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**SEROPREVALENCE OF FOOT AND MOUTH DISEASE AND INDIGENOUS  
DISEASE MANAGEMENT PRACTICES IN SMALLHOLDER AND  
INTENSIVE DAIRY CATTLE PRODUCTION SYSTEMS IN JIMMA CITY,  
SOUTHWEST ETHIOPIA**



**MSc THESIS**

**ADDIS ABABA UNIVERSITY  
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE  
DEPARTMENT OF CLINICAL STUDIES**

**BY**

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**JUNE 2024**

**BISHOFTU, ETHIOPIA**

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DISEASE MANAGEMENT PRACTICES IN SMALLHOLDER AND  
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**A Thesis Submitted to Addis Ababa University, College of Veterinary Medicine and  
Agriculture in Partial Fulfillment of the Requirements for the Degree of Master of  
Veterinary Science in Veterinary Epidemiology**

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**JUNE 2024**

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As MSc research advisors, we hereby confirm that we have read and reviewed the Thesis written under our supervision by **Emraphel Ali**, entitled “**Seroprevalence of Foot and Mouth Disease and Indigenous Disease Management Practices in Smallholder and Intensive Dairy Cattle Production Systems in Jimma City, Southwest Ethiopia**”. We recommend that this research can be submitted as fulfilling the MSc Thesis requirement.

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## STATEMENT OF THE AUTHOR

First, I declare that this thesis research 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 MSc degree in Veterinary Epidemiology at Addis Ababa University, College of Veterinary Medicine and Agriculture. The thesis is stored in the University/College Library to be made available to borrowers in accordance with the rules of the library. I further solemnly declare that this thesis has not been submitted to any other institution anywhere for the purpose of receiving any academic degree, diploma or certificate.

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## **LIST OF ABBREVIATIONS**

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
LPBE	Liquid Phase Blocking Elisa
MCL	Mixed Crop Livestock
NSP	Nonstructural Protein
RNA	Ribonucleic Acid
SPCE	Solid Phase Competitive Elisa
TAD	Trans boundary Animal Disease
VNT	Virus Neutralization Test
VP	Viral Protein
WOAH	World Organization of Animal Health

## ABSTRACT

Foot and mouth disease (FMD) is a highly contagious viral disease that has a significant economic loss in the livestock sector in Ethiopia. A cross-sectional study was conducted to determine the seroprevalence of FMD, the associated risk factor for its occurrence, and FMDV serotypes circulating in the area. Moreover, efforts were made to assess the traditional FMD management practices in the study area. A total of 384 blood samples were collected from dairy cattle managed under various production systems and subjected to a 3ABC enzyme-linked immune-sorbent assay (ELISA) test to detect antibodies against non-structural proteins of FMD virus. A total of 22 serum samples exhibiting higher antibody titer were further tested using solid-phase competitive ELISA to identify the serotypes circulating in the area. Semi structured questionnaires were used to collect information on potential risk factors and to extract knowledge, attitudes, and practices among respondents pertinent to the epidemiology of FMD. In this study, the overall seroprevalence of FMDV in dairy cattle of Jimma City was 29.9% (95% CI: 25.4 - 34.8). A multivariable logistic regression analysis revealed that the risk of being FMDV seropositive was four times higher in crossbred cattle (OR = 4.15; 95% CI: 1.67 - 10.3; P = 0.002), three times higher in animals managed extensively (OR = 3.22; 95% CI: 1.33 - 7.83; P = 0.01), three times higher in animals uses communal grazing and watering points (OR = 2.5; 95% CI: 1.28-4.86; P 0.007), and two times higher in larger herd sizes (OR = 2.05; 95% CI: 1.059-4.17; P = 0.039). In this study, four FMDV serotypes were identified in which serotype O was the predominant serotype (33%), followed by SAT-2 (27%), serotype A (23%), and SAT-1 (17%). Of the 104 respondents, the majority (84.6%) knew about FMD. Additionally, the indigenous FMD management practice of the respondents demonstrated extensive use of indigenous treatments and herbal remedies such as *Solanum Nigrum* ('*Embuay*') (9.1%), *Urtica pilulifera L* ("*Sama*") (15.2%), suspension of honey with red pepper (42.4%), and tobacco leaves powder (12.1), and salt (21.2%) in managing FMD in cattle. This study demonstrated a high prevalence of FMD in the study area.

**Keywords;** *Jimma City, Cattle, Indigenous practice, FMD, Seroprevalence, Serotypes*

## 1. INTRODUCTION

Ethiopia is home to the largest cattle population with an estimated 70.3 million cattle (CSA, 2021). The livestock industry makes up roughly 45% of the agricultural GDP, 18.7% of the overall GDP, and 16 - 19% of the nation's foreign exchange profits (Behnke and Metaferia, 2011). However, the livestock potential is not exploited because of the multifaceted challenges of the sector. Among them, transboundary animal diseases provide significant issues for the livestock industry (Otte *et al.*, 2004). Among transboundary viral diseases affecting cloven-hoofed animals, foot and mouth disease (FMD) is a particularly significant disease that hampers the trade of animals and animal products to the international market.

Foot and Mouth Disease Virus (FMDV), the causal agent, is a member of the *Aphthovirus* genus and family of *Picornaviridae* viruses. It has seven different serotypes such as serotype A, O, C, South African Territories 1 (SAT-1), SAT-2, SAT-3, and Asia-1. Many biotypical strains and topotypes are included in each serotype, and little cross-protection has been shown between them (OIE, 2012; Brito *et al.*, 2016). Serotypes O and A are most common in Africa (Rweyemamu *et al.*, 2008; Jemberu *et al.*, 2016), although serotype C has seen a decline in reported cases in recent years. Several investigations have identified five out of the seven FMDV serotypes (O, A, C, SAT-1, and SAT-2) are reported in Ethiopia (Gelaye *et al.*, 2009; Urge and Gizaw, 2018; Menda *et al.*, 2014).

Foot and Mouth Disease (FMD) outbreaks have a significant impact on the cattle agribusiness chain, leading to a fall in the number of cattle, an increase in the importation of cattle and beef, a decrease in the consumption of beef, and significant financial losses (Knight-Jones *et al.*, 2017; Benjamin *et al.*, 2023). When FMD outbreaks occur in areas or nations that are FMD-free, severe and expensive control measures are implemented, including as trade prohibitions, movement restrictions, and culling, until the disease is completely eradicated and the FMD-free status is restored (Pendell and Cho, 2012; Sinkala *et al.*, 2014). Additionally, FMD reduces the trading potential of low- and middle-income nations, which are home to the majority of the

world's cattle population but only contribute a small portion of the world's livestock exports (Sinkala *et al.*, 2014).

Wildlife can serve as maintenance or bridge hosts for the spread of the disease. Sharing grazing or drinking areas, or breaching fences, can bring livestock and wildlife into touch (Abdela, 2017; Gortázar *et al.*, 2022). FMD epidemics are more likely as a result, particularly during the dry season when pasture and water are in short supply. During the dry season, the virus can also thrive more readily in the surroundings (Hemida *et al.*, 2018).

Ethiopia has recently increased efforts to control the disease with the goal of boosting live animal and meat exports as FMD is acknowledged as the main obstacle to international commerce in livestock and meat (MOARD, 2006). However, in endemic and low-income countries like Ethiopia, managing FMD presents serious difficulties. These difficulties include the extensive prevalence of several viral serotypes and subtypes, the large variety of hosts that can become infected, the uncontrolled movement of animals, and the scarcity of reasonably priced and potent FMD vaccinations. Moreover, to successfully control FMD in endemic nations like Ethiopia, the need for implementing a long-term, progressive risk reduction plan (Paton *et al.*, 2009; Rweyemamu *et al.*, 2008b).

Jimma is an important urban hub in Ethiopia. The Jimma dairy farms are important since they are the main providers of milk to the city and its neighboring areas. Diseases such as FMD have an effect not just on individual farms, but also on the whole milk value chain, which puts the residents of this crowded metropolis in southwest Oromia at risk. Limited information is available on the status and recurrence of FMD in Jimma City and all districts in Jimma Zone. The epidemiology and economic impact of FMD should be studied on a regular basis for the purposes of prevention, control, mitigation, and eradication. For effective control and prevention, all the serotypes circulating in the area should be identified. Understanding farmers' indigenous practices towards FMD is essential for developing effective disease management strategies that are practical, feasible, and acceptable to farmers.

## **General Objective**

- ❖ To determine the epidemiology of FMDV in the dairy production system and to assess the indigenous knowledge and FMD management practices of the livestock owners and attendants in Jimma City, Oromia regional state, Ethiopia.

## **Specific Objectives**

- To investigate the seroprevalence of FMDV in various production systems
- To identify the FMD serotypes circulating in the districts
- To assess the knowledge and Indigenous management practice towards FMD in the study area
- To identify risk factor for the occurrence of FMD in the study area

## 2. LITERATURE REVIEW

The most severe and infectious Trans border Animal Disease (TAD) affecting cloven-hoofed wild and domestic animals is Foot-and-Mouth Disease (FMD) (Sumption *et al.*, 2007). It is one of the most important animal diseases for commerce worldwide (Knight-Jones and Rushton, 2013).

### 2.1. Etiology

Foot and mouth disease (FMDV), an infectious viral infection caused by the FMD virus (FMDV) of the genus Aphthovirus in the Picornaviridae family, can affect animals with cloven hooves (Quinn *et al.*, 2011). A polypeptide that can split into four structural proteins (SPs) and numerous nonstructural proteins (NSPs) is encoded by the 8,400 nucleotides of single stranded ribonucleic acid (ssRNA) that make up the FMDV genome (Jamal and Belsham, 2013). There are seven serotypes of FMDV: SAT 1, SAT 2, SAT 3, O, A, C, and Asia 1. Based on the genetic and antigenic variety of the capsid genes, particularly the VP1 region, these serotypes exhibit distinct sub-lineages or topotypes. Although serotypes differ by 30–50% in the VP1 region, serotypes generally have 86% of the same characteristics. New strains of FMDV are continuously emerging in the field, with the potential to trigger outbreaks and spread to new regions. Based on their geographic distribution and distinctive characteristics, FMDV serotypes and their sub-lineages, or topotypes, are categorized into seven virus pools (Hammond *et al.*, 2021). These pools are dispersed throughout the Americas, Asia, Africa, the Middle East, and Europe (Hammond *et al.*, 2021; Yang *et al.*, 2008).

The most significant structural protein of FMDV is viral protein 1 (VP1), which controls the host's immunological response and serotype. Part of the virus's exterior covering, the capsid, which interacts with host cell receptors and shields its genetic material, contains VP1. On the capsid surface, VP1 is the most exposed and changeable protein. It has the FMDV main antigenic site, which the host's antibodies can identify. Additionally, VP1 plays a role in the attachment of FMDV to integrins and heparan sulfate proteoglycans, two host cell receptors. To

evade the host's immune response and infect fresh cells, VP1 can alter its antigenicity and structure (Domingo *et al.*, 2005; Knowles *et al.*, 2003).

The most crucial target for developing vaccines against FMDV and selecting against it is viral protein 1 (VP1). Vaccines are drugs that, without actually spreading illness, encourage the body to create antibodies against a particular virus. Vaccines can shield the host from diseases in the future caused by the same or related pathogen strains. To be effective, vaccinations must, however, match the circulating strains of FMDV due to the virus's considerable diversity, particularly in the VP1 region. Thus, it is crucial to keep an eye on how FMDV serotypes and their sub-lineages, or topotypes, are evolving and spreading in order to update vaccines appropriately (Paton *et al.*, 2009).

## **2.2. Epidemiology of Foot and Mouth Diseases**

### *2.2.1. Transmission*

FMD viruses can spread in two ways: they can be detected in all bodily fluids and excretions, including expired air, of animals that are seriously ill. Though occasionally infections are transferred by carrier animals and susceptible individuals, the majority of illnesses are most likely spread by direct contact between acutely unwell and fragile individuals. It is widely accepted that the most common way that FMD spreads is through physical contact between susceptible animals and infected animals, often as a result of infected animals moving around (Pharo and Cobb, 2011). Cattle and pigs are known to get diseases primarily through inhalation and eating, respectively (Savill *et al.*, 2006).

Inhaling virus particles from the breath of an infected animal is the main way that FMD is disseminated. However, the disease can also spread through the air under some situations. Control measures are difficult since the virus can travel via the air and infect animals outside of quarantine zones in the correct conditions (Brown *et al.*, 2022). Pigs can get the disease through their digestive tracts from contaminated food, water, or

fomites (Souley *et al.*, 2018), but cattle are mostly impacted by the respiratory or airborne routes.

If the environment becomes contaminated by infected animals, the virus can also spread indirectly. Favorable conditions, such as temperatures below 50 °C, relative humidity above 55%, and neutral pH, allow the virus to persist for an extended period of time (Colenutt *et al.*, 2018; Colenutt *et al.*, 2020). Because the virus can infect people through many sources, this complicates the epidemiology and control of FMD. According to Colenutt *et al.* (2018), animal excretions and secretions can spread the disease and be used as noninvasive samples for monitoring and diagnosis.

### 2.2.2. FMD risk factors across species

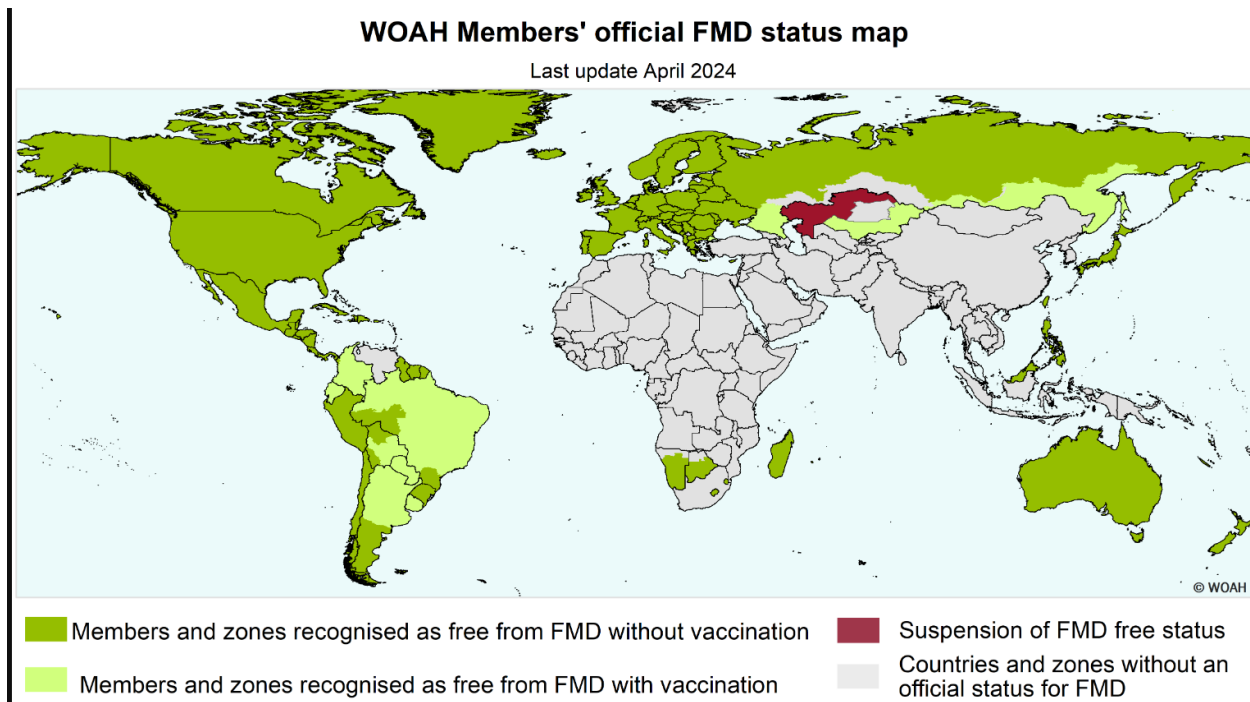
One of risk factor for FMD occurrence is contact with wildlife (Dubie, 2021); nevertheless, the African buffalo, or *Syncerus caffer*, is the only known wild-life reservoir (WOAH, 2023). In addition to being impacted by FMD outbreaks, wildlife can serve as maintenance or bridge hosts for the spread of the illness. Sharing grazing or drinking areas, or breaching fences, can bring livestock and wildlife into touch (Abdela, 2017; Gortázar *et al.*, 2022). FMD epidemics are more likely as a result, particularly during the dry season when pasture and water are in short supply. During the dry season, the virus can also thrive more readily in the surroundings (Hemida *et al.*, 2018).

Since different species may differ in their susceptibilities and levels of immunity to the virus, keeping different species of livestock together can raise the risk of FMD transmission. Sheep and goats, for instance, have the ability to transmit FMD to cattle silently and without any noticeable symptoms. Due to genetic characteristics that alter their immunological response to the virus, some breeds of cattle are more resistant to FMD than others. According to Hemida *et al.* (2018), Borana cattle in Ethiopia, for example, have a lower infection rate and exhibit milder symptoms than cattle of other breeds.

### 2.2.3. *Host range and geographic distribution*

A variety of livestock species are prone to infection and can disseminate the disease, including cattle, pigs, sheep, goats, and water buffalo (*Bubalus bubalis*). Cattle, sheep, and goat breeds native to Africa and Asia, where foot and mouth disease (FMD) is widespread, have much less evident or sub-clinical disease. It is thought that these animals are the source of infection for formerly disease-free nations (Sumption *et al.*, 2007).

Through trade and migration, FMDV may have emerged in the Middle Ages and spread from the Mediterranean region to other areas of the world. When conditions are right, FMDV can live for a very long time. There are seven serotypes of FMDV, and each has unique evolutionary links and molecular traits. Additionally, FMDV frequently recombines, which increases its genetic variety and complicates the development and selection of vaccines (Hammond *et al.*, 2021). Across the world, FMD affects 77% of livestock, mostly in Africa, the Middle East, Asia, and some parts of South America. Even after vaccination, foot and mouth disease (FMD) can still spread to countries where the virus is not yet present because certain vaccinated animals may become virus carriers or remain prone to infection. For example, in 2019, Mongolia had a recurrence of FMD despite having implemented a vaccine program and been clear of the disease since 2017. The outbreak was caused by a new strain of FMDV that was not shielded by the vaccination (WOAH, 2023). The cost of FMD prevention and control is borne by most low- and lower-middle-income countries (75%). According to WOA (2023), the regions with the highest costs are Africa and Eurasia, which account for half and a third of the worldwide total, respectively.



**Figure 1:** World FMD Status Map:

Adopted from <https://www.woah.org/app/uploads/2023/05/fmd-world-eng.png>

According to official data, China has experienced 140 FMD outbreaks since 2010. 109 FMD outbreaks affecting sheep, goats, pigs, and cattle were reported in 24 Chinese provinces between 2010 and 2019 (WOAH, 2023; Chen *et al.*, 2020). The most recent event, which impacted 76 animals, took place in October 2020 in Heshuo County, Xinjiang Uyghur Autonomous Region. Moreover, the prevalence of small-scale farming and grazing in these regions may increase the likelihood of FMD transmission (Haoran *et al.*, 2021). Brazil and Kenya were the final two countries in which serotype C of the 2004 FMD virus was discovered. Serotype C has been considered extinct ever then (Jamal and Belsham, 2013)

In order to reduce FMD, the FAO and OIE introduced a 15-year global strategy in 2012. The plan's objective is to mitigate the detrimental effects of FMD on economic growth, animal welfare, and food security. The plan calls for the implementation of progressive control measures based on risk assessment and surveillance as well as the improvement of veterinary

services (FAO, 2023). By using zoning and vaccination tactics, most of the countries in Latin America have been FMD-free. While FMD is not present in some other parts of the world, it can nonetheless sporadically appear there. The World Health Organization (WOAH) has a list of official nations that are free of foot and mouth disease (FMD) over their whole borders or in particular zones and sections of their territory (WOAH 2023). Early detection, warning systems, and surveillance are critical for the disease's prevention.

#### *2.2.4. Molecular epidemiology of FMDV*

Understanding FMDV distribution and disease conditions, as well as creating efficient control strategies, depend on molecular epidemiology. It deduces the origin, propagation, and relatedness of FMDV strains using their genetic sequences, especially the VP1 gene, which encodes the main capsid protein and antigenic determinant of the virus. In addition, VP1 can be used to track the spatiotemporal distribution of viruses linked to FMD outbreaks, animal movements, transcontinental introductions, and interspecies transmission events (Ularamu *et al.*, 2017). A more effective technique for tracking transmission both within and between herds is whole-genome sequencing of FMDVs that produce subclinical infections, which has been documented in a small number of investigations (Ramirez-Carvajal *et al.*, 2018).

New FMDV variants that could endanger animal health and commerce can also be monitored by molecular epidemiology (Kassaw *et al.*, 2013; Brito *et al.*, 2018). According to Kassaw *et al.*, (2013), the primary antigenic sites of the FMDV capsid have been identified in order to track down virus strains and their modes of transmission. In addition, formal and informal cattle migrations, geographic relatedness among FMD viral isolates, and interspecies transmission all impact the incidence of FMD. According to Cottam *et al.* (2008), whole genome sequencing can be used to rebuild transmission paths between farms during epidemics and differentiate between closely related viruses. Although there is 86% similarity among virus serotypes, there is a substantial genetic variability due to the high rate of replication errors in the FMD viral RNA replication process (Belsham, 2020). FMDV's main immunogenic epitopes are found on the variable protein VP1. There are 30–50% differences between the seven serotypes (Ularamu *et al.*, 2017).

Variation, genetic linkages, and geographic distributions among various FMDV serotypes can be discovered using phylogenetic analysis of VP1 nucleotide sequences (Pybus and Rambaut, 2009). Consequently, molecular diversity analyses that identify lineages and topo-types can benefit from the VP1 genomic area. However, because complete genome sequences have amino acids specific to specific serotypes, which enable serotype differentiation (Knowles *et al.*, 2016), they can offer additional insights into the evolution and virulence of circulating strains (Naqvi *et al.*, 2022). The whole FMDV genome sequence, particularly the ORF region, can provide information about amino acid alterations that impact the immunogenicity, antigenicity, disease prognosis, and viral transmission specific to a certain serotype (Hassan *et al.*, 2022). Analyzing the FMDV capsid proteins molecularly can yield comprehensive details regarding the antigenic and genomic properties of the virus serotype (Hagag *et al.*, 2023). Animal migration, vaccination- and infection-induced partial antibody responses in some regions, and a high population vulnerable to FMD all contribute to the formation and dissemination of FMDV genetic lineages (Subramaniam *et al.*, 2022).

#### *2.2.5. The economic consequences of FMD*

##### *2.2.5.1. Global impact of FMD*

Foot and Mouth Disease (FMD) outbreaks have a significant impact on the cattle agribusiness chain, leading to a fall in the number of cattle, an increase in the importation of cattle and beef, a decrease in the consumption of beef, and significant financial losses (Knight-Jones *et al.*, 2017; Benjamin *et al.*, 2023). Livestock owners who are affected by FMD also face serious social repercussions, including disease, stress, depression, stroke, divorce, and even suicide. When FMD outbreaks occur in areas or nations that are FMD-free, severe and expensive control measures are implemented, including as trade prohibitions, movement restrictions, and culling, until the disease is completely eradicated and the FMD-free status is restored. For example, the UK, Japan, and South Korea FMD outbreaks have demonstrated the susceptibility of even highly developed biosecurity systems to the introduction and spread of FMD and the ensuing losses in both home and export markets (Pendell and Cho, 2012; Sinkala *et al.*, 2014).

A significant outbreak of FMD struck the United Kingdom in 2001, killing approximately six

million animals at an estimated cost of £8 billion (Donaldson *et al.*, 2001). India is among the nations where FMD has the greatest economic impact because of the country's high cattle population and low vaccination rate. According to Knight-Jones and Rushton (2013), FMD-related losses in India are projected to be around USD 4.5 billion annually. Additionally, FMD reduces the trading potential of low- and middle-income nations, which are home to the majority of the world's cattle population but only contribute a small portion of the world's livestock exports. For instance, Uganda is unable to reach profitable export markets due to its endemic FMD status (Baluka, 2016). Due to import restrictions linked to FMD, Zambia loses \$1.6 billion annually in beef and sable antelope exports to Botswana and South Africa (Sinkala *et al.*, 2014).

In order to control FMD, public funding for veterinary services and surveillance networks both within and across nations is needed. These measures may assist in the control of other livestock disease. By strengthening FMD control in endemic areas and defending FMD-free areas, the Global FMD Control Strategy seeks to benefit developing nations and the animal trade (FAO, 2023). Controlling FMD can lessen the disease's direct consequences on animal productivity and production, which in endemic areas can cost anywhere from USD 6.5 to USD 21 billion annually (Knight-Jones and Rushto, 2013; Abao *et al.*, 2013). The implementation of control measures for livestock disease (FMD) can help prevent or limit export losses resulting from trade restrictions on livestock and livestock products. These losses can account for a significant portion of the overall expenses associated with an FMD outbreak, depending on the country (Marschik *et al.*, 2021).

#### *2.2.5.2. Economic losses of FMD in Ethiopia*

Lower milk production, problems with reproduction, early culling, and calf mortality are all consequences of the disease that reduce animal productivity. According to Tadesse *et al.* (2020), trade barriers, restrictions on travel and unfavorable opinions also limit market access and tourism potential. The export embargo between 2005 and 2006 cost the nation more than 14 million USD (Pal, 2018). Ethiopia is home to a large number of people affected by the deadly foot and mouth disease (FMD), which has a negative impact on livestock owners, attendants, and

other industry participants' livelihoods (Yang *et al.*, 2020). Comparably, a bull shipment that was turned down by a foreign market in 2011 was said to have caused an economic loss of 3,322,269 USD (Alemayehu *et al.*, 2014). The economic losses in northwest Ethiopia caused by FMD varied according to the industrial system. Mixed crop-livestock (MCL) systems experienced losses of USD 34 per affected herd, as opposed to USD 459.1 per impacted farm for commercial dairy farms (Tadesse *et al.*, 2020).

The average losses under the MCL system were 9.8 USD per animal and 76 USD per herd. In the pastoral system, the average losses were 5.3 USD per animal and 174 USD per herd. Jemberu *et al.* (2014) reported that the MCL system experienced animal-level mortality losses of 129 USD, whereas the pastoral system experienced losses of 151 USD. Together with export losses and control costs, there was an estimated 1,350 million ETB annual economic loss. But the production losses were the most costly. Furthermore, Jemberu *et al.* (2016) reported that animal death accounted for almost 300 million ETB of the annual loss caused by FMD export expenses.

#### *2.2.6. Morbidity and mortality*

Although foot and mouth disease has a low death rate, it has a high morbidity rate. Some of the primary factors associated with the morbidity and death rate of the disease are breed type, production system, age group, no mobility limitation, animal density, use of shared pasture and watering locations, and season (Jemberu *et al.*, 2016a).

### **2.3. Diagnosis of FMD**

A reliable detection of FMDV infection is essential for both control and eradication efforts in places where FMD is endemic (Longjam *et al.*, 2011). Elevated body temperature, increased salivation, vesicle formation on the nose, mouth mucosa, interdigital spaces, and foot coronary bands are clinical markers of the syndrome. However, clinically speaking, it is impossible to distinguish virally-caused vesicular illnesses such as foot and mouth ailment from one another. To differentiate swine vesicular disease, vesicular exanthema, and vesicular stomatitis from foot and mouth disease, laboratory tests were required (Jamal and Belsham, 2013).

Tissue epithelium or vesicular fluid obtained from an unruptured or freshly ruptured vesicle typically from the tongue, buccal mucosa, or feet is the preferred sample for laboratory diagnosis. Probang cups are used to collect samples of oropharyngeal fluid (or, in the case of pigs, swabbing the throat) in order to diagnose situations where epithelial tissue is not available, such as in advanced or convalescent cases or where infection is suspected despite the lack of clinical signs. Based on the spike of antibodies against a certain serotype, serum samples are also utilized for FMD diagnosis (Musser, 2004). According to Grubman and Baxt (2004), the laboratory methods for diagnosing foot and mouth disease (FMD) include virus isolation, demonstrating the presence of FMD viral antigens or nucleic acid in a sample tissue or fluid, and detecting antibodies specific to the virus.

### *2.3.1. Virus isolation*

Isolation and virus characterization represent the "golden standard" for diagnosing viral infections (Admassu *et al.*, 2015). An infectious virus must exist in order to isolate one, and sample quality establishes this. If the virus is present in low concentrations, it may take up to 4 days to manifest its presence (Jamal and Belsham, 2013). Sensitive cell culture systems include primary thyroid cells from cows and kidney cells from pigs, calves, or lambs. Established cell lines such as IBRS-2 and BHK-21 (baby hamster kidney) are often less sensitive than primary cells for detecting low levels of infectivity, yet they can still be used. The sensitivity of any cells used should be evaluated using standard FMDV preparations (Sissay *et al.*, 2017).

According to Bouma *et al.* (2001), pig cell lines were not always appropriate for isolating FMDV originating from other species. It appears that the BHK-21 cell line is less species-dependent. For a full 48 hours, the cell cultures should be observed for the Cytopathic Effect (CPE). The cells should be used to inoculate new cultures after being frozen and thawed. If CPE is not found, the cells must be observed for a full 48 hours. By freeing the virus from immune complexes, pretreatment with an equivalent volume of chloro-fluoro-carbons may increase the rate of virus detection in OP fluids (Sissay *et al.*, 2017).

### 2.3.2. Serological approaches

The amount of antibodies produced when animals are exposed to FMDV or get FMDV vaccinations is commonly measured using serological assays. Complement fixation tests (CFT) are still employed in a small number of labs despite the usage of Enzyme Linked Immunosorbent Assays (ELISAs) and Virus Neutralization Tests (VNTs) as the primary procedures. Antibodies to FMDV structural proteins are useful in the diagnosis of past or current infections. These assays include the tried-and-true serotype-specific Virus Neutralization Tests (VNT), the Solid-phase Competition ELISA (SPCE) and Liquid-phase Blocking ELISA (LPBE) ELISAs (Deb *et al.*, 2013).

These serological tests are specifically intended to identify animals that are FMDV-positive within a vaccinated herd. This concept is called DIVA (differentiating infected from vaccinated animals), and it is based on variations in the antibody (humoral) responses produced in vaccinated animals compared to animals that are naturally infected with FMDV (independent of vaccination status). The majority of the viral non-structural proteins (NSP) have been extracted using the purified structural protein (SP) present in high-quality FMDV vaccinations. On the other hand, when FMDV infection occurs spontaneously, viral NSP are expressed and cause a corresponding immune response that may be detected using diagnostic methods (Ludi *et al.*, 2017).

### 2.3.3. Nucleic acid recognition method

The most popular nucleic acid-based diagnostic approach for swiftly detecting the FMD virus is PCR, as reported by Xu *et al.* (2013). A specific reverse transcriptase polymerase chain reaction was developed and shown to be 1000 times more sensitive than isolating a single passage virus in order to identify the FMD polymerase gene (3D) (Longjam *et al.*, 2011). The World Organization for Animal Health (WOAH) suggests employing fluorescently tagged probes and universal primers in real-time PCR experiments to identify FMDV. These probes are intended to detect conserved areas in the 5' UTR or within the RNA-dependent RNA polymerase gene (Reid *et al.*, 2000). The most rapid and precise method for determining nucleic acid is this one. The viral RNA is constantly detectable over a seven-log range, with the lowest log range

corresponding to as few as 10-100 RNA per volume tested. Using portable equipment, the test can be finished in two hours or less while the sample is kept at room temperature. While real-time chemistry makes it possible to identify PCR amplification early in the reaction, real-time PCR monitors the progress of a PCR reaction in real-time. Furthermore, it is feasible to quantify a relatively small quantity of PCR products (DNA or cDNA) (Shaw *et al.*, 2007).

#### **2.4. Prevention and Control**

Livestock and wildlife animals are susceptible to the extremely contagious viral disease (Foot and Mouth Disease) (Paton *et al.*, 2009). Countries must evaluate their disease risk and take the necessary steps to lower it in order to control FMD. The Progressive Control Pathway for FMD (PCP-FMD), which directs nations to progressively and methodically improve their FMD condition, is one framework for doing this (FAO, 2023). Vaccinating susceptible animals, limiting the transportation of animals and animal products, and eliminating diseased and exposed animals are some of the strategies that nations might employ to prevent FMD. These precautions are limited, though, as the FMD virus is multitudinous and highly transmissible (Kamel *et al.*, 2019). Thus, it's critical to enhance veterinary services and laboratories, develop better diagnostic and vaccine technologies, plan national and regional initiatives, and encourage legislators, traders, consumers, and livestock owners to embrace best practices (Paton *et al.*, 2009).

Since FMD is a viral infection, there is no known cure for it. Thus, in the event of an outbreak, the following preventive measures should be put into place while the disease is not yet present: monitoring national borders to prevent significant animal and livestock product movement; banning the import of animals and livestock products from countries where the disease is endemic; and putting emergency protocols in place that include taking immediate action to kill infected animals and infected animals in contact, as well as thoroughly cleaning and disinfecting them to lower the risk of re-infection. In addition, emergency immunization needs to be considered (Ding *et al.*, 2013).

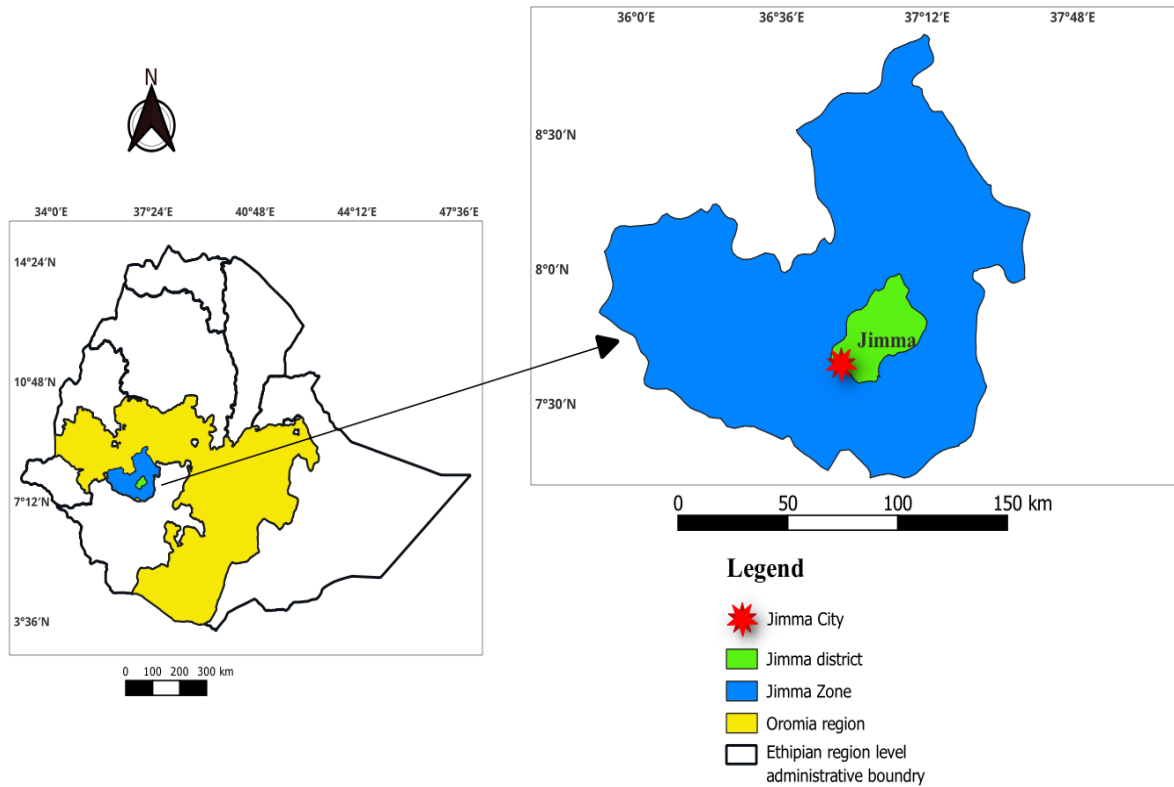
To prevent Foot and Mouth Disease (FMD), Ethiopia uses vaccination campaigns, quarantines, livestock separation, proper disposal of contaminated carcasses, and other financially feasible

methods (Admassu *et al.*, 2015). There are two reasons that could account for the rise in FMD outbreaks in some endemic areas, such Ethiopia. The poor level of immunity caused by inefficient vaccination programs (in terms of timing, coverage, and quality) is one factor. The other is uncontrolled animal movement and products. Animal diseases like FMD can only be effectively controlled with a strong regional focus and coordinated regional activities to strengthen biosecurity and regulatory oversight of the movement of animals between and within nations (Ayelet *et al.*, 2012).

### 3. MATERIAL AND METHODS

#### 3.1. Description of the Study Area

The study was conducted in the southwest of Ethiopia's Oromia Region, Jimma zone, which includes the area around Jimma Town. Jimma Town is located at 7°40'N, 36°50'E latitude and longitude. Every year, the area has a long wet season that lasts from March to October. Jimma experiences daily maximum temperatures usually between 24 and 27 °C and has annual average rainfall of 1500mm throughout the year. Research districts were chosen based on a number of criteria, including a history of FMD outbreaks, a sizable population of cattle under varying management levels (extensive, semi-intensive, and intense), and a sizable amount of livestock exchange with other areas.



**Figure 2:** Map of the study area that showed Jimma City, Ethiopia.

### **3.2. Study Population**

Dairy cattle of all ages and breeds managed under extensive, semi-intensive and intensive production in Jimma City. This study targeted dairy cattle that were not vaccinated and older than six months. Local cattle breeds as well as their crosses with Jersey and Holstein Friesians breeds make up the study population. Smallholder livestock owners mostly rely on extensive production systems, whereas commercial dairy farms use intensive and semi-intensive management systems. The animals were divided into three age groups: calf (6 months to 2 years old), young (2 years to 4 years old), and adult (above 4 years old) based on some modification of Chowdhury *et al.* (2020). The classification of the herd size was based on Seifu *et al.* (2023) with minor modifications that small (< 15 cattle), medium (15 – 35 cattle), and large (> 35 cattle) herd sizes.

### **3.3. Study Design and sampling strategy**

A cross-sectional study coupled with a structured questionnaire survey was conducted from November 2023 to May 2024. Multistage sampling strategy that encompasses purpose sampling of Kebeles with higher cattle population followed by a stratified sampling of herds based on production systems. Then simple random sampling of cattle from each herd was followed in recruiting sampling elements for serological survey. Animals with recent vaccination history and calves less than 6 months of age were excluded from the sampling unit.

A questionnaire survey was also conducted to get relevant data on the awareness of livestock owners and attendants on FMD. The potential risk factors for both individual animals and herds, such as breed, age, sex, farm size, communal grazing, and other characteristics were documented. Data on livestock owners' and attendants' knowledge and traditional methods of managing the disease were gathered using a pretested, semi-structured questionnaire (Annex 5). In each farm, in-person interviews were done with respondents using the local language.

### 3.4. Sample size determination

The sample size was calculated for the purpose of estimating FMD seroprevalence using the formula provided by Thrusfield (2017) for a hypothetically infinite population. Therefore, 384 dairy cattle sera were sampled using the following criteria: 50% expected prevalence (P), 95% confidence interval (Z), and 5% desired precision (d) (Quinn *et al.*, 2011).

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

Concurrent with the collection of blood samples, a knowledge survey of livestock owners and attendants was conducted in the district. A sample of 104 livestock owners and attendants were chosen at random. With a 95% confidence interval and a standard error (SE) of 0.05, the sample size was calculated using the formula ( $n = 0.25/SE^2$ ) as per Arsham (2002).

### 3.5. Data collection

#### 3.5.1. Blood sample collection

From each animal, 10 milliliters of blood were drawn from the jugular vein using a needle and plain vacutainer tubes under aseptic condition. Each vacutainer tube had a label with information on it, including the type of farm, the sampling location, the date, and identifying codes. Following a night of upright storage at room temperature, the serum samples were separated into 2-milliliter sterile cryovials. The serum was then transported using an icebox containing icepacks to the Animal Health Institute (AHI), Sebeta, Ethiopia. The samples were stored at -20 °C until processed.

#### 3.5.2. Questionnaire Survey

To evaluate the knowledge, practices, and attitudes of dairy cattle owners and attendants regarding FMD, an interview utilizing a semi-structured questionnaire was conducted. The purpose of the questionnaires was to gather data on the type of farm, size of the herd,

management techniques, frequency of FMD, and native disease control methods. Verbal consent was sought from the farmer prior to presenting the questionnaire. The permission outlined the requirements for secrecy as well as the option to decline.

### **3.6. Serological test**

A commercial 3ABC ELISA kit (ID.vet, France) was used in a serological test to identify antibodies against the 3ABC non-structural proteins of FMD viruses, allowing the distinction between animals that were infected and those that were vaccinated. According to the manufacturer's instructions, the assay has a diagnostic sensitivity of 100% and specificity of 95-98%. The assay protocol prescribed by the manufacturer was followed as described in Annex 3. The quantification of antibodies was done using a spectrophotometer wavelength of 450 nm in terms of optical density (OD). If the mean value of the positive control OD was less than 30% of the ODNC and the mean value of the negative control OD was more than 0.7, the test was considered validated. The competition proportion (S/N%) served as the basis for the interpretation of each sample:

$$\frac{S}{N}\% = OD \frac{\text{sample}}{NC} \times 100$$

Positive samples were defined as having a S/N% less than or equal to 50%, whereas negative samples had a S/N% larger than 50%.

### **3.7. Serotyping of FMD Virus**

Solid phase competitive ELISA (SPCE) which measures antibodies specific to each FMDV serotype was used for serotyping. This test targets serotypes such as type O, A, SAT- 1, and SAT-2 and was manufactured to detect and type antibodies against FMD virus. SPCE was performed on a total of 22 strong positive (OD < 0.2) serum samples. To assess antibodies against serotypes, this assay employs unique neutralizing anti-FMDV monoclonal antibodies that are tailored to individual FMDV serotypes. The tests were done following the procedure suggested by the manufacturer. At 450nm in wavelength, the optical density (OD) measurement was taken with a spectrophotometer. The % inhibition of positive control and test sera are used to calculate the results.

% inhibition = 100-(serum OD/Reference OD\*) 100.

Reference OD = mean OD of four wells processed with negative control.

### **3.8. Data Management and Analysis**

A Microsoft Excel spreadsheet was developed using the information gathered from the laboratory and field studies. The data was appropriately coded, checked, and cleaned. After that, the data were loaded into STATA statistical software, version 14.1, in order to conduct statistical analysis. Using descriptive statistics, the data were compiled. The relationship between different risk factors and FMD seropositivity was determined using a univariable logistic regression analysis. To determine the independent relationship between risk factors and the occurrence of FMD at 95% CI, all risk factors that shown a significant association with FMD positivity ( $P < 0.25$ ) at univariable analysis were incorporated in a multivariable logistic regression model. When the 95% confidence interval's p-value was less than 0.05, the results were deemed statistically significant. The questionnaire survey was analyzed using the Chi-square ( $\chi^2$ ) test. None of the independent variables exhibit multicollinearity. The final model's further variable selection procedure, which was based on a backward stepwise selection technique, excluded less significant variables. The Receiver Operator Curve (ROC) analysis was used to evaluate the goodness of fit of the logistic regression models. Acceptable models were those with ROC area under the curve (AUC) values greater than 0.7.

### **3.9. Ethical Considerations**

The Animal Research Ethical Review Committee of Addis Ababa University College of Veterinary Medicine and Agriculture accepted the study and issued an ethical clearance certificate under the Thematic Project (VM/ERC/04/16/024). Animal owners granted their approval for their animal to be included in the study before the research began, after being told of its goals and advantages.

## **4. RESULTS**

### **4.1. Seroprevalence of FMD**

The study's animal level seroprevalence of FMDV was determined to be 29.94% (95%CI: 25.4-34.8) and 77.5% (95% CI: 63.94-91.02) at herd level seroprevalence.

### **4.2. Risk factors associated with FMD seropositivity**

#### **Univariate logistic regression analysis**

The variables age, breed, farm management, farm size, and grazing system exhibited a significant association with FMDV prevalence ( $p < 0.05$ ) (Table 1). The seroprevalence of FMD was notably higher in older (32.2%) animals compared to young ones (8.3%), Adult (>4yrs) cattle demonstrated a 5.24 times higher likelihood (95% CI =1.20 - 22.71;  $p = 0.027$ ) of acquiring FMD than young cattle (6m-2yrs). Similarly, Crossbred cattle had a 4.25 times higher frequency of harboring FMD (33.3%) than indigenous breed cattle (10.5%), revealed statically significant outcome (95% CI= 1.76-10.21,  $p=0.001$ ) (Table 1).

This finding also exhibited that Cattle kept under extensive (43.4%) and semi-intensive system (33.3%) were found to be significantly 6.35 and 4.13 times more prone to FMD than intensive system (10.8%) (95% CI =2.06 – 13.32;  $p = 0.00$ ) respectively (Table 1).

In this study, it was observed that animals in large-sized farms (42.35%) (95% CI=1.58-5.34,  $P=0.001$ ) and medium-sized farms (31.1%) (95% CI=1.04-3.07,  $P=0.033$ ) were 2.91 and 1.79 times more prone to acquiring FMDV compared to those in small-sized farms (20.15%) respectively, regardless of other factors, and this difference was statistically significant ( $p < 0.05$ ). Likewise, cattle managed under communal grazing and watering (42.2%) were evinced significantly 3.91 times more likely harboring FMD than not sharing water and grazing land (15.7%) (95% CI=2.40- 6.38,  $P=0.00$ ) (Table 1).

This finding demonstrated that even though slightly higher FMD seroprevalence was obtained among male cattle (31.4%) than female cattle (27.44%), and cattle herd management groups with mixed herd composition (33.8%) than non-mixed herd composition (27.6%), overall outcome were statistically not significant ( $p>0.05$ ) (Table 1).

**Table 1:** Univariable logistics regression analysis of potential risk factors associated with FMD seropositivity

<b>Variable</b>	<b>Category</b>	<b>No of tested</b>	<b>No. positive %</b>	<b>OR(95% CI)</b>	<b>P-value</b>
Sex	Female	349	27.4	Ref	
	Male	35	31.4	1.079(0.51-2.28)	0.8417
Breed	Local	57	10.5	Ref	
	Cross	327	33.3	4.25(1.76 - 10.21)	0.001
Age	6 months – 2 years	24	8.3	Ref	
	2-4 years	47	25.5	3.77(0.76- 18.47)	0.102
	>4 years	313	32.2	5.24(1.20- 22.71)	0.027
Farm management	Intensive	102	10.8	Ref	
	Semi-intensive	183	33.3	4.13(2.06-8.3)	0.000
	Extensive	99	43.4	6.35(3.02-13.32)	0.000
Herd composition	Not mixed	239	27.6	Ref	
	Mixed	145	33.8	1.338(0.85 - 2.08)	0.201
Communal grazing and watering	No	206	42.2	Ref	
	Yes	178	15.7	3.91(2.40- 6.38)	0.000
Farm size	Small	129	20.15	Ref	
	Medium	170	31.1	1.79(1.04-3.07)	0.033
	Large	85	42.35	2.91(1.58-5.34)	0.001

/

## Multivariable logistic regression analysis

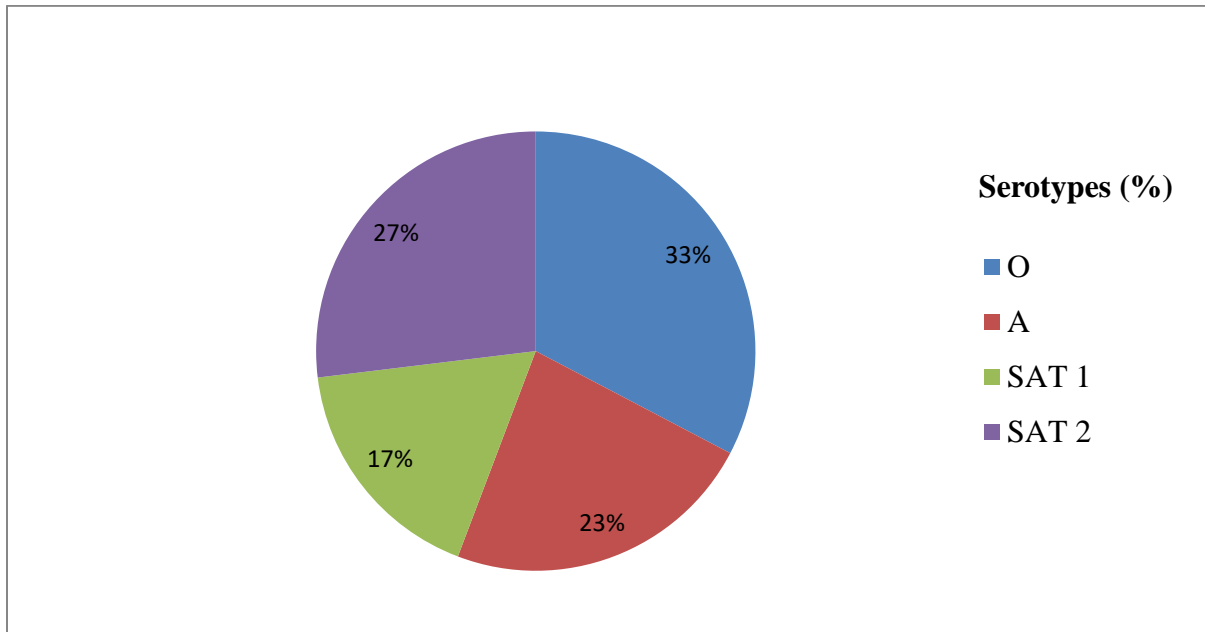
The final multivariable logistic regression model's outcome shows that the predictor variable and FMD seroprevalence are related. The risk of being FMDV seropositive was four times higher in crossbred cattle (OR = 4.15; 95% CI: 1.67 - 10.3; P = 0.002) compared to local breed cattle. Animals managed extensively had three times higher risk of FMD seropositivity (OR = 3.22; 95%CI: 1.33 -7.83; P = 0.01) compared to intensive production systems. Dairy cattle using communal grazing and watering points had three times higher risk of FMD seropositivity (OR = 2.5; 95% CI: 1.28 - 4.86; P 0.007) compared with those not used communal points and two times higher in larger herd sizes (OR = 2.05; 95% CI: 0.001-0.04; P = 0.039) compared to small herd size (Table 2).

**Table 2:** Multivariable logistics regression analysis of Factors associated with the prevalence of FMD in cattle

Variable	Categories	OR (95% CI)	P value
Breed	Local	Ref	
	Cross	4.15 (1.67-10.3)	0.002
Age	6 months – 2 years	Ref	
	2 – 4 years	2.108 (0.46-13.2)	0.249
	> 4 years	3.776 (0.81-17.56)	0.09
Farm management	Intensive	Ref	
	Semi-intensive	1.722 (0.69-4.27)	0.241
	Extensive	3.22 (1.33-7.83)	0.01
Herd Composition	Not mixed	Ref	
	Mixed	1.26 (0.76-2.06)	0.367
Communal grazing and watering	No	Ref	
	Yes	2.501 (1.28-4.86)	0.007
Farm size	Small	Ref	
	Medium	1.63 (0.89-2.96)	0.109
	Large	2.055 (1.059-4.17)	0.039

### 4.3. Serotypes of FMDV in the Study Areas

In this study, among the 22 serum samples exhibiting strong antibody positivity and analyzed using FMDV Solid Phase Competitive ELISA for antibodies specific to serotypes O, A, SAT 1, and SAT 2 were detected. Serotype O was the predominant serotype (33%), followed by serotype SAT 2 at (27%), serotype A (23%), and serotype SAT 1 (17%) (Figure 3).



**Figure 3:** Summary of percentage (n =22) of serotypes of FMDV in the study area

### 4.4. Questionnaire survey

#### 4.4.1. Sociodemography characteristics of respondents

The sociodemographic characteristics of the respondents showed that 47.1% were aged 25-40, 33.7% were aged 41-50, and the remaining 19.2% were above 51 years old. Similarly, 23.1% were illiterate, 39.4% had primary school education, 29.8% had secondary school education, and 7.7% had a degree or higher. In terms of experience in the livestock sector, 39.4% had less than 10 years, 49.1% had 11-20 years, and 11.5% had over 21 years. The majority (72.8%) owned small farms, while 22.3% owned medium-sized farms, and 4.8% owned large farms. Regarding livestock breeds, 19.52% owned crossbreeds, 34.86% owned local breeds, and 46.2% owned

both. Management systems varied, with 34.6% employing an extensive system, 56.2% utilizing semi-intensive methods, and only 9.2% adopting intensive farming setups (Table 3).

**Table 3:** Socio-demography characteristics of respondents in the study area.

<b>Variables</b>		<b>Response</b>	<b>Percentage %</b>
Age of respondent	<40 years	49	47.1
	41-50 years	35	33.7
	>51years	20	19.2
Education level	Illiterate	24	23.1
	Primary	41	39.4
	Secondary	31	29.8
	Certificate and above	8	7.7
Work experience	<10 years	41	39.4
	11-20 years	51	49.1
	>21 years	12	11.5
Farm size	Small	76	72.8
	Medium	23	22.3
	large	5	4.8
Breed	Local	49	47.5
	Cross	40	39.8
	Both	15	14.5
Management system	Extensive	36	34.6
	Semi-intensive	58	56.2
	Intensive	10	9.2

#### *4.4.2. Knowledge, attitude, and practice of the respondents*

The evaluation of knowledge, attitudes, and practices towards FMD demonstrated that farmers practice communal watering (57.2%) of livestock. Zero-grazing, grazing outside, and within the farm were reported by 13.5%, 59.6%, and 26.9% of respondents, respectively (Table 4).

Furthermore, 80.77% reported sharing equipment, 71.15% shared workers from neighboring farms, and 24.42 % shared bulls from neighboring farms as a breeding method. Of the 104 dairy livestock owners and attendants interviewed, the majority 84.6% of the respondents knew about FMD (Table 4).

**Table 4:** Knowledge, attitudes, and practices of respondents towards FMD

<b>Variable</b>		<b>Response</b>	<b>Percentage</b>
Share workers	Yes	74	71.15
	No	30	28.85
Share equipment	Yes	84	80.77
	No	20	19.23
Breeding method	AI	45	43.27
	Own bull	34	32.31
	Shared bull	25	24.42
Communal watering	Yes	59	57.2
	No	45	42.8
Grazing	Zero grazing	14	13.5
	Grazing outside	62	59.6
	Grazing within the farm	28	26.9
Know FMD	Yes	88	84.6
	No	16	15.4
Action about the disease	Call veterinarian	55/88	62.5
	Treat using traditional medicine	33/88	62.5
Report FMD to authority	Yes	12/88	13.6
	No	76/88	86.4

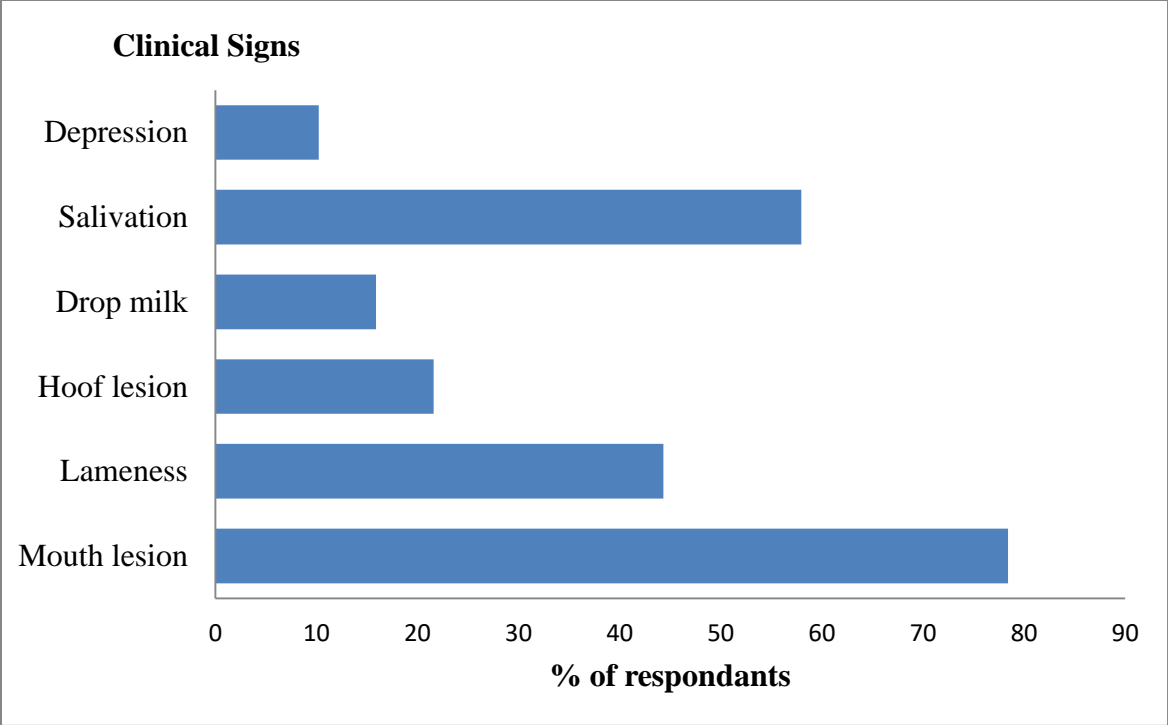
This study assesses the socio-demographic characteristics of respondents concerning their knowledge of Foot-and-Mouth Disease (FMD) and their attitudes towards implementing various management practices for preventing FMD. The findings reveal that respondents' knowledge and awareness of FMD were significantly higher among old age groups (<40 years: 73%, 41-50

years: 94%, >51 years: 95%), as well as among those with higher levels of education (Illiterate: 70%, Primary education: 80%, Secondary education: 97%, Above certificate: 100%), and greater work experience in dairy farming (<10 years: 88%, 11-20 years: 98%, >20 years: 100%) (p<0.05). Interestingly, illiterate respondents were found to have a significantly higher frequency of sharing equipment and workers compared to educated respondents (those with primary school, secondary school, or above certificate education) (p<0.05).

**Table 5:** Association of sociodemography of the respondent with KPA

		Share Worker			Share Equipment			Know FMD		
		%	X <sup>2</sup>	P	%	X <sup>2</sup>	P	%	X <sup>2</sup>	P
Age of respondent	<40 yrs.	76	1.255	0.534	90	4.96	0.084	73	8.84	0.012
	41-50 yrs.	66			71			94		
	>51 yrs.	65			75			95		
	Illiterate	96	22.98	0.00	91	12.4	0.006	70	9.01	0.029
Education status	Primary	80			90			80		
	Secondary	45			90			97		
	Above Certificate	38			50			100		
Work experience	<10 yrs.	80	3.6	0.164	93	11.2	0.004	88	23.39	0.00
	11-20 yrs.	65			78			98		
	>20 yrs.	58			50			100		

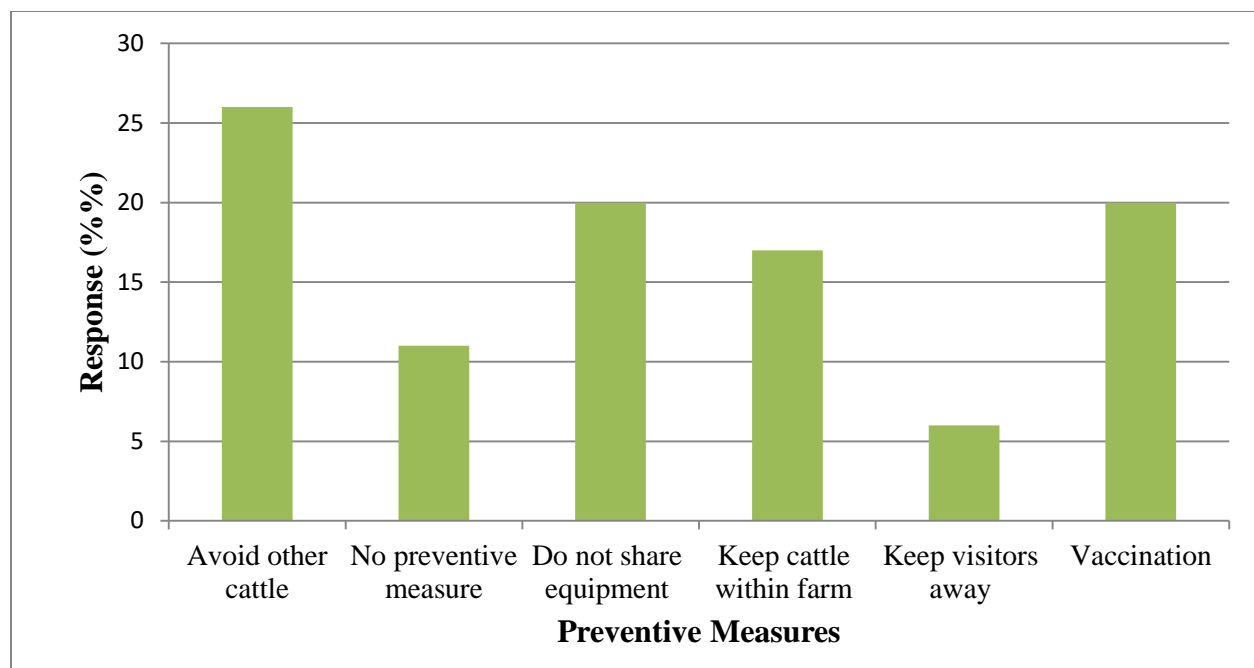
Livestock owners and attendants who were familiar with FMD were queried about their ability to recognize its clinical signs. The prevalent clinical indicators they cited were mouth lesions (78%), followed by hyper-salivation (58%), lameness (44%), and hoof lesions (22%). Additionally, they mentioned depression (10%) and a reduction in milk production (16%) (Figure 4).



**Figure 4:** Respondents knowledge about the clinical signs of FMD

*4.4.3. Prevention measures*

Among the 88 respondents who knows FMD they mentioned different prevent measures, 20% did not share equipment and 17% confining cattle within the farm compound and 26% prevented other cattle from entering the premises. Merely 20%, and 6% of dairy livestock owners and attendants cited vaccination and restricting farm visitors as preventive measures, respectively. However, despite being aware of FMD, 20% of livestock owners and attendants did not implement any disease prevention measures (Figure 5).



**Figure 5:** Summary of livestock owners and attendant response on the knowledge of prevention measures against FMD

#### 4.4.4. Indigenous disease treatment practice towards FMD

The Indigenous FMD management practice of the respondents demonstrated extensive use of indigenous treatments and herbal remedies such as *Solanum Nigrum* ('Embuay') (9.1%), *Urtica pilulifera L* ("Sama") (15.2%), suspension of honey with red pepper (42.4%), and tobacco leaves powder with salt (12.1), and salted warm water (21.2%) in managing FMD in cattle (Table 6).

**Table 6:** Summary of Indigenous remedies and methods of use against FMD in the study area

<b>Traditional treatment</b>	<b>No. of respondents (%)</b>	<b>How to use</b>
<i>Solanum nigrum (Hiddii)</i>	3/33(9.1%)	Grind the fruit, apply it on the wound, and bandage it on the tail of infected cattle
<i>Urtica pilulifera L (Dobii)</i>	5/33(15.2%)	Boil the leaf and apply on the lesion wound
Honey with red pepper	14/33(42.4%)	Mix and apply on the wound
Tobacco with salt	4/33(12.1)	Grind the leaf and then apply it on the wound
Salt	7/33(21.2)	Add the salt to warm water and wash the wound

## 5. DISCUSSION

The study revealed an overall prevalence of FMD at 29.94%, which aligns with findings from similar studies conducted across Ethiopia. Notably, the Guji zone of the Oromia region reported an overall prevalence of 32.7%, while the Yeka district city of Addis Ababa recorded 30% (Ayelet *et al.*, 2012). In other regions, such as the central Tigray zone (Ayelet *et al.*, 2012) and the South Omo zone (Megersa *et al.*, 2009), seroprevalence rates of 26.6% and 23.9% were documented, respectively. However, certain areas exhibited higher seroprevalence rates compared to this study. For instance, Adiss Abeba reported 72.1% (Awel and Dilba, 2021), the Gamo zone recorded 49.2%, Tigray's Eastern zone (Ayelet *et al.*, 2012) documented 41.5%, and the West Shewa Zone (Ahmed *et al.*, 2020) reported 40.4%. Conversely, Southern Ethiopia displayed the lowest seroprevalence rates at 9.5%, with rates of 6.9% in the Gamo Gofa zone and 5.9% in the Sidama zone respectively (Megersa *et al.*, 2009). The reasons for these differences in prevalence could be due to different study designs, sample strategies, or the existence or lack of extrinsic risk factors such agro-ecology, animal mobility, communal grazing, and communal watering.

In this study, there was a notable variation in FMD prevalence across different age groups, with statistically significant differences observed. The seroprevalence of FMD was higher in older animals (32.2%) compared to younger ones (8.3%). Adult cattle (>4 years) exhibited a 3.77 times higher likelihood of FMD acquisition compared to young cattle (6 months to 2 years) ( $P < 0.05$ ). This finding aligns with previous studies by Dubie and Negash (2021) in Afar region, as well as findings by Awel and Dilba (2020) in Addis Ababa, Megersa *et al.* (2009) in Southern Ethiopia, Sulayeman *et al.* (2018) in central Ethiopia, Gelana (2016) in Western Oromiya, and Abunna *et al.* (2013) in Dire Dawa. The observed variations in prevalence between age groups could be explained by the aging process of animals' increased exposure to risk factors for disease. Calves in the study area were mainly housed in barns until they were old enough to be let out on communal grazing, which minimized their exposure to the disease. Furthermore, passive maternal immunization shields calves under a year old from the illness (Tesfaye *et al.*, 2016).

According to the current study, there is a statistically significant difference in the prevalence of FMD in animals that are crossbred and native breeds. This finding is consistent with earlier research by Sulayeman *et al.* (2018), Urge *et al.* (2020), and Ahmed *et al.* (2020), who also reported significant differences in prevalence estimates between local and crossbred breeds in central Ethiopia, the Welmera district of the Oromia region, and the West Showa zone, respectively. Crossbred cattle had a 4.15 times higher frequency of harboring FMD (33.3%) than indigenous breed cattle (10.5%). The present investigation also suggests that crossbreeds are more susceptible than native breeds, supporting the conclusions stated by Sulayeman *et al.* (2018), Urge *et al.* (2020), and Ahmed *et al.* (2020).

This finding also indicates that cattle kept under extensive (43.4%) and semi-intensive systems (33.3%) were significantly 3.22 and 1.72 times more susceptible to FMD compared to those under intensive systems. This aligns with previous studies by Sulayeman *et al.* (2018). Due to interaction with infected cattle as well as same grazing and watering techniques, livestock fed off the farm may be more susceptible to the virus.

In this study, it was observed that animals in large-sized farms (42.35%) and medium-sized farms (31.1%) were significantly 2.05 and 1.63 times more prone to acquiring FMDV compared to those in small-sized farms (20.15%) respectively ( $P < 0.05$ ). Likewise, cattle managed under communal grazing and watering (42.2%) were significantly 2.5 times more likely harboring FMD than not sharing water and grazing land (15.7%). In agreement with Seifu *et al.* (2023), displayed significantly high rate of prone to FMD in extensive farms and semi-intensive farms, large size farms. This might be due to communal grazing and watering (free movement of animals among different farms) and large size herd may contribute for distribution and transmission of circulating serotype from infected cattle to healthy one.

In contrast to the findings of Gelaye *et al.* (2009), Sulayeman *et al.* (2018) which showed significantly higher FMD prevalence among male cattle compared to females, and higher prevalence in mixed herd compositions compared to non-mixed herds, the present study revealed slightly higher seroprevalence rates among male cattle (31.4%) compared to females (27.44%), and among cattle in mixed herd compositions (33.8%) compared to non-mixed herds (27.6%).

However, these differences were not statistically significant ( $p>0.05$ ). The lack of significance on sex of animals may be due to the disproportionate allocation of sample sizes between males and females in our study, where the sample size of female cattle exceeded that of males. Insignificance finding among the herd composition might be due to FMD is prevalent in all production herd composition, able to transmit from cattle to cattle as well as cattle to small ruminants and vice versa.

In the present finding, serotypes O, SAT2, A, and SAT1 were identified in varying proportions. Serotype O was the most prevalent, accounting for 33%, followed by serotype SAT 2 at 27%, serotype A at 23%, and serotype SAT1 at 17%. This finding is consistent with reports by Awel *et al.* (2021), Negussie *et al.* (2009), Ayelet *et al.* (2012) and Gizaw *et al.* (2020). A retrospective analysis by Ayelet *et al.* (2012) covering the years 2008–2018 also found that serotype O was the most identified, followed by serotypes SAT 2 and A then SAT1. Regular monitoring of the circulating FMDV serotypes in livestock may possibly be important for the selection of appropriate vaccine strains for strategic control and prevention practices in the study area.

The current study examined the association between the disease and farm size, finding that seroprevalence of FMDV antibodies increased as farm size increased. This may indicate the infectious character of the disease and its route of transmission, which is linked to animal crowding caused by rising herding sizes. This can increase the likelihood of FMD transmission by allowing for more frequent direct contact. The results corroborated the findings of Bayissa *et al.* (2011), who found a positive correlation between herd size and FMD seroprevalence.

The study's questionnaire survey revealed that dairy farmers knew enough about FMD. The farmers provided a detailed description of the clinical indications. The three clinical indicators that were most frequently reported were lameness, excessive salivation, and oral lesions. It was also shown that the majority of farm workers predominantly detected these signals as clinical indicators. According to this, it is too late to detect an FMD infection by looking for sores in the oral cavity and excessive salivation in order to effectively stop the virus from spreading. The attention paid to the animals and the level of FMD knowledge among farm personnel varied, as did their ability to identify the clinical indications. 59/104 [57.2%] of the respondents watered

the farm communally, while 62/104 (59.6) of them grazed outside. Free animal movement in pursuit of food and water is a known risk factor for the development of FMD, according to a study conducted by Kebede *et al.* (2018). In addition, a significant proportion of farmers (80%) share workers and equipment (71%). FMDV transmission is accelerated by unrestricted livestock movement and personnel and equipment sharing (Moroga *et al.*, 2013). This implied that appropriate biosecurity precautions ought to be put in place.

Farmers in the research areas mostly relied on AI (43.3%), their own bull (32.3%), and shared bulls from nearby farms (24.42%) for their breeding practices. While direct touch is the primary method of FMD transmission, infected animals' semen can also spread the disease (Sharama *et al.*, 2012). Merely 20% of farmers employ immunization to prevent foot and mouth disease (FMD), which could be as a result of vaccine's expensive cost and limited availability. It's surprising to see that 20% of farmers don't take any preventative action even when they are aware of FMD. This might be the result of a lack of understanding, an assumption that preventive care is hard to put into practice, or an underestimate of the risk of disease. This implies that farmers need to be trained in FMD prevention techniques, including biosecurity and vaccination protocols.

The survey results indicate that individuals with a modern level of education comprised the majority of respondents. Specifically, 39.4% had primary school education, 29.8% had secondary school education, and 7.7% held a certificates or higher. Notably, the younger age groups, particularly those aged 25-40 (47.1%) and 41-50 (33.7%), were more prominent among the respondents. Moreover, those with experience in dairy husbandry showed a higher percentage compared to those who were illiterate (23.1%). This data underscores the significance of education in disseminating information swiftly, especially in educating and training farmers extensively on recommended prevention and control measures for Foot-and-Mouth Disease (FMD). Such measures are crucial for reducing the seroprevalence and economic impact of FMD on livestock within and around Jimma town. The findings suggest that higher levels of education and young adult age positively influence knowledge, management practices, and attitudes towards veterinary disease controls. Furthermore, individuals with higher education levels and young adults typically have better access to information, possess critical thinking skills, and can

comprehend scientific concepts more effectively. Consequently, this leads to a greater depth of knowledge, improved management practices, and more favorable attitudes towards disease control measures (Amemiya *et al.*, 2023; Seifu *et al.*, 2023; Tufa *et al.*, 2023). Previous studies by Acosta *et al.* (2022) and Campbell *et al.* (2018) have also explored the impact of education level on healthcare practices. They concluded that education can enhance knowledge and attitudes, yet achieving behavioral change may require additional interventions.

This study identified some of indigenous treatment of FMD like lesion practiced by the farmers in the districts. Honey with red pepper is applied mostly. They mix honey with the red pepper and applied on the mouth lesion. Even before bacteria were found, honey was used as a wound cure as far back as 2,000 years ago. According to recent reports, honey has the ability to inhibit around sixty different types of bacteria (Molan, 1992). *Solanum nigrum* (Embuay), *Urtica pilulifera* L (Sama) and Tobacco with salt were also applied to treat the lesion. The farmers also respond they washed the wound with salt and warm water mixture. In Kenya, study conducted by Gakuya *et al.* (2011) reports the traditional usage of Soda ash, honey and millet for the management of FMD is supported by the rapid healing of the FMD lesions observed. The usage of these traditional remedies is therefore recommended as these products are cheap, locally available and easy to apply at farm level. However, further studies on these products are necessary to experimentally evaluate their potency.

## 6. CONCLUSION AND RECOMMENDATIONS

In this study, the seroprevalence of FMD in dairy cattle of Jimma City was 30% (95% CI: 25.4-34.8). Four serotypes of FMDV such as serotype O, A, SAT-1, and SAT-2 were identified. Risk factors such as farm size, age of cattle, and the management system were significantly associated with FMD seropositivity. Interestingly, the majority of cattle owners and attendants were aware of FMD. However, the majority uses traditional practices to alleviate the symptoms of FMD. This study identified significant gaps in cattle vaccination against FMD and biosecurity protocols. Considering the high presence of the virus with diverse serotypes in the city, the following are recommended to prevent and mitigate the impact of FMD in the emerging dairy industry of Jimma City:

- Intensive awareness creation among farm owners on biosecurity measures and scheduled vaccination
- More work is needed on serotype identification, molecular epidemiology, and vaccine development against the circulating serotypes
- Further research on the effectiveness of traditional FMD disease management practices is required

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## 8. ANNEXES

### ANNEX 1: Data Recording Format for Blood sampled Cattle

Animal ID	age	Sex	breed	Production system	Herd size

### ANNEX 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. 8ml to 10ml blood was collected and keep overnight at room temperature
5. Carefully decant the serum into 2ml cryovials which is labeled with identification code, if the serum was not separated within overnight, centrifuge with manual centrifuge.
6. Transport the serum with the ice to Animal Health Institute (AHI) then Keep under -20°C until laboratory diagnosis

### ANNEX 3: laboratory procedure for 3ABC ELISA

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°C for 2 hours, 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.

#### **ANNEX 4:** laboratory procedure for Solid Phase Competitive ELISA

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. 50µl of negative control dispensed in each 4 wells A1-D1 then 67.5µl of ELISA buffer was added in well E1 and 50µl in F1. 7.5µl of positive control serum were added in well E1 to obtain the positive serum dilution 1/10 and well mixed. 25µl of positive serum taken and added to F1 and mixed using monochanal pipette to obtain 1/30 dilution of positive control. After mixing 25µl of the dilution discarded from F1 to obtain 50µl volume. For the left 90 wells 45µl of ELISA buffer and 5 µl of test sera were dispensed. After an hr. incubation 25µl of diluted HRPO-conjugate were added in each plate. And then incubated for 1 hour again at room temperature. After an hour the plate were washed and 50µl of substrate were added and put it in a dark place at room temperature for 20 minutes. When the time relapsed 50µl of stop solution were added and then the result were seen using 450nm wavelength on microplate reader.

#### **ANNEX 5:** Questionnaire Survey for the Assessment of knowledge and indigenous disease management practice of the livestock owners and attendant towards FMD.

Questionnaire Survey for assessment of major risk factors, knowledge and disease management practice among livestock owners and attendant.

### **I. General Information**

1. Name of the respondent: \_\_\_\_\_

2. Age of the respondent: \_\_\_\_\_

3. Position at the farm: (please circle the correct choice)

- a. Owner
- b. Employee
- c. Milkier
- d. Herdsman
- e. Manager
- f. Other

4. Level of education?

- a. Illiterate
- b. Primary
- c. Secondary
- d. Certificate and above

5. District: \_\_\_\_\_

\_\_\_\_\_

6. How many years have you been doing dairy farming?

## **II. Livestock Production**

7. What is the size of farm (where the cattle are) in acres?

- a. Small (<15 cattle)
- b. Medium (15 – 35 cattle)
- c. Large (> 35 cattle)

8. What other species are found in your farm (state number):

- a. Goats \_\_\_\_\_
- b. Sheep \_\_\_\_\_
- c. Pigs \_\_\_\_\_
- d. Equine \_\_\_\_\_
- e. None

9. Which breed do you keep:

- a. Local
- b. Exotic
- c. Cross
- d. More than one breeds

### **III. Risk Factors**

10. What grazing methods do you use for your animals?

- a. Stall feeding
- b. Semi grazing (Animals allowed to graze but additional food is given by stallfeeding)
- c. Pasture grazing only

11. Do you use communal grazing fields to feed your animals?

- a. Yes
- b. No
- c. Don't know

12. Do you use communal watering facilities to water your animals?

- a. Yes
- b. No

13. If you do semi or full time grazing, do you ever graze your animals in (yes or No):

- a. Forests
- b. Within towns
- c. By roadsides
- d. Near livestock markets or slaughterhouses

14. Do you share workers with other surrounding farms?

- a. Yes
- b. No
- c. Don't know

15. Do you share equipment with surrounding farms?

- a. Yes
- b. No
- c. Don't know

16. Breeding methods,

- a. AI
- b. Own bull
- c. Use of a shared bull with surrounding livestock owners and attendant

#### **IV. Knowledge And Occurrence Of FMD**

17. Have you heard of foot and mouth disease?

- a. Yes (go to question 23)
- b. No (go to question 24)

18. Have you encountered an animal with two or more of the following signs?

- Excessive salivation

- Hoof lesions
- Mouth lesions
- Lameness
- Drop in milk production
- Lesions on teats
- Death in adult cattle
- Other (specify)

19. What do you call the disease condition with two more signs in #18

---

20. When was the last time you observe the cases in #18

- a. At present
- b. Last three week
- c. Before three month
- d. Before six month

21. What were your action in #18 cases

- a. Call a veterinarian
- b. Treat using traditional medicine

22. When there is a foot and mouth disease case do you report it to the veterinary authorities?

- a. Yes
- b. No
- c. Don't know

23. What have you done to protect your herd from FMD?

- Vaccination
- Keep cattle within farm compound
- Avoid other cattle from entering farm compound
- Keep cattle away from farm compound boundaries
- Do not bring in new cattle
- Avoid use of communal dips
- Do not share equipment with surrounding farms
- Keep visitors away from cattle
- Do not do any preventive measure
- Other (specify)

24. Is your herd being vaccinated against FMD?

- a. Yes                      b. No

25. Who vaccinates – This asked who vaccinated their cattle:

- a. Myself
- b. Private veterinarian
- c. Private animal health assistant
- d. Government veterinarian
- e. Government animal health assistant
- f. Other non-veterinarian

26. How many times in a year do you usually do this?

---

27. At what age (in months) do you start vaccinating your animals?

\_\_\_\_\_months

28. When did you last vaccinate for FMD? (mm/yy)?

\_\_\_\_\_

29. Did you notice the disease in vaccinated animals?

a. Yes

b. No

30. Have you ever used herbs to treat FMD wound?

a. Yes

b. No

31. Which herbes do you use and how?

**ANNEX 6: Figures during study periods**

**Appendix Figure 1: During serum preparation from the whole blood**



**Appendix Figure 2: During reading the result**



**Appendix Figure 3:** During laboratory procedure



**ANNEX 7: Ethical clearance certificate**

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ADDIS ABABA UNIVERSITY  
College of Veterinary Medicine  
and Agriculture  
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Animal Research Ethical Review Committee

*Ethical clearance certificate*

Certificate Ref. No: VM/ERC/04/20/16/2024

Name of Applicant: **Emraphe Ali (DVM, MSc student)**

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


Title of the project: *Epidemiology of FMD virus and indigenous knowledge of disease management practices among smallholder and intensive dairy cattle production in Jimma City, Oromia Regional state-Ethiopia*

Date of application: **December, 2023**  
Nature of the project: **Farm investigation and questionnaire survey**  
Target animal species: **dairy cattle**  
Number of animals involved: **384**  
Study area: **Jimma, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/16/024, 16/05/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of **Emraphe Ali**.

Professor Getachew Terefe (DVM, PhD)  
Chairman

  
Signature

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