a cadaver, what you are going to do?

Do you know FMD?

Is there a history of diseases outbreak in your farm?

If yes in Qno 2, can you describe the symptom of these diseases you have observed?

Is there other animal disease in your farm before or after FMD outbreak?

If yes, what was the sign and symptom?

Total Number of animal's suffered in foot and mouth diseases outbreak?

Number of calf suffered with this outbreak?

Number of dry cows suffered with this outbreak?

Number of milking cow suffered with this outbreak?

Number of oxen suffered with this outbreak?

Number of goat suffered with this outbreak?

Number of sheep suffered with this outbreak?

Is there died milking cow?

Is there died shout?

Is there died dry cow?

Is there died oxen?

Number of died cow?

Number of died shout?

Number of died oxen?

Number of died calf with outbreak?

Cost of oxen before outbreak?

Cost of oxen after outbreak?

Cost of cow before outbreak?

Cost of cow after outbreak?

Cost of calf befor outbreak?

Cost of calf after outbreak?

Is there retained animal from market?

How many of them retained?

Any measure taken during outbreak?

Number of animal culled?

Number of animals sold with lower price than market price and cost of each diseased animal

Cost of treated per animal

Cost of vaccination per animal

Total number of treated animal

Total number of vaccinated animal

How many litters of milk you get in average per cow per day before diseases occurrence (milk/cow/day)

During outbreak, what are the average milk yields per cow per day you get?

Average price of milk per litter in your area?

For how many days the outbreak occurs in the dairy farm

How many animals infected in your farm?

Is your animal retained from selling or export?

How many of animal's retained from selling or export?

Cost for additional feeding and other facilities?

Average weight of animal in beef farm

Average price of beef per kg?

How many of sheep and goat retained from export?

Average weight of an each shoat in kg?

How many shout retained if there is?

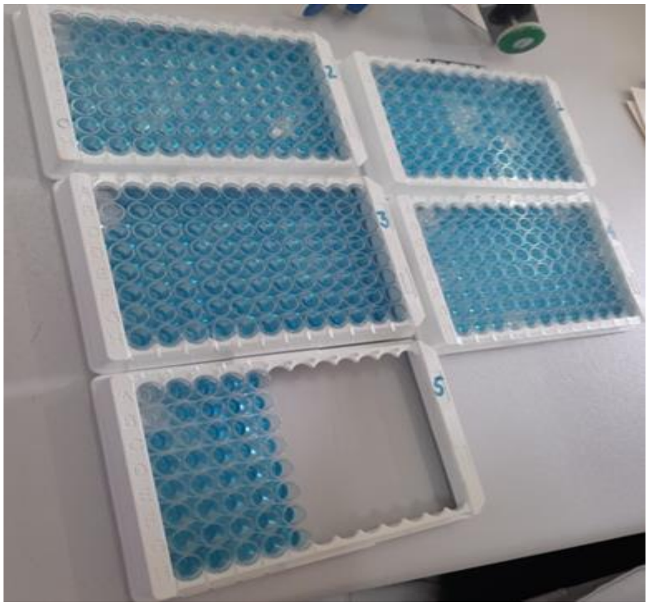
Average price of shoat per kg?

What is the mechanism to mitigate effect of FMD?

Appendix 8: Figures during study periods

Appendix Figure 1: During sampling observe FMDV infected cattle

Appendix Figure 2: Photo during serum separation from blood



Appendix Figure 3: Serological test result before stop solution added

Appendix Figure 4: Reagents used during serum test

Appendix 8: 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: Certificate Ref. No: VM/ERC/07/04/13/2021

Name of Applicant: **Yasmin Jibril** **(DVM, MSc, PhD)**

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

Title of the project: *Investigation of major trans-boundary animal diseases affecting export trade and improvement of healthcare decision making and veterinary medicinal products usage reporting system in Ethiopia*

Date of application:	**April, 2021**
Nature of the project:	**Mildly invasive /little stress**
Target animal species:	**Domestic Ruminants**
Number of animals involved:	**900**
Study area:	**Different parts of Ethiopia**

Minutes No. and date of review: **VM/ERC/04/13/021, 21/04/2021**

The above mentioned 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

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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