

WALLAGA UNIVERSITY

POST GRADUATE STUDIES

SCHOOL OF VETERINARY MEDICINE

ISOLATION, SEROTPYING, AND MOLECULAR DETECTION OF BOVINE FOOT AND MOUTH DISEASE VIRUS FROM OUTBREAK CASES IN SELECTED AREAS OF KELLEM WOLLEGA, WEST, AND EAST WOLLEGA ZONES, WESTERN ETHIOPIA.

MSc THESIS

FIKADU ITEFA GUYA

JUNE, 2026

NEKEMTE, ETHIOPIA



WALLAGA UNIVERSITY

POST GRADUATE STUDIES

SCHOOL OF VETERINARY MEDICINE

DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND PUBLIC HEALTH

ISOLATION, SEROTPYING AND MOLECULAR DETECTION OF BOVINE FOOT AND MOUTH DISEASE VIRUS FROM OUTBREAK CASES IN SELECTED AREAS OF KELLEM WOLLEGA, WEST, AND EAST WOLLEGA ZONES, WESTERN ETHIOPIA.

A Thesis Submitted to the School of Veterinary Medicine Wallaga University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Microbiology

BY

FIKADU ITEFA GUYA

PROGRAM: MSc IN VETERINARY MICROBIOLOGY

ADVISOR: YOBSAN TAMIRU (DVM, MVSc, ASSISTANT PROFESSOR OF VET-MICROBIOLOGY)

JUNE, 2026

NEKEMTE, ETHIOPIA

WALLAGAUNIVERSITY, SCHOOL OF GRADUATE STUDIES

P.O. BOX: 395, Nekemte, ETHIOPIA

APPROVAL SHEET FOR SUBMITTING FINAL THESIS

As members of the Board of Examining of the Final MSc. thesis open defense, we certify that we have read and evaluated the thesis prepared by **Fikadu Itefa Guya** under the title "**Isolation Serotyping and Molecular Detection of Bovine Foot and Mouth Disease Virus From Outbreak Cases In selected areas of Kellem Wollega, West and East Wollega zones, Western Ethiopia** " and recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Veterinary Microbiology.

_____	_____	_____
Chairperson	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

Final Approval and Acceptance

Thesis Approved by:

_____	_____	_____
Department PGC	Signature	Date
_____	_____	_____
Dean of College	Signature	Date

Certification of the Final Thesis I hereby certify that all the correction and recommendation suggested by the board of examiners are incorporated into the final thesis entitled "**Isolation, Serotyping and Molecular Detection of Foot and Mouth Disease Virus Outbreak Cases from Cattle In selected areas of Kellem Wollega and West Wollega Zone, Western Ethiopia."**

_____	_____	_____
Dean of SGS	Signature	Date

DECLARATION

This is to certify that this thesis, entitled: "**Isolation Serotyping and Molecular Detection of Bovine Foot and Mouth Disease Virus From Outbreak Cases In selected areas of Kellem Wollega, West and East Wollega zones, Western Ethiopia**" was accepted in partial fulfilment of the requirements for the award of the degree of Master of Science by the school of graduate studies, Wallaga University, through the school of Veterinary Medicine; done by **Fikadu Itefa Guya,** was a genuine work carried out by him under my guidance. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the research thesis requirements.

Student: Name: **Fikadu Itefa Guya**

Signature: _____

Date: _____

Major Advisor: Yobsan Tamiru (DVM, MVSc, Assistant Professor)

Signature _____

Date _____

Co-Advisor: Mr. Damasa Negassu (BVSc, MVSc)

Signature _____

Date _____

TABLE OF CONTENTS

Contents **page**

DECLARATION ii

TABLE OF CONTENTS iii

STATEMENT OF THE AUTHOR v

ACKNOWLEDGEMENTS vi

LIST OF TABLES vii

LIST OF APPENDICES ix

LIST OF ABREBRATIONS x

ABSTRACT xi

1. INTRODUCTION 1

2. FOOT AND MOUTH DISEASE 3

2.1. Etiology 3

2.1.1. Genomic structure of foot and mouth disease virus 3

2.1.2. Physicochemical properties of FMDV 3

2.1.3. Antigenic variation in foot and mouth diseases virus 4

2.1.4. Serotypes and Genotypes of FMDV 5

2.2. Mode of transmission 6

2.3. Epidemiology 6

2.3.1. Disease distribution 7

2.3.2. Host range 7

2.3.3. Carrier State 8

2.4. Clinical Sign and Incubation period 8

2.5. Diagnostic methods 9

2.5.1. Virus Isolation 9

2.5.2. Serological Test 10

2.5.3. Nucleic acid detection method 10

2.6. Prevention and Control 10

2.7. Distribution of Foot and Mouth diseases virus in Ethiopia 11

2.7.1 Current status of Foot and Mouth diseases virus in Ethiopia 11

3. MARIALS AND METHODS 12

3.1. Study Area 12

3.2. Study Population 13

3.3. Study and sampling techniques 14

3.4. Sample size, Sample collection, storage and transportation 14
3.5. Sample Processing.....15
3.6. Laboratory investigation 15
3.6.1. FMD Virus isolation.....15
3.6.2. Serotyping of Foot and mouth disease (FMDV) Isolates 16
3.6.3 RNA Extraction.....17
3.6.4. Detection of FMDV using real time RT- PCR.....17
3.7. Ethical clearance 18
3.8. Data Management and Analysis 18
4. RESULT.....19
4.1. Clinical Examination of FMD outbreak cases.....19
4.2. Molecular Detection of Foot and Mouth Disease virus.....19
4.3. Serotyping by Sandwich ELISA 21
4.4. Virus isolation on cell culture.....22
5. DISCUSSION.....23
6. CONCLUSION AND RECOMMENDATIONS.....26
7. REFERENCES.....27
8. LIST OF APPENDICES 37

STATEMENT OF THE AUTHOR

Fikadu Itefa Guya here by declare and affirm that the thesis entitled "Isolation, Serotyping and Molecular Detection of Bovine Foot and Mouth Disease Virus in selected areas of Kellem Wollega, West and East Wollega zone Western Ethiopia**"** is my own work conducted under the supervision of Dr. Yobsan Tamiru. I have followed all the ethical principles of scholarship in the preparation, data collection, data analysis and completion of this thesis. All scholarly matter that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and I have not misrepresented, fabricated, or falsified any idea / data / fact / source in my submission. This thesis is submitted in partial fulfillment of the requirement for a degree from the Post Graduate Studies at Wallaga University. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. I understand that any violation of the above will be cause for disciplinary action by the University and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Name: Fikadu Itefa Guya Signature: _____

Date: 05/07/2018

Department: Veterinary Microbiology, Immunology and Public Health

School: Veterinary Medicine

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to the God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I am sincerely thankful to my advisor Dr. Yobsan Tamiru for all of his aid and support starting from the developments of proposal and until final thesis. This could not have been completed without their kind attention, unconditional advice, unwavering supervision, insightful counsel, helpful recommendations, and encouragement.

I would like to thanks also Animal Health Institutes (AHI), Sebata and its staff members for their kindness, encouragement, collaboration and sustained support on various occasions during my study time.

I am especially very thankful to Mr. Abdi Aliy, Mr. Damasa Negassu, Sr. Analem Fentie, Dr. Daniel Gizaw, and others staff members of AHI workers for their helping and providing technical guidance during every stage of the laboratory work throughout my study period.

I would like to express my heartfelt thanks to all staff of Wallaga University School of Veterinary Medicine who have directly or indirectly contributed to the completion of my study.

My sincere thanks also extend to Kellem Wollega zone (Gawo Kebe, Sayo, and Hewa Gelan districts) , West Wollega zone (Gimbi district), and East Wollega zone (Guto Gida district) Animal Health Workers and Cattle owners for their kindness and cooperation during sample collection for my study.

Finally, I would like to express my heartfelt thanks my mother, directly or indirectly contributed to the completion of my study. Especially I would like to extend my sincere appreciation to my wife Galane Tasfaye Gerba for their moral and constructive advice It fills my heart with joy unspeakable to express my gratitude to everybody who contributed in the successful accomplishment of this thesis.

LIST OF TABLES **PAGE**

Table 1 Distribution of FMD in Ethiopia from 2020 to 2025.....11
Table 2: Population description of the study areas. 13
Table 3: Results of real time RT-PCR and virus isolation across the sample type and study area. 20
Table 4: Real time RT-PCR and virus isolation findings per sample types 21
Table 5: Identification of serotypes of FMD virus from four Outbreaks areas 21

LIST OF FIGURES **PAGE**

Figure 1: Foot-and-mouth disease virus genome....4
Figure 2: Routes of FMDV transimission....6
Figure 3: FMD clinical manifestations....9
Figure 4: Map of the study area....12
Figure 5: Clinical sign of Foot-and -Mouth diseases observed in affected cattle....19
Figure 6: Real time-PCR amplification of FMD virus....20
Figure 7: Picture showing FMDV on BHK cell....22

LIST OF APPENDICES **PAGE**

Appendix 1: FMD outbreak case for of sample collection from field 37
Appendix 2: Preparation of BHK -21 for virus inoculation 37
Appendix 3: Virus isolation procedure on BHK-21 cell 38
Appendix 4: RNA extraction protocol 38
Appendix 5: Plate layout for antigen detection sandwich ELISA 39
Appendix 6: Interpretation of OD values of Sandwich ELISA 41
Appendix 7: Master Mix preparation 41
APPENDIX 8: Field examination 42
Appendix 9: RT-PCR positive and Negative Ct value of FMDV tested. 43
Appendix 10: Miscellaneous pictures. 44
Appendix 11: Ethical Clearance 47

LIST OF ABREBRATIONS

AHI	Animal Health Institute
CSA	Central Statistical Agency
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FMD	Foot-and-Mouth Disease
GDP	Gross Domestic Product
MEGA	Molecular Evolutionary Genetics Analysis software
OIE	Office International des Epizooties
PCR	Polymers chain reaction
RNA	Ribo Nucleic acid
SAT	South African Territories
TAD	Transboundary Animal Disease
VTM	Viral Transport Medium

ABSTRACT

Foot-and-Mouth Disease is a highly contagious and economically important trans boundary viral disease affecting domestic and wild cloven-hoofed animals. An outbreak-based study design was conducted from September 2025 to May, 2026 to isolate, molecularly detect, and identify the circulating serotypes of FMDV in cattle populations from outbreak cases in selected areas of Kellem Wollega, West Wollega and East Wollega zone, Western Ethiopia. Purposive sampling was used to select outbreak sites based on occurrence of clinically suspected FMD cases during the study period. Samples were collected from clinically affected and unvaccinated cattle exhibiting characteristic signs of FMD infection. A total of 32 clinical samples (13 epithelial tissues and 19 oral swab samples) were collected considering their outbreak sites and animal factors. One-step rRT-PCR test was employed to detect 3D (pol) gene of FMDV and antigen detection was performed using sandwich ELISA. PCR positive samples were further inoculated on Baby Hamster Kidney (BHK-21) monolayer cell for virus isolation. Descriptive statistics were applied to calculate the percentages. Out of the 32 samples tested; 10(31.25%) samples where positive for the FMDV using real time PCR targeting 3D gene. From the total of PCR positive samples, 70% (7/10) and (3 /10, 30%) were epithelial tissue and oral swab samples, respectively. Furthermore, serotype O was identified in all rRT-PCR positive samples subjected to antigen detection by using sandwich ELISA. 90% (9/10) of the PCR positive samples were able to show cytopathic effects up on adapting them onto BHK-21 cell line. This study revealed that FMD serotypes O were the causality for the outbreak cases of the FMD virus in the study settings. The virus was detected mainly from clinically affected cattle that had no vaccination history, indicating that disease is circulating in cattle in these areas and they are highly susceptible to FMD infection. Therefore, regular and timely vaccination strategy is highly important for effective control. Further phylogenetic analysis of the isolates should be carried for effective vaccine development to control of the disease.

Key words: *Isolation, Serotype , Molecular, FMDV, Ethiopia*

1. INTRODUCTION

Ethiopia possesses the largest livestock sector in the African continent, comprising approximately 70 million cattle, 42.9 million sheep, 52.5 million goats, and 57 million poultry, according to recent estimates (CSA, 2021). The sector constitutes a cornerstone of the national economy, contributing an estimated 19% to the overall Gross Domestic Product (GDP), 40% to the agricultural GDP, and approximately 20% to foreign exchange earnings through exports (Woldesenbet *et al*., 2023). Livestock production, particularly cattle rearing, serves as a primary livelihood strategy for millions of smallholder farmers, especially in rural communities, underpinning household income, food and nutritional security, and resilience to climatic shocks such as droughts. Commodities derived from livestock meat, skins, hides, and live animals are pivotal to Ethiopia's trade portfolio and play a significant role in securing international market linkages (Dubie and Amare, 2020).

Despite its economic and social importance, the livestock sector is beset by systemic challenges, chief among them the prevalence of transboundary and endemic infectious diseases. This virus is a single-stranded, non-enveloped RNA virus that encodes both structural and non-structural proteins (Jamal and Belsham, 2013). The virus comprises seven antigenically different serotypes (A, O, C, Asia1, SAT1, SAT2, and SAT3) within which numerous subtypes are found (WOAH, 2008). Four of them (A, O, SAT1, and SAT2) are currently endemic in Ethiopia (Gelaye *et al*.,2009). Although the government has expressed a strong commitment to improving animal health and boosting meat and live animal exports in Ethiopia, there is currently no formal policy in place to control Foot and Mouth Disease (FMD) except vaccination practice (Dubie *et al*., 2020). The absence of well-structured official vaccination strategies combined with unrestricted animal movement without proper health certification, are key factors contributing to the spread the disease. Moreover, the evidence of constantly increasing outbreak cases in different parts of the country due to high numbers of susceptible animals, presence of reservoir hosts, and common grazing and watering points makes the spatio-temporal distribution of the disease complex (Wubshet *et al*., 2019). The reservoir host, African buffalo, may enter a carrier state, silently harboring the virus and complicating eradication efforts (Arzt *et al*., 2018; Stenfeldt *et al*., 2020). In East Africa and Ethiopia in particular,wher there is extensive traditional farming system and unlimited movement control of animals, the role of this wild life in disseminating the virus is imminent (Ayelet *et al*., 2012).

Foot and Mouth Disease outbreaks result in substantial economic losses, including reduced

milk and meat production, decreased draft power, and severe trade restrictions, which collectively impose a heavy burden on livestock-dependent communities (Perry and Grace, 2009; FAO, 2021). Moreover, existing vaccines provide short-term immunity and may not protect against all circulating serotypes or strains, while inadequate vaccination coverage and weak surveillance in endemic regions, particularly in parts of Africa and Ethiopia in particular, allow the disease to persist (Paton *et al*., 2021; WRLFMD, 2023). Although various molecular studies on FMD have been conducted in different parts of Ethiopia (Sulayeman *et al*.,2018; Tesfaye *et al*.,2020; Demessa *et al*.,2023), there is currently no documented information on the status of the disease in Western Oromia specifically in the Kellem Wollega, West Wollega and some parts of East Wollega Zone. The disease is currently causing a serious impact in those study area, affecting livestock health, reducing productivity, and threatening the livelihoods of farmers. Given that the area is known for its high cattle population, outbreak investigations and characterization is essential for generating baseline data needed to confirm the nature of specific serotype circulating. Hence, the findings of this study will support the development and expansion of safer and more acquainted vaccines, through the genetic analysis of serotype circulating in the study area.

General Objective

- To isolate and molecularly detect the causal serotypes of FMD virus from outbreak cases in cattle populations in selected areas of Kellem Wollega, West Wollega, and East Wollega Zones, Western Ethiopia.

Specific Objectives

- To isolate and molecularly detect the Foot and mouth disease virus from the collected samples.
- To identify the causal serotypes of FMD Virus outbreaks in the study area

2. FOOT AND MOUTH DISEASE

2.1. Etiology

Foot-and-Mouth Disease (FMD) is the most contagious and acute Transboundary Animal Disease (TAD) affecting cloven hoofed wild and domestic animals (Sumption *et al*., 2007). It is one of the most economically important animal diseases in the world (Wang *et al.*,2020).

2.1.1. Genomic structure of foot and mouth disease virus

The virus is stable at temperatures below freezing point, but it can be readily rendered inactive by UV-light, heat, gamma radiation, disinfectants and other chemicals. In cold, wet organic matter, the virus can survive for several days or even weeks. It is possible to inactivate the virus found in milk and meat by heating at 70°C for at least 30 minutes (Tomasula and Konstance,2004).

2.1.2. Physicochemical properties of FMDV

The genome of FMDV is a single-stranded positive-sense RNA of 8.3 kb. The virus particle has a diameter of around 25-30 nm and a roughly spherical shape. It is made up of the 60 copies of the capsomers that make up the capsid and the RNA genome (Jamal and Belsham, 2013). Moreover, it has three portions: Single open reading frame (coding region (ORF), 3' untranslated region (24 or 25 nucleotides) (3'UTR), and 5' untranslated region (1,300 nucleotides) (5' UTR) (Knowles and Samuel,2003; Firth and Brierley, 2012). The 5'end of the genome is linked to a small non-structural protein (NSP) called viral protein linked genome (VPg), which has roughly 24 nucleotides. The primer for replicating the genome is this protein (Longjam *et al.,* 2011). Inside the FMD virus sit four capsid-making structural proteins - named VP1, VP2, VP3, and VP4 - that come directly from the virus's genetic code. Sitting on the outer shell are VP1 through VP3, whereas VP4 hides within the core (Ashfaq *et al*., 2015; Bari *et al*., 2015; Mahapatra *et al*., 2017), alongside ten non-structural players: L, 2A, 2B, 2C, 3A, three versions of 3B, plus 3C and 3D (Jamal and Belsham, 2013; Gao *et al*., 2016). Because its copying tool - the RNA-based RdRp enzyme - can’t spot mistakes while duplicating, mutations pile up fast. That faulty copy process swaps out nucleotides too often, pushing mutation levels skyward (Longjam *et al.,* 2011; Lordwin, 2011).

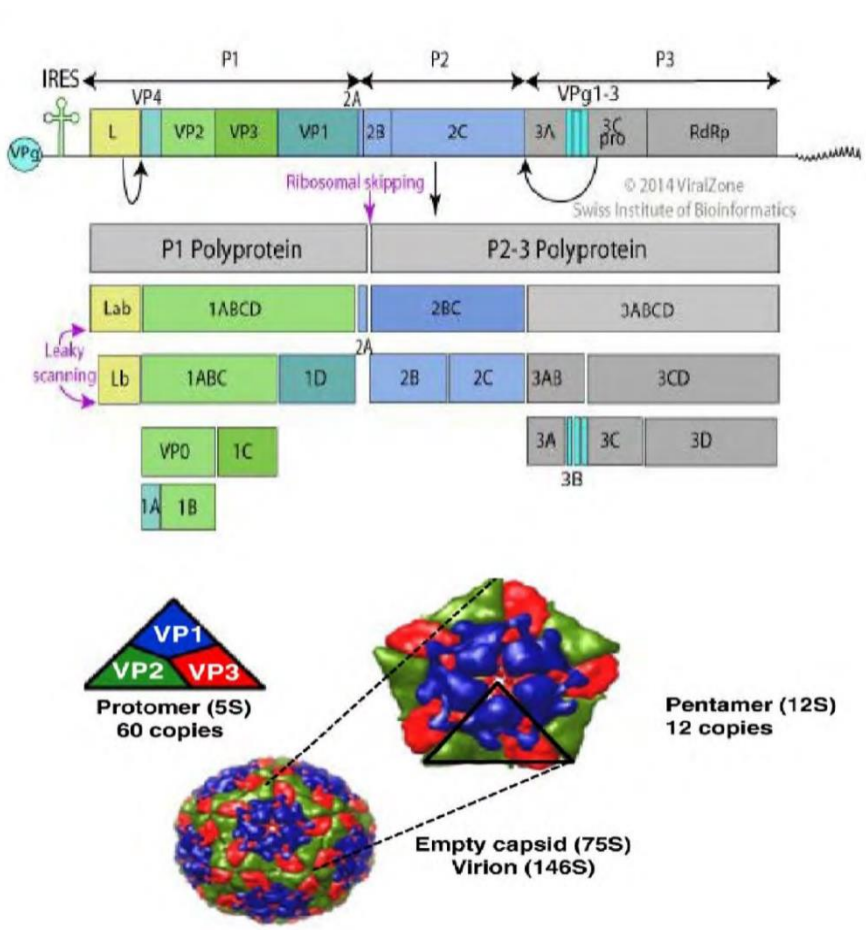


Figure 1: Foot-and-mouth disease virus genome

Source: Sulayeman *et al*.,2018.

2.1.3. Antigenic variation in foot and mouth diseases virus

Foot and Mouth Disease Virus is particularly adept at changing the structure of its surface proteins, a mechanism it uses to avoid being detected by the host's immune defenses. This ability is closely tied to how the immune system responds and is especially influenced by the high rate of mutations in viral protein 1 (VP1). When the immune system mounts a response against the virus, it puts pressure on VP1, one of the most exposed parts of the virus. In response, the virus rapidly accumulates mutations, particularly in the highly changeable sections of VP1 known as hypervariable regions. As the virus replicates, these mutations alter the sequence of amino acids in VP1, helping the virus evade immune detection. This ongoing tug-of-war between the immune response and the virus's adaptations leads to significant variability, especially in the G-H loop .A key factor in this variability is antigenic shift, where sudden and substantial changes occur in the virus's surface structure. These shifts are driven by mutations in areas that influence B-cell epitopes the regions the immune system uses to

identify threats . These changes can allow the virus to slip past existing immunity in a host population, all while continuing to infect the same species. Most of this diversity comes from genetic changes in the virus's capsid protein genes, especially those responsible for its outer coat like VP1. Experiments using direct gene editing have shown that even minor alterations in the G-H loop of VP1 can significantly affect how well antibodies can bind to and neutralize the virus. These findings demonstrate how even small mutations in key antigenic regions can give rise to new viral variants that are capable of escaping immune detection. This highlights the close relationship between the immune system's response, the virus's mutation rate, and the emergence of new forms of the virus (Ding *et al*., 2013; Chakraborty *et al*., 2014).

One of the most defining traits of FMDV is its high capacity for antigenic variation. The virus frequently modifies its outer surface proteins to stay one step ahead of the host's immune system. These changes mostly occur through amino acid substitutions, which introduce genetic mutations. To monitor how different virus strains evolve, researchers often rely on nucleotide sequencing to study the genetic links between current isolates and earlier strains (Maree *et al.,* 2014). Changes in antigenic properties are a key driver behind the appearance of new virus strains, the severity of outbreaks, and how vaccines are selected. However, there's not always a direct connection between genetic similarity and antigenic similarity in different geographic areas, suggesting that other environmental or biological factors may be involved. Phylogenetic studies have revealed that viruses from different serotypes, such as A and SAT1, have exchanged genetic material, resulting in mosaic genomes (Udreshappa *et al*., 2012) These recombination events are often seen in non-structural genes like 3D^pol and 2C, which play critical roles in viral replication. This type of genetic mixing is made more likely by shared environments and overlapping transmission cycles involving both domestic livestock and wild animals (Verma and Kumar, 2012).

2.1.4. Serotypes and Genotypes of FMDV

The virus exists as seven immunologically distinct serotypes: O, A, C, Asia 1, Southern African Territory (SAT)-1, SAT-2 and SAT-3 (Kasambula *et al*., 2012). There are seven immunologically different serotypes of FMDV that infect cloven-hoofed animals worldwide: O, A, C, SAT (Southern African Territories) 1, 2, and 3, and Asia 1 (Kasambula *et al*., 2012). Although there are numerous sub-types, some of which are extremely different from other strains of the same serotype, a large number of samples from all over the world have been

examined, but the existence of another serotype has not been identified (Jamal and Belsham, 2013).

2.2. Mode of transmission

Inhaling viral particles from infected animals' breath is the primary way that FMD is spread. But under some circumstances, the illness can also spread through the air. Control efforts are challenging since the virus can spread through the air under the right circumstances and infect animals outside of quarantine zones (Zewdie *et al.,* 2023). Physical contact between infected and susceptible animals, frequently as a result of infected animals. moving, is largely acknowledged as the most common method of FMD transmission (Pharo and Cobb, 2011). When susceptible animals come into direct contact with infected animals, the virus can enter the host through cuts, abrasions, mucous membranes, damaged epithelium, or the deposition of droplets in the respiratory tract, as illustrated in the figure below (Paton, 2018).

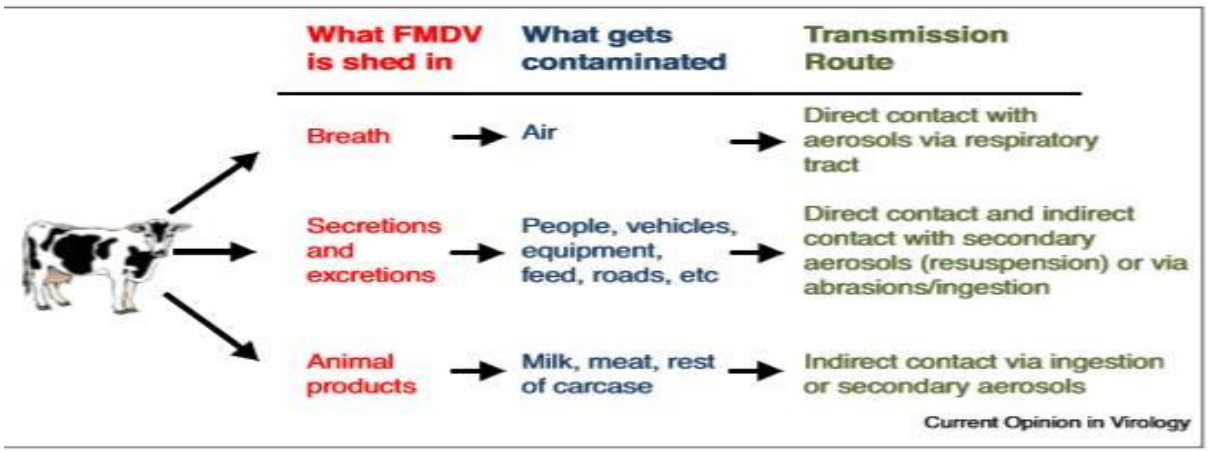


Figure 2: Routes of FMDV transimission

Source: Paton, 2018.

2.3. Epidemiology

FMD is one of the world's most widely distributed diseases, endemic to South America, Asia, the Middle East and sub-Saharan Africa. Out of the 178 countries making up the World Organisation for Animal Health, only 66 are free of FMD, with 10 of these having FMD-free zones (Depa *et al*., 2012). The seven serotypes of the virus do not show any obvious global distribution. O and A serotypes show the greatest distribution and persistent circulation throughout most of the endemic areas including Africa, South Asia, the Far East and South

America (Gorna *et al*., 2014). Asia 1 generally only occurs in the South East and Asia, whilst type C appears widely distributed throughout the Indian sub-continent. Types O, A, SAT 1, SAT 2 and SAT 3 appear to be circulating throughout the African continent, with only type Asia 1 not being found there. The African pools involve different viruses, with the following circulating: Pools 4 (O, A, SAT1, SAT2 and SAT3), Pool 5 (O, A, SAT1 and SAT2) and Pool 6 (SAT1, SAT2 and SAT3), but Pool 7 (South America) seems to only contain viruses of type O and A (Logan, 2017).

2.3.1. Disease distribution

In Africa, with up to 75% of cattle raised via extensive farming methods supporting rural livelihoods and female and child-centric economies, FMD is highly prevalent. Two different cycles govern the epidemiology of FMD in Africa: those sustained autonomously in the domestic animal population and the wildlife (mainly African buffalo) hosted cycle (Thomson et al., 2003). In addition to the maintenance of three SAT serotypes in the African buffalo herd, difficulty in controlling FMD results from the presence of inadequate veterinary resources, lack of trained veterinary personnel and restrictive movements or lack of proper vaccines (Ayebazibwe et al., 2010). Different patterns of FMD epidemiological behaviour are attributable to the wide distribution of FMDVs. Six serotypes (O, A, C, SAT1, SAT2, and SAT3) of FMDVs have been reported in Africa; a summary of the cumulative incidences for each of these serotypes (Paton *et al*., 2009).

2.3.2. Host range

Foot-and-mouth disease has a broad host spectrum, affecting domestic cloven-hoofed animals such as cattle, sheep, goats, and pigs, as well as various wild ruminants. Cattle serve as the primary hosts and are most commonly affected (Arzt *et al*., 2018; WRLFMD, 2023). After infection, cattle can become carriers, meaning they continue to harbor FMDV in the esophageal-pharyngeal (OP) region for more than 28 days post-infection (Juleff *et al*., 2008; Wang *et al*.,2016). While most cattle eventually clear the virus within six months, some rare cases show persistence for up to three years. Sheep and goats can also harbor the virus but usually do so only for a few months (Knight-Jones *et al*., 2017).

Though pigs are susceptible to infection particularly to cell culture-adapted strains of serotype O they are not natural long-term hosts and quickly eliminate the virus after infection

(Zewdie et al., 2023). African buffaloes, in contrast, are well-known long-term carriers. Individual buffalo have been shown to carry the virus for up to five years, and in some herds, circulation has been observed for as long as 24 years (Stenfeldt *et al*., 2020; WOAH, 2022). Although direct transmission from these carriers to domestic livestock has not been conclusively proven, especially in the case of buffaloes, it remains a concern for epidemiologists (Stenfeldt *et al*., 2016; WRLFMD, 2023).

Other wild species such as feral pigs, antelopes, and deer can also harbor strains of FMDV capable of infecting cattle, suggesting that wildlife may occasionally contribute to new outbreaks. Nevertheless, infections in species like elephants and giraffes are often incidental and not deemed significant for broader disease dynamics (Zewdie *et al*., 2023). While FMDV is not considered a food safety or public health hazard, international guidelines still mandate that only animals showing no clinical signs be approved for human consumption (WOAH, 2023). Although the virus poses little zoonotic risk, its impact on livestock health and productivity is profound. Outbreaks can severely disrupt rural economies, reduce household income, and threaten national food security frameworks (FAO, 2023).

2.3.3. Carrier State

FMD carrier animals are those that harbor infectious FMD virus in the nasopharynx beyond 28 days after infection (Stenfeldt *et al*., 2016; WRLFMD, 2023). In the case of Cattle it may goes even up to 2–3 years; Sheep/goats: ~9 months, and African buffalo up to 5 years (Arzt *et al*., 2018; WRLFMD, 2023). Carrier animals show no clinical signs, but buffalo act as long-term reservoirs in endemic areas. Cattle and small ruminants pose a low transmission risk under natural conditions (Stenfeldt *et al*., 2020).

2.4. Clinical Sign and Incubation period

Pyrexia, salivation, and the cattle, lesions are frequently first noticed as blanched regions before developing into fluid-filled vesicles. However, lambs and goats rarely have these vesicles in their mouths; this could be because the lingual epithelium is weak, which results in superficial lesions to break early, leaving behind shallow erosions that typically go away in a few days (Ranjan *et al.,* 2016). The most noticeable and common symptoms in cattle are dribbling saliva from oral lesions and inappetence. In mild cases the lesions are superficial and transient, and heal rapidly (Nsamba, 2015). The deep structure of the foot and mouth may

be severely affected by secondary bacterial invasion of the ruptured lesions, which could hinder healing (Mitiku *et al.,* 2016). About 24 hours prior to the onset of clinical symptoms, virus shedding begins during the incubation stage. The incubation period varies from two to fourteen days, depending on the virus strain that enters the animal, the host's sensitivity, and the infecting dose (Mitiku *et al*.,2016).



Salivation (A), oral mucosal erosion (B), and interdigital space erosion (C).

Figure 3: FMD clinical manifestations

Source: El-Khabaz , 2017

2.5. Diagnostic methods

Infected animals may develop fever, reduced appetite, weight loss, lameness, excessive drooling, and signs of depression. Characteristic blister-like lesions often appear in the mouth, on the teats, and around the hooves (WOAH, 2023). Rapid diagnostic testing to confirm initial clinical suspicions plays a vital role in controlling FMD outbreaks. Diagnosis typically combines observation of clinical symptoms with laboratory analysis to identify the specific serotype of the virus (Admassu *et al.,* 2015). While the symptoms are often clearly identifiable in highly susceptible animals, in regions where the disease is endemic, partial immunity either from prior exposure or vaccination can result in milder or atypical presentations (Yalew, 2019,Verma and Kumar, 2012). Given the speed at which FMD spreads, diagnosis must be carried out quickly using accurate and reliable methods in specialized laboratories equipped to handle high-risk pathogens safely. Preliminary assessments are primarily based on clinical observations (Senawi, 2012; OIE, 2012).

2.5.1. Virus Isolation

Among established diagnostic methods, virus isolation (VI) in cell culture is regarded as the gold standard, as outlined by OIE (2012). However, freezing bovine thyroid cells immediately after trypsinization diminishes their susceptibility to the virus. Although cell

lines are generally less sensitive than primary cells, they are more practical for routine diagnostics. Of the available cell lines, the baby hamster kidney (*BHK-21*) line is preferred due to efficient viral replication and suitability for large-scale use. FMDV is cytocidal, leading to visible changes in infected cells (Verma and Kumar, 2012). When CPE is not initially observed, the culture is frozen and thawed before being transferred to fresh cells, then monitored for another 48 hours. If no CPE appears after three consecutive blind passages, the sample is considered negative for viable virus. Specifically, maintaining a cell culture facility for virus isolation can be challenging and costly, with testing typically taking between 4 to 6 days (Jae *et al*., 2009).

2.5.2. Serological Test

To differentiate with other disease at a later stage, as part of disease control scheme, to certify animals for import or export, to demonstrate freedom of the animal population from infection and to provide proof of vaccine effectiveness, serological diagnosis may be required. Serological testing may be used as a way of identifying virus infections and these are usually based upon reacting to a specific virus protein. The most accurate of the above methods would have to be virus neutralisation test as the most accurate for identifying antibodies to the structural proteins of FMDV (Deb *et al*., 2013). Through Antigen ELISA (Sandwich ELISA) from samples collected from actively infected outbreaks, virus presence can be detected to establish what types of FMDV are present at high concentrations (Foot, 2012).

2.5.3. Nucleic acid detection method

Polymerase chain reaction (PCR) is the most widely used nucleic acid-based diagnostic method for quickly identifying the FMD virus (Xu *et al*., 2013, Reid *et al*., 2000). This method of identifying nucleic acid is the fastest and most sensitive. Over a seven-log range, the viral RNA can be consistently detected; the lowest corresponds to as little as 10-100 RNA per volume analyzed. Using a portable equipment, the test can be finished in two hours or less, and the sample can be stored at room temperature. A small quantity of PCR products (DNA or cDNA) can be measured concurrently (Shaw *et al*., 2007).

2.6. Prevention and Control

The two main methods used to control FMD are vaccination and animal movement control (Mekonen et al., 2011). In Ethiopia, FMD is controlled through quarantine, isolation of sick

animals, vaccination programmes, correct disposal of infected carcasses, and other procedures that are economically possible ((Radostits *et al*., 2007; Gakuya *et al*., 2011; Admassu *et al*., 2015). Two variables could explain the recent increase in FMD outbreaks in endemic locations such as Ethiopia. One factor is a lack of immunity as a result of ineffective vaccination techniques (quality, coverage, and timing). Uncontrolled animal and product mobility is the other problem. Only with a strong regional emphasis and integrated regional policies to improve biosecurity and regulatory monitoring of animal movement between and within nations can animal diseases like FMD be effectively managed (Ayelet *et al*., 2012).

2.7. Distribution of Foot and Mouth diseases virus in Ethiopia

2.7.1 Current status of Foot and Mouth diseases virus in Ethiopia

Ethiopia is home to a large number of domestic and wild animals. Foot-and-Mouth Disease (FMD) is one of the most significant challenge in the country having a significant socioeconomic impact where it is mostly uncontrolled due to frequently movements of cattle without being certified, a lack of Vaccination (Quality, coverage and timing) as well as high vaccine cost for preventive measures, with the exception of a small number of dairy herds that are having exotic animals (Zewdie *et al.,* 2023). Trade animal movements may be connected to the high FMD in central Ethiopia. Ethiopia's urban centres, the greatest of which is Addis Ababa, are more expensive, and cattle regularly move there from other regions of the nation. The country's southern and southeastern regions are the main sources of export animals and cow pastoral lands. Intense animal movement as part of the daily routine of the pastoral farming system may be responsible for the higher prevalence in these areas commercial purposes (Jemberu *et al*., 2016a).

Table 1 Distribution of FMD in Ethiopia from 2020 to 2025

Study area	Serotypes identified	Diagnostic test	Authors
Central Ethiopia	O, A, SAT1, SAT2	sandwich ELISA	(Negessu *et al*., 2023).
Wolaita Zone	SAT-2	sandwich ELISA	Bandaw *et al*., (2024)
Central Ethiopia	O	RT-PCR .	Awel *et al*., (2021).
Different part Ethiopia	O,A,&SAT2	RT-PCR	(Gizaw *et al*., 2020).
Western Amhara		sandwich ELISA	Demil & Tadesse (2025)
Southeastern Ethiopia	O,A, SAT1, SAT2	sandwich ELISA	Gizaw *et al*., (2025)

3. MARIALS AND METHODS

3.1. Study Area

The research was carried out in regions where FMD outbreak cases occurred between September 2025 and June 2026.in Kellem Wollega, East and West Wollega zones, which are located in the Oromia Regional State Western Ethiopia (Figure 4 and Table 2). Kellem Wollega is located at a distance of 650 KM from Addis Ababa, the capital city of the country. This zone has 12 districts and one zonal town. The West Wollega zone is located at a distance of 440 KM from Addis Ababa. This zone had 19 districts and 2 zonal towns. The East Wollega area is located at a distance of 320 KM from Addis Ababa. This zone had 20 districts and one zonal town.

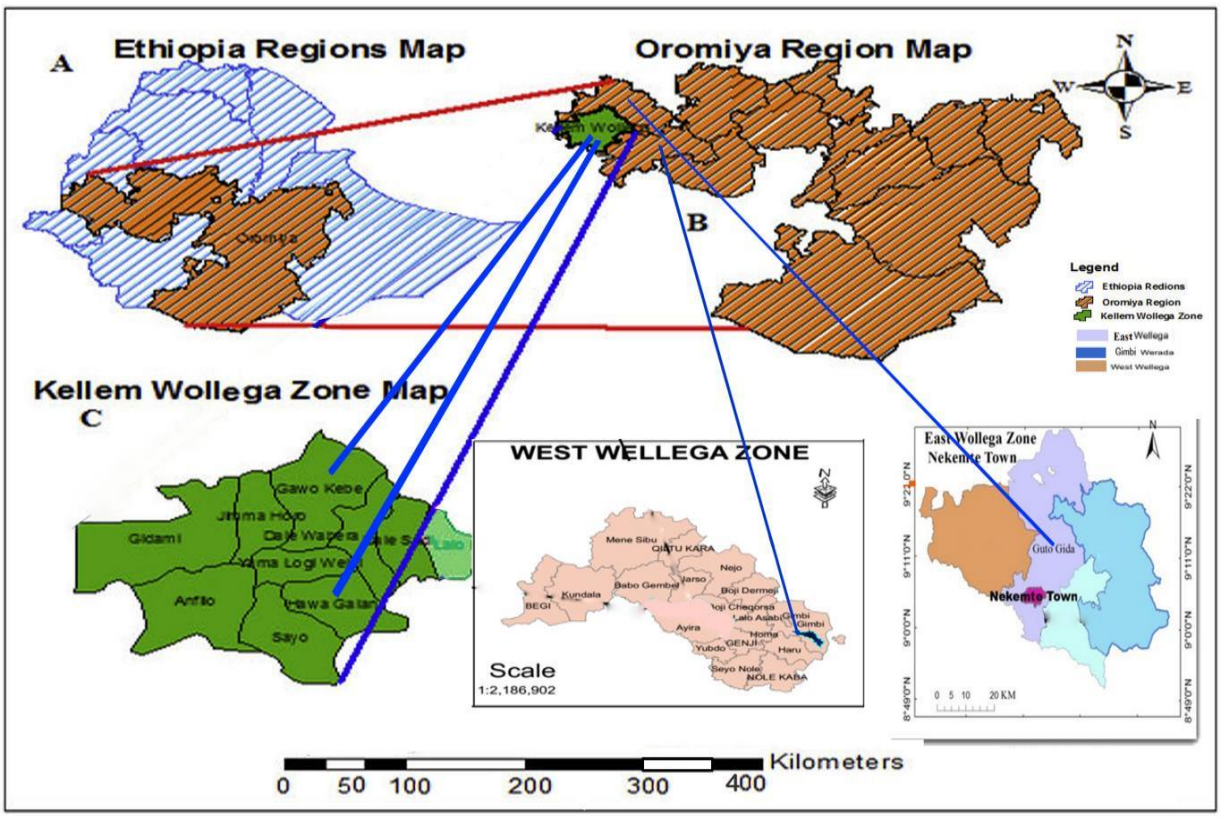


Figure 4: Map of the study area

Source: ArcGIS software, 2026

Table 2: Population description of the study areas.

Districts	Cattle	Sheep	Goats	Equine	Poultry	Swine	Pet animals	References
Kellem Wollega	1579446	927278	799820	241243	4363525	325	49249	(KWAO, 2026)
West Wollega	1734352	820539	633657	326759	4523486	0	45742	(WWAO,2026)
East Wollega	1345497	624578	743285	295924	5364770	0	48538	(EWAO,2026)

Key: Kellem Wollega Agriculture Office, (KWAO, 2026) , West Wollega Agriculture Office, (WWAO,2026), East Wollega Agriculture Office, (EWAO,2026).

3.2. Study Population

The study population was cattle that had experienced an outbreak case of FMD Virus and showed typical clinical signs of the disease (FMD) in the study area during the study period. The study animals were local indigenous breed (*Bos indicus*) and totally managed under extensive type. Moreover, according to information obtained from the veterinary professionals working in the study areas, the animals had not been vaccinated against FMD for three years.

During active outbreaks of foot and mouth disease virus, animal factors including, age, sex, and clinical features of the disease, were documented, and appropriate representative samples were collected. The age of animals was categorized as adult ($\geq$ 3 years old) and young (< 3 years old), according to Mohammed *et al*. (2022). The study animals were closely observed to confirm they showed clear clinical symptoms and increased body temperature (Dubie and Amare, 2020; WOAH, 2018).

3.3. Study and sampling techniques

An outbreak-based study was conducted from September 2025 to June 2026. A purposive sampling technique was used to select districts, towns, and kebeles based on the outbreak cases. Information channels provided to Animal Health professionals were used, and information on FMD outbreak cases was acquired via mobile phone communication. Following the reporting of an active FMD outbreak, a field investigation at the outbreak location within the study districts was carried out for each outbreak that occurred. All animals exhibiting suggestive clinical indications were examined in detail. Animals that were salivating with their mouths opened wide had their tongues, dental pads, and oral mucosa checked for erosions, ulcers, and intact or burst vesicles. Similarly, animals that had lameness had their hooves washed with water to inspect for lesions on the coronary bands and interdigital spaces.

3.4. Sample size, Sample collection, storage and transportation

Epithelial tissue samples were aseptically collected using sterile forceps and surgical scissors. Each specimen was transferred immediately into sterile universal containers containing viral transport medium, with a target ratio of 10% tissue volume relative to the total volume. The VTM consisted of an equal mixture of glycerol and 0.04 M phosphate-buffered saline (PBS), supplemented with 1 µg/ml streptomycin (Certa, Braine l'Alleud, Belgium), 1 mg/ml kanamycin (Sigma, St. Louis, MO, USA), 1000 U/ml penicillin (Continental Pharma, Puurs, Belgium), and 5 µg/ml amphotericin B (Bristol-Myers Squibb, New York, USA), in accordance with the guidelines set by the World Organisation for Animal Health (WOAH, 2022). In addition, oral swab samples were collected using sterile cotton-tipped swabs by gently swabbing the sublingual region and the interface between the lower gums and the inner surface of the lower lip. The swabs were then placed into sterile screw-cap polypropylene tubes containing 2 ml of VTM.

To prevent cross-contamination and transmission of Foot-and-Mouth Disease Virus (FMDV), strict biosecurity protocols were implemented. These included the use of disposable gloves and disinfectants, which were replaced between each household visit to ensure personnel safety and uphold animal welfare standards. All suspected FMDV clinical specimens were maintained under cold chain conditions. Initially, samples were stored at −20°C at the School of Veterinary Medicine, Department of Veterinary Microbiology Laboratory, at Wollega

University, Main Campus just for a week. A total of 32 (13 epithelial tissue samples and 19 oral swab) samples were collected. All collected samples were labeled with an identification number, date of collection, sex, breed, age, type of sample, and other relevant information as outlined in the sampling method (Appendix 1). All clinical collected samples were then packed and transported under a cold chain to the Virology Laboratory at the Animal Health Institute (AHI) in Sebeta. Upon arrival, samples were logged, assigned unique identification codes, and stored at −80°C until further analysis.

3.5. Sample Processing

Sample processing followed WOAH (2022) guidelines. Epithelial tissues were first removed from the transport medium and gently dried using sterile absorbent paper. Approximately 1 g of tissue was homogenized using a sterile mortar and pestle with sterile, acid-washed sand, along with a minimal volume of tissue culture medium of Dulbecco's Modified Eagle Medium (DMEM) and antimicrobial agents (amphotericin B, streptomycin, and penicillin). The homogenate was diluted with DMEM to achieve a 10% (w/v) suspension and was centrifuged at 2000 rpm for 10 minutes using a table-top centrifuge to separate cellular debris. The resulting supernatant was filtered through a 0.22 μm pore-size Millipore membrane and was aliquoted into sterile cryovials. These vials were labeled and preserved at −80°C until further virological analysis. For the oral swab samples, 1 ml of the suspension fluid was vortexed and centrifuged at 2000 rpm for 15 minutes. The supernatant was collected and used for FMDV detection via subsequent molecular tests. All the procedures were conducted inside Biosafety level 2.

3.6. Laboratory investigation

3.6.1. FMD Virus isolation

Baby Hamster Kidney-21(*BHK-21*) cell line was used for isolation of the samples for FMDV. A suspension of the samples was prepared in sterile phosphate buffer saline (PBS)supplemented with antibiotics and it was transferred into a sterile tube and centrifuged. supernatants where then harvest and inoculated onto confluent primary BHK-21 cell line in maintenance Dulbecco's modified Eagle Medium (DMEM)containing 2%bovine fetal calf serum and incubated at 37ºC. The monolayers from the cultures were examined twice daily under an inverted microscope for cytopathic effects (CPEs). The mono layer cell was then maintained in humidified incubator at 37°C and 5%CO2 (Appendix 3). Conversely, the

uninfected cell line was used as the negative control. The plates were examined every day under inverted microscope for cytopathic effect and virus isolates were harvested when > 85% over a period of 24 to 72 hours (1 to 3 days) maximum 3 days for the features rounding of the cell, cell death, disruption of the monolayer cell, aggregation and separation of the cell from the flask were used to determine the cytopathic effect (CPE) (Appendix 3). Samples that did not exhibit cytopathic effects (CPE) in the first passage were stored at -80oC subjected to repeated freeze-thaw cycles, and re-inoculated onto fresh monolayer cell lines. Samples that didn't showing any cytopathic effects (CPE) after 72 hours in the third blind passage were considered negative (OEI, 2012, Ding *et al*., 2013; Gorna *et al*., 2014). The characteristic changes in infected monolayers where carefully examined in each passage. The time for the appearance and density of CPEs was also recorded in each passage. samples that did not develop CPE until the third blind passage were considered negative.

3.6.2. Serotyping of Foot and mouth disease (FMDV) Isolates

Serotyping of FMDV isolates was performed using antigen detection sandwich ELISA and a panel of serotype-specific primers. Sandwich ELISA utilized specific combination of anti-FMDV of monoclonal antibodies (MAbs) as both capture and detection antibodies. To ensure comprehensive serotype coverage, a pan-FMDV test was included in the assay kit to detect any viral isolates belonging to serotypes O, A, C, Asia1, SAT-1, SAT-2, and SAT-3, following the World Organisation for Animal Health (WOAH, 2022) guidelines.

For all Realtime PCR positive sample, sandwich ELISA was conducted using a 96-well microplate format. Each well received 25 µl of diluted ELISA buffer. Subsequently, 25 µl of each sample diluted in ELISA buffer and ready-to-use controls were added to pre-coated wells containing recombinant FMDV antibodies. For each serotype (O, A, C, SAT-1, SAT-2), one positive control and one negative control were included on each plate. The microplates were sealed with adhesive plate sealers and incubated at room temperature (20–25°C) for 1 hour. Following incubation, the wells were washed three times using 200 µl of the supplied washing solution, allowing a 3-minute incubation at room temperature during each cycle. Any residual fluid was removed using clean absorbent paper. Next, 50 µl of conjugate A was added to columns 1–8, and 50 µl of conjugate B was added to columns 9–12. Plates were incubated again at room temperature for 1 hour. Afterward, 50 µl of substrate solution was added to each well, and the plates were incubated in the dark at room temperature for 20

minutes. The reaction was stopped by adding 50 µl of sulfuric acid (H_2SO_4) stop solution per well. Immediately after stopping the reaction, optical density (OD) was measured at 450 nm using a microplate reader (Dubie and Amare, 2020, Mohammed *et al*., 2022). The result was interpreted according to the kit protocol as depicted in Appendix 5.

3.6.3 RNA Extraction

RNA extraction was performed at the Animal Health Institute (AHI) using processed epithelial tissue and oral swab suspensions. Viral RNA was isolated using the Qiagen Viral RNA Mini Kit (Qiagen, Crawley, West Sussex, UK) according to the manufacturer's recommendations and procedures previously described by Tikuye (2019).

Briefly, 140 µl of each prepared sample suspension was mixed with buffer AVL containing carrier RNA to facilitate viral lysis. The mixture was vortexed for approximately 15 seconds and incubated at room temperature for 10 minutes. Following brief centrifugation, 560 µl of 96% ethanol was added to enhance RNA binding efficiency. The solution was mixed thoroughly and transferred into QIAamp Mini spin columns in sequential steps, followed by centrifugation at 8000 rpm for 1 minute.

The columns were subsequently washed using buffer AW1 and buffer AW2 according to the kit procedure to remove impurities and contaminants. Final centrifugation was carried out at 14,000 rpm for 3 minutes to ensure complete drying of the membrane. RNA was then eluted using 60 µl of buffer AVE after incubation at room temperature for 1 minute. The purified RNA obtained was used for downstream molecular analysis, particularly real-time RT-PCR, and samples were temporarily stored at +4°C prior to analysis.

3.6.4. Detection of FMDV using real time RT- PCR

A one-step real-time reverse transcription polymerase chain reaction (rRT-PCR) assay was being employed to detect the presence of FMDV in all field collected samples. RNA by targeting a conserved region of the viral genome. Specifically, primers and a TaqMan probe that amplify 3D(pol)gene of the virus were employed following the protocol described by Mohammed *et al*. (2022). The assay utilized the following universal primers and probe: FMD 3D-forward (5′-ACT GGG TTT TAC AAA CCT GTGA-3′), FMD 3D-reverse (5′-GCG AGT CCT GCC ACG GA-3′), and the TaqMan probe (5′-[6FAM] TCC TTT GCA CGC CGT GGG AC [TAM]-3′). Real-time RT-PCR was performed using the SuperScript™ III Platinum® One-Step rRT-PCR Kit (Invitrogen™, USA). A total volume of 25 µl reaction

were used. Each 25 µl reaction mixture consisted of 5 µl of extracted RNA template, 12.5 µl of 2× reaction mix, 0.5 µl of SuperScript III/Platinum Taq enzyme mix, 2 µl each of forward and reverse primers, 1.5 µl of the TaqMan probe, and 1.5 µl of nuclease-free water (Appendix 7).

Each PCR run included both a positive control (FMDV field isolate) and a negative control (nuclease-free water) to validate the assay. Thermal cycling conditions was set as follows: reverse transcription at 50°C for 30 minutes, initial denaturation at 95°C for 10 minutes, followed by 50 amplification cycles consisting of denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute, and extension at 72°C for 30 seconds.

Interpretation of real-time RT-PCR

PCR Amplification results were analyzed based on the threshold cycle (Ct) values. A Ct value of less than 32 was considered positive for FMDV; values between 32 and 40 was considered doubtful and subject to retesting, while samples with undetectable Ct values was classified as negative; which underwent a repeat test (Howson *et al*., 2017), and samples having Ct value <24.62 were taken as strong positive, indicated that Ct value is inversely proportional to the concentration of nucleic acid in the samples as it is shown in Appendix 8.

3.7. Ethical clearance

Ethical approval for this research was obtained from Wallaga University, animal research ethical review committee (Ref. No: WU/REC/819/), Nekemte Campus (indicated in Appendix XI). Oral informed consent was also obtained from the animal owners. All the Sampling process from the animals was undertaken as per the standards approved by the animal welfare and research ethics committee at the university, which is in accordance with the international guidelines for animal care and welfare.

3.8. Data Management and Analysis

Data collected from both field and laboratory investigations were coded and entered into Microsoft Excel 2010 spreadsheets for organization and analysis. Descriptive statistical methods were used to determine proportions. Results obtained from serotype identification and RT-qPCR assays were further categorized and presented in tabulated.

4. RESULT

4.1. Clinical Examination of FMD outbreak cases

During the outbreak investigation, animals showed typical clinical manifestations of FMDV infection, including salivation, lameness, and vesicle formation in the oral cavity and interdigital spaces were observed. Oral lesions consisted of erosions on the dental pads and tongue, while foot lesions involved abrasions in the interdigital areas. Clinically affected cattle were reluctant to move and they were also unable to take feed (Figure 5).



A B

Figure 5: Clinical sign of Foot-and -Mouth diseases observed in affected cattle.

(The arrow in A interdigital vesicle and B indicate wound on upper gum).

4.2. Molecular Detection of Foot and Mouth Disease virus

Out of the 32 samples analyzed, 10 samples (31.25%) were positive for FMDV. The cycle threshold (Ct) values of the positive samples ranged between 22.67 and 31.69 (Figure 6).

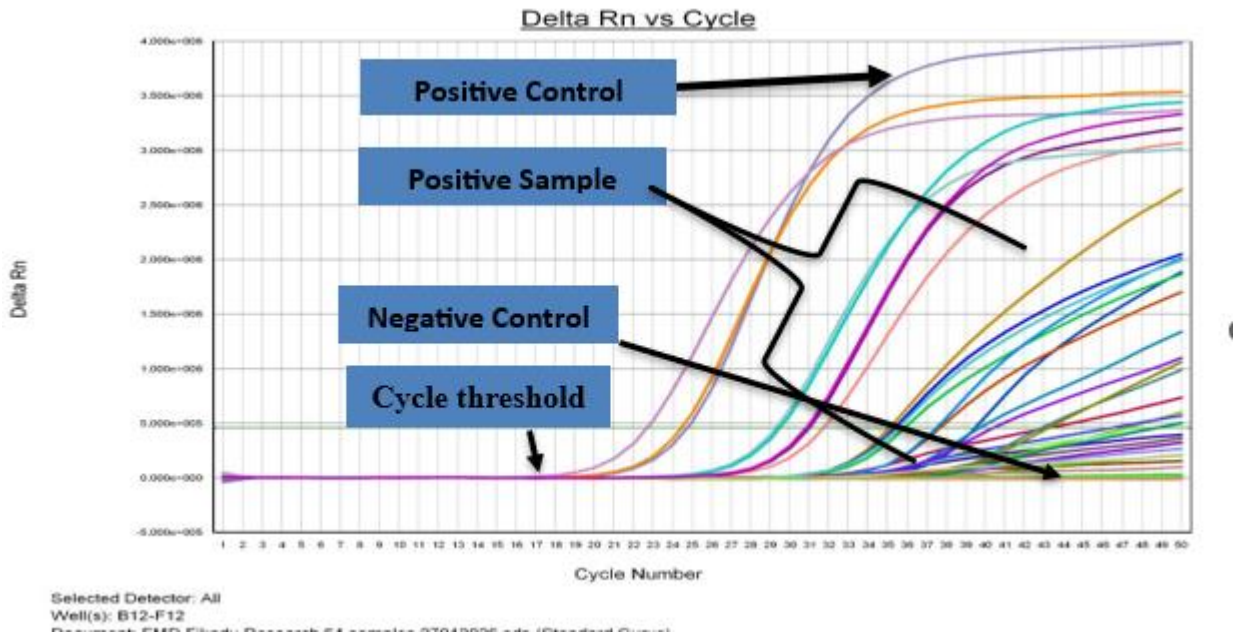


Figure 6: Real time-PCR amplification of FMD virus

Molecular detection of FMDV based on the outbreak area indicated that, the highest number of RT-qPCR positive samples were obtained from Guto Gida district (3,60%), East Wollega zone, followed by Gawo Kebe district (3; 30% and Hawa Gelan (2; 28.57%) from Kelem Wollega zone (Table 3).

Table 3: Results of real time RT-PCR and virus isolation across the sample type and study area.

Zone	Districts	Sample type	No of sample tested	Real time RT-PCR positive	Virus isolation
Kellem Wollega	Gawo Kebe	Swab	5	1 (20%)	0%
		Tissue	5	2 (40%)	2(100%)
	Sayo	Swab	4	0(0%)	NT
		Tissue	1	1(100%)	1(100%)
	Hawa Gelan	Swab	6	1(16.67%)	1(100%)
		Tissue	1	1(100%)	1(100%)
West Wollega	Gimbi	Swab	3	0(0%)	NT
		Tissue	2	1(50%)	1(100%)
East Wollega	Guto Gida	Swab	1	1(100%)	1(100%)
		Tissue	4	2(50%)	2(100%)
	Total		**32**	**10(31.25%))**	**9 (90%)**

Where; NT: Not Tested

Based on the type of sample, from the total of 32 collected samples, 19 samples were swab type. Among these, only 3(15.79%) sample was found positive using Real time RT-PCR. Whereas, from the total of 13 epithelial tissues collected, 7(53.85%) samples were found positive (Table 4).

Table 4: Real time RT-PCR and virus isolation findings per sample types

Sample type	**No of sample tested**	**Real time RT-PCR positive**	**Virus isolation**
Swab	19	3(15.79%)	2(166.67%)
Epithelial tissue	13	7(53.85%)	7(100%)
Total	**32**	**10(31.25%)**	**9(90%)**

4.3. Serotyping by Sandwich ELISA

To identify the circulating serotypes in the study areas, antigen detection was performed using sandwich ELISA on samples that were positive by real-time reverse transcriptase polymerase chain reaction (RT-qPCR). The findings indicated that serotype O was the only serotype detected as the causative agent of the outbreak across all study sites (Table 5).

Table 5: Identification of serotypes of FMD virus from four Outbreaks areas

Outbreak area		**Sample Types**	**Animal Factors**		**Identified Serotypes**
Zone	**District**		**Sex**	**Age**	
Kellem	Gawo Kebe	Tissue	Male	young	O
Wollega		Tissue	Female	Adult	O
		Swab	Male	Adult	O
		Tissue	Female	Young	O
	Sayo	Tissue	Male	Adult	O
	Hawa Gelan	Tissue	Male	Adult	O
		Swab	Female	Adult	O
West Wollega	Gimbi	Tissue	Male	Adult	O
East Wollega	Guto Gida	Tissue	Female	Young	O
		Tissue	Female	Young	O
		Swab	Male	Adult	O

4.4. Virus isolation on cell culture

All the real time RT-PCR positive samples were inoculated on to Baby hamster kidney (BHK-21). From ten positive samples inoculated on this cell line, 9(90%) showed cytopathic effects (CPE). The observed CPEs were destruction of monolayer cells and round shaped appearance. Additionally, there were complete destruction of the cells and their detachments and swelling which were mostly seen within 48hrs of inoculations (Figure7).

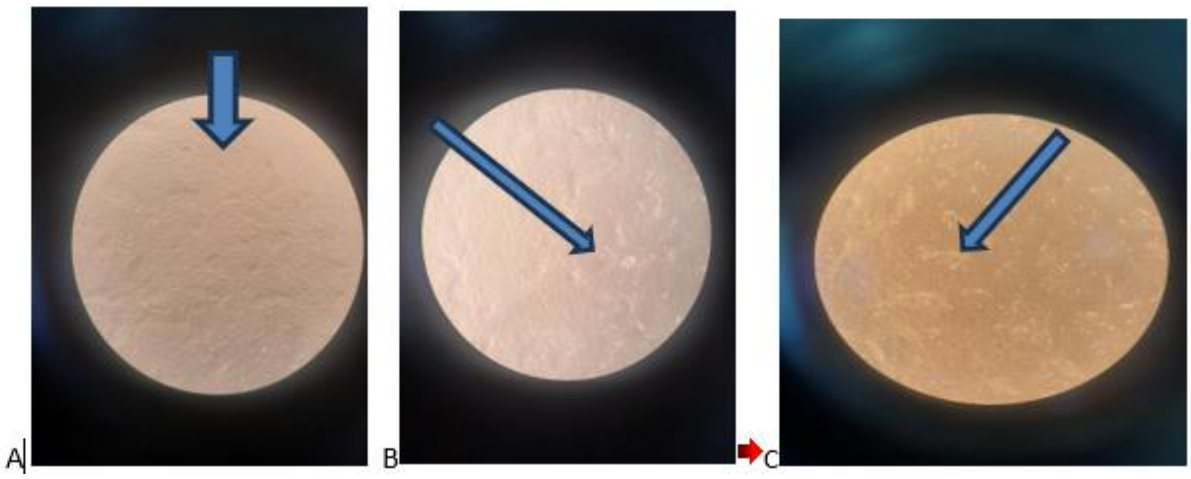


Figure 7: Picture showing FMDV on BHK cell

The arrow in **A** shows BHK-21 cell control (un inoculated), **B**; shows induced cell rounding and swelling and the arrow in **C**; shows Induced cell detachment, massive cell death and granule formation.

5. DISCUSSION

Foot-and-Mouth Disease (FMD) is a highly contagious viral infection that affects cloven-hoofed animals, including cattle, sheep, goats, and pigs. It is a trans-boundary disease, easily spreading across borders and causing disruptions in international trade (Betelihem, 2021). The disease is prevalent in most regions of sub-Saharan Africa, where six out of seven serotypes exist, with the virus transmitting between wild animals and domesticated livestock (Ayelet *et al*., 2009). Ethiopia is home to a significant population of livestock, which includes both domestic and wild species. In Ethiopia, FMD is recognized as one of the key livestock diseases with considerable socio-economic implications. The disease continues to be endemic in various parts of the world, such as Ethiopia, presenting significant challenges for commercial trade with countries that are free from Foot-and-Mouth Disease (FMD). Therefore, it is crucial to manage FMD by minimizing the spread of the virus to unaffected regions and employing customized vaccines that have antigenic compatibility with the strain involved in the outbreak (Wubshet *et al*., 2019; Zewdie *et al*., 2023).

In the present outbreak investigation, clinical signs indicative of FMD, particularly vesicles were mainly found in the oral cavity and abrasions were found in the interdigital areas. This finding aligns with the previous researches conducted by various scholars (Tesfaye *et al*., 2020; Woldesenbet *et al*., 2023; Bandaw *et al*., 2024). Further studies reported by Tindashe (2018) in Oromia, Amhara, and Addis Ababa; Tikuye (2019) in Tigray, Oromia, and SNNP regional states; Mutal (2019) in Oromia, Amhara, and SNNP; Betelihem (2021) in Western Amhara ; Awel *et al*.(2021) and Moahammed *et al*.(2022) in Central Ethiopia; Tegegne *et al.* (2024) in Jimma Zone and Southwest Ethiopia, Bandaw *et al*.(2024) in Wolaita Zone, and Yirdaw *et al*.(2024) in western Amhara regional state corroborate the present findings.

This research presents the molecular evidence of Foot-and-Mouth Disease (FMDV) circulation across the three zones of Wollega. Out of the 32 samples analyzed, 10 (31.25%) samples were positive for Foot-and-Mouth Disease (FMDV) through real-time reverse transcriptase polymerase chain reaction RT-PCR targeting the 3D gene. This detection rate is consistent with the previous findings from other outbreak studies in Ethiopia (Sulayeman *et al*., 2018, Awel *et al*., 2021,Gizaw *et al*.,2020; Negessu *et al*.,2023). Paixao *et al*.(2021) also corroborates the current finding; reporting that real time-PCR targeting 3D region of the viral genome is a powerful technique for reliable detection of FMDV from the field samples.

The present study also revealed considerable variations in FMDV gene detection among the districts. Consequently, the highest proportion of FMDV gene was recorded in Guto Gida district from East Wollega Zone. The variations among the districts might be due to the difference in agro-ecological and management practices. Hence, the samples were preserved for long time at −20°C until the transportation of the samples from the collection site to the laboratory which could significantly cause deterioration of the sample (OIE, 2012; Mohammed *et al*., 2022; Yirdaw *et al*., 2024).

The current finding also showed that, from the total of 10 real time RT-PCR positive samples, higher number of the positive samples were obtained from epithelial tissues than from oral swab sample. This finding substantiated the earlier studies conducted by Urge *et al*. (2020) and Ayele *et al.* (2009), reporting the presence of higher levels of viral RNA in the epithelial tissues. In support of this observation, OIE (Now a days called WOAH) (2012) conveyed that the ideal sample for virus detection is the epithelial tissue. In agreement with this, Reid *et al*. (2003) also indicated epithelial tissue samples from the vesicular lesions could be used as the sample of choice for FMDV detection.

In the current study, serotypes O were identified using sandwich ELISA. This finding was in agreement with previous studies indicating that serotype O was highly dominant serotype circulating in Ethiopia, primarily due to its significant genetic variability, characterized by the presence of multiple topotypes and the emergence of new variants (Ayelet *et al*.,2009; Negessu *et al*.,2023; Seifu *et al*.,2023. Furthermore, the Outbreaks reported in different parts of Ethiopia (Tesfaye *et al*., 2021; Jemberu *et al*., 2022.), and other studies reported from different African countries (Souley Kouato *et al*., 2018 in Nigeria; Josiah *et al*., 2024 in Kenya) were largely attributed to this serotype, which is considered the most parts of the continent. The exclusive detection of serotype O has important implications for control and prevention. The Ethiopian National Veterinary Institute produces a trivalent vaccine containing serotypes O, A, and SAT2. Since serotype O was the only one identified, the current vaccines should, in principle, offer protection. However, the effectiveness of the vaccine is contingent upon a close antigenic match between the vaccine strain and circulating field strain. Foot-and-Mouth Disease (FMDV), particularly serotype O, exhibits high genetic and antigenic diversity due to its RNA-dependent RNA polymerase's lack of proofreading ability (Jamal and Belsham, 2013; Longjam *et al*.,2011).

Studies in Ethiopia have already shown that field strains can differ significantly from vaccine strains, potentially leading to vaccine failure (Tesfaye *et al*.,2021; Jemberu *et al*.,2022). The

rapid spread of serotype O demonstrates the need to have regular surveillance and monitoring systems to determine and respond effectively to FMDV in endemic areas. In this study, among RT-PCR positive samples that were subjected to Baby Hamster Kidney (BHK-21) monolayer cell line adaptation, almost near to all (90%) samples were showed cytopathic effects (CPE) such as cell rounding followed by rapid cell distraction, detachment of cell from the flask's surface and total/massive cell death. This finding was in line with study findings by Tesfaye *et al*. (2016), Sulayeman *et al*. (2018), Negessu *et al*., 2023.and Metages (2018) in which infected cells in both study results showed round and sloughing as well as monolayer detachment from the wall of the cell-culture flask. Other authors also described that FMDV isolated from clinical samples and inoculated on BHK-21 cell-line results in infected cells that showed specific CPE within 24–48-hour postinfection was characterized by rounding of cells and distortion of the monolayer and cell detachments (Shawky *et al*.,2013).

6. CONCLUSION AND RECOMMENDATIONS

The present study primarily focused on molecular detection and isolation of the presence of Foot and Mouth Disease Virus and its specific serotype circulating in the study areas from the outbreak cases. Molecular analysis conducted using real-time PCR confirmed the presence of Foot and Mouth Disease Virus (FMDV) gene in both epithelial tissue and oral swab samples. More importantly, epithelial tissue samples demonstrated a higher proportion of positive results than oral swabs, indicating a greater viral RNA concentration and suitability for the molecular detection of Foot and Mouth Disease Virus (FMDV). Furthermore, virus isolation on BHK-21 cells demonstrated that almost near to all PCR positive samples showed cytopathic effect.

Based on the above conclusions, the following recommendations are forwarded:

- Further epidemiological investigation complemented with genetic analysis of the FMD virus should be carried out to obtain detailed information on the serotypes and topotypes of the virus circulating within the study areas
- Effective control strategies of the disease should be implemented.

7. REFERENCES.

Admassu, B., Getnet, K., Shite, A., Mohammed, S. (2015): Review on Foot and Mouth Disease : Distribution and Economic Significance. *Academic Journal of Animal Diseases*, **4**(**3**): 160-169.

Alexandersen, S., Zhang, Z., Donaldson, A. I., & Garland, A. J. (2003). The pathogenesis and properties of FMDV. *Veterinary Journal*, **165**(**2**): 146–169.

ArcGIS software, 2026

Arzt, J., Pacheco, J. M., and Rodriguez, L. (2018). The risk of FMDV transmission from carriers. *mBio*, **9**(**3**): e00579-18.

Ashfaq, M., Razzaq, A. and Muhammad, G. (2015). Economic analysis of dairy animal diseases in Punjab: a case study of Faisalabad district. *J. Anim. Plant Sci.,* **25**:1482-1495.

Awel, S. M. (2021). Seroprevalence and molecular detection of foot-and-mouth disease virus in dairy cattle around Addis Ababa, central Ethiopia. Veterinary Medicine: Research and Reports, **12**: 181–191.

Ayebazibwe, C., Mwiine, F.N., Tjornehoj, K. (2010): The role of African buffalos (Syncerus caffer) in the maintenance of foot-and-mouth disease in Uganda. *BMC Veterinary Research*, **6**:54.

Ayelet, G., Mahapatra, M., Gelaye, E., Egziabher, B. G., Rufeal, T., Sahle, M., ... & Knowles, N. J. (2012). *Journal of Clinical Microbiology*, **52**(**9**): 2893-2901.

Ayelet, G., Mahapatra, M., Gelaye, E., Egziabher, B.G., Rufeal, T., Sahle, M., Ferris, N.P., Wadsworth, J., Hutchings, GH. and Knowles, NJ.(2009). Genetic characterization of foot-and-mouth disease viruses, Ethiopia, 1981–2007. *Emerg. Infect. Dis*.;**15**(9):1409. doi: 10.3201/eid1509.09009.

Ayelet, G., Mahapatra, M., Gelaye, E., Egziabher, B., Rufeal, T., Sahle, M. (2009): Genetic characterization of foot and mouth disease viruses, Ethiopia, 1981– 2007. *EmergingInfectious Disease,* **15:** 1409.

Bandaw, T., Gebremeskel, H. F., Muluneh, A., Mengistu, T. S., Kebede, I. A., & others. (2024). Seroprevalence and molecular detection of foot and mouth disease virus in cattle in selected districts of Wolaita Zone, Southern Ethiopia. *Scientific Reports*, **14**:7929.

Bari FD, Parida S, Tekleghiorghis T, Dekker A, Sangula A, Reeve R, Haydon DT, Paton DJ, Mahapatra M (2014) Genetic and antigenic characterisation of serotype A FMD viruses from East Africa to select new vaccine strains. *Vaccine* **32**:5794–5800.

Bari, F., Parida, S., Asfor, A., Haydon, D., Reeve, R., Paton, D. and Mahapatra, M. (2015).Prediction and characterization of novel epitopes of serotype A foot and mouth disease viruses circulating in East Africa using site-directed mutagenesis. *J. Gen. Virol.*, **96**:1033.

Betelihem, Y. G. (2021). Epidemiology and economic impact of foot and mouth disease in domestic ruminants in western amhara regional state, north western Ethiopia (Doctoral dissertation, Addis Ababa University).

Carrillo, C., Kutish, G., and Rock, D. (2005). Comparative Genomics of Foot and Mouth Disease Virus. *J. Virol*., **79**: 6487-6504.

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

CSA, (2021). Agricultural sample survey, 2020/21 Volume II, Report on livestock and livestock characteristics (private peasant holdings). Central Statistical Agency (CSA), Addis Ababa, Ethiopia.

Curry, S., Fry, E., and Blakemore, W. (2015). Foot-and-Mouth Disease Virus Capsid Stability. *Journal of Virology*, **89**(**3**):1751–1761.

Deb. R, Chakraborty. S., Veeregowda. B., Verma AK., Tiwari. R, and Dhama K.(2013). Monoclonal antibody and its use in the diagnosis of livestock diseases. *Adv Biosci Biotechnol*; **4**:50-62.

Demessa, B., Leta, S., & Amenu, K. (2023). Frontiers in Veterinary Science, **10 :**1124456.

Demil, E., & Tadese, T. (2025). Spatio-temporal distribution of foot-and-mouth disease outbreaks (January 2018–June 2023) in Western Amhara region, Ethiopia. PLoS ONE, **20**(**5**):e0313305.

Depa, P. M., Dimri, U., Sharma, M. C. and Tiwari R. (2012). Update on epidemiology and control of Foot and Mouth Disease-A menace to international trade and global animal enterprise. Veterinary world, **5**: 694-704.

Ding, Y. Z., Chen, H. T., Zhang, J., Zhou, J. H., Ma, L. N., Zhang, L. and Liu, Y. S. (2013).An overview of control strategy and diagnostic technology for foot-and-mouth disease in China. *Virology journal*, **10**(**1**): 1-6.

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. *Veterinay medicine international*,**2020**(**1**), 8847728.

El-Khabaz, K. A. S., & Al-Hosary, A. A. T. (2017). Detection and identification of Foot and Mouth disease virus serotypes in Assiut governorate, Egypt. *Journal of Advanced Veterinary and Animal Research*, ***4***(**1**): 32-38.

FAO (2021). Global FMD control strategy – progress report.

FAO. The global foot and mouth disease control strategy." Food and Agriculture Organization of the United Nations. https://www.fao.org/ in-action/kore/goodpractices/good-practices-details/en/c/11373 39/ (accessed 29 Nov 2023, 2023)

Food and Agriculture Organization of the United Nations (FAO). (2023). Foot-and-mouth disease: Quarterly report (Apr–Jun 2023). FAO. https://openknowledge.fao.orgfoot-and-mouth disease on livelihoods in the Borena Plateau of Ethiopia. *Pastoralism: Research, Policy and Practice*, ***3***(**1**):1-11.

Foot, M. (2012). Foot-and-mouth disease virus: Structure, pathogenesis and control. *Veterinary Microbiology Journal*, **156**(**3**–**4**): 123–130.

Gakuye, A., Tolosa, T. and Regassa, F. (2011). *Ethiopian Veterinary Journa*l, **15**(**2**): 45-55.

Gammada I., Morshed, M.M., Rabby, T.R. and Hossain, M.I. (2022). The prevalence of lumpy skin disease in the cattle population: a brief study. *Int. J. Agric. Vet. Sci.*, **4**(**3**): 55-67.

Gao, Y., Sun, S. and Guo, H. (2016). Biological function of foot and mouth disease virus non-structural proteins and non-coding elements. *Virol. J.*, **13**:107.

Gelaye, E., Ayelet, G., Abera, T., and Asmare, K. (2009). Sero prevalence of foot and mouth disease in Bench Maji zone, south western Ethiopia. *J Vet Med Anim Health*., **1**(1) :5-10.

Gizaw, D., Beyene, G., Ashenafi, H., Legesse, M., & Kassa, T. (2024). Seroprevalence and serotype distribution of foot and mouth disease virus and associated risk factors in cattle across various export livestock-sourcing districts of Bale Zone, Ethiopia. *BMC Veterinary Research*, **20**(**1**): 382.

Gizaw, D., Beyene, G., Ashenafi, H., Legesse, M., & Kassa, T. (2025). Spatial and temporal patterns of foot-and-mouth disease outbreaks (2011–2022) in cattle export-sourcing areas of southeastern Ethiopia. BMC Veterinary Research, **21**(**1**):11.

Gizaw, D., Tesfaye, Y., Wood, B. A., Di Nardo, A., Shegu, D., Muluneh, A. and King, D. P. (2020). Molecular characterization of foot-and-mouth disease viruses circulating in

Ethiopia between 2008 and 2019. *Transboundary and emerging diseases*, 67(**6**), 2983-2992.

Gorna, K., Houndje, E., Romey, A., Relmy, A., Blaise-Boisseau, S., Kpodekon, M., Saegerman, C., Moutou, F., Zientara, S. and Bakkali, K. L. (2014). First isolation and molecular characterization of foot-and-mouth disease virus in Benin. *Veterinary Microbiology*, **171**: 175-181.

Grace, D., Alonso, S., Mutua, F., Roesel, K., & Lindahl, J. (2016). Food safety and zoonoses in developing countries: The role of livestock. *International Livestock Research Institute*.

Graham., B, Anette., B, Louise L Foot-and-Mouth Disease in Animals. https://www.msdvetmanual.com/generalized-conditions/foot-andmouth-disease/foot-and-mouth-disease-in-animals (accessed 07 June, 2023).

Howson, E. L. A., Armson, B., Madi, M., Kasanga, C. J., Kandusi, S., Sallu, R.and Fowler,V.L. (2017). Evaluation of two lyophilized molecular assays to rapidly detect foot-and mouth disease virus directly from clinical samples in field settings. *Transboundary and Emerging Diseases*, **64**(**3**) :861-871

International Committee on Taxonomy of Viruses. (2012). Virus taxonomy: 2012 release. *ICTV*. https://ictv.global/report/2012.

Jae, Y. J., Park, J. H., and Ko, Y. J. (2009). Molecular characterization of foot-and-mouth disease virus and its epidemiology. *Journal of Veterinary Science*, **10**(**3**): 203–210.

Jamal S. and Belsham GJ. (2013). Foot and mouth disease: past, present and future. *Vet Res.* **44**:116.

Jemberu, W. T., Molla, W., Fentie, T., and Knight-Jones, T. J (2022). Assessment of effectiveness of a foot-and-mouth disease vaccine in cattle in Ethiopia. Paper presented at the 16th International Symposium of Veterinary Epidemiology and Economics, Halifax, Canada. International Livestock Research Institute (ILRI). https://www.ilri.org/knowledge/publications/assessment-effectiveness-foot-and-mouth-disease-vaccine-cattle-ethiopia

Jemberu, W. T., Mourits, M. C. M., Sahle, M., Siraw, B., Vernooij, J. C. M., & Hogeveen, H. (2016). Epidemiology of foot-and-mouth disease in Ethiopia: A retrospective analysis of district-level outbreaks, 2007–2012. *Transboundary and Emerging Diseases*, **63**(**6**): 246–e259.

Jemberu, W. T., Selassie, M., and Gebremedhin, E. Z. (2020). *Preventive Veterinary Medicine*, **174** :104808.

Josiah, J. M., Nyamache, A. K., Woldemariyam, F. T., Kariuki, C. K., Paeshuyse, J.,and Kamau, J. M. (2024). Serotype Diversity of Foot and Mouth Disease Virus and Molecular Characterization of Serotype O Strains from 2019 and 2020 Outbreaks in Kenya. *Benha Veterinary Medical Journal*, **46**(**1**):81-86.

Kasambula, L., Belsham, G., Siegismund, H., Muwanika, V., Ademun-Okurut, A. and Masembe, C. (2012). Serotype identification and VP1 coding sequence analysis of foot and mouth disease viruses from outbreaks in eastern and northern Uganda in 2008/9. *Transbound. Emerg. Dis*., **59**:323–330.

Kim, S. M., Joo, H. D. and Lee, K. (2024). Calcium chloride enhances FMD vaccine stability. *Vaccines*, **12**(**4**) :367.

Klein, J.(2009). Understanding the molecular epidemiology of foot and mouth disease virus. *J. Mol. Epi. Evol. Genet. Infect. Dis*., **9**: 153-161.

Knight-Jones TJD, McLaws M, Rushton J. Foot-and-mouth disease impact on smallholders - what do we know, what don't we know and how can we fnd out more? *Transbound Emerg Dis*. 2017;**64**(**4**):1079– 94.

Logan G. (2017). The molecular and genetic evolution of foot-and-mouth disease virus. PhD Thesis, University of Glasgow.

Longjam, N., Deb, R., Sarmah, A., Tayo, T., Awachat, V. and Saxena, V. (2011). A brief review on diagnosis of foot and mouth disease of livestock: conventional to molecular tools. *Vet. Med. Int.*, **2011**: 1-17.

Lordwin, K. (2011). Inter-epidemic molecular characterization of foot and mouth disease Viruses in eastern and northern Uganda between 2008 and 2009 (doctoral dissertation, Makeree University, Uganda)

Mahapatra, M., Upadhyaya, S., Aviso, S., Babu, A., Hutchings, G. and Parida, S. (2017). Selection of vaccine strains for serotype O foot and mouth disease viruses (2007–2012) circulating in Southeast Asia, east Asia and far east. *Vaccine*, **35**:7147–7153.

Maree, F.F., Kasanga, C.J., Scott, K.A., Opperman, P.A., Chitray, M., Sangula, A.K., Sallu, R., King, D.P., Paton, D.J.and Rweyemamu, M.M. (2014). Challenges and prospects for the control of foot- and-mouth disease: an African perspective. Veterinary Medicine: *Research and Reports*, **5**: 119–138.

Mekonnen, G., Ayelet, G., & Hussein, A. (2011). *Tropical Animal Health and Production*, **43**(**4**): 789-795.

Mitiku, T., Garedew, L., Zemene, Mebrie, Dagnaw, M., Admassu, B.,and Getaneh, G. (2016). A Review on Foot And Mouth Disease And Its Current Status In Ethiopia. *Researcher*, **8**: 42-51.

Mohammed, Z.,Aliy, A., Jibril ,Y.and Negussie, H.,(2022).Epidemiological and clinical characteristics of the foot and mouth disease outbreaks in cattle in central Ethiopia.**26**(**1**):105-121.

Mutal. H. (2019).serological investigation of foot and mouth disease in cattle and pigs inselected commercial farms and molecular characterization from active outbreak cases (Doctoral dissertation, College of Veterinary Medicine and Agriculture, Addis AbabaUniversity).

Negessu, D., Ebisa, I.,Mohamad, A., Bilata, T., Muluneh, A., Shagu, D. and Woldemariyam, F. T. (2023). Isolation, Molecular Detection and Genotyping of Foot-and-Mouth Disease Virus from Naturally Infected Cattle in Central Ethiopia. *American Journal of Life Sciences*, **11**(**3**):36-43.

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

Nsamba, P., Mwiine, F. N., & Okurut, A. R. A. (2015). *Journal of Advanced Veterinary and Animal Research*, **2**(**4**): 407-413.

OIE Foot-and-Mouth Disease (Infection with Foot and Mouth disease viruses) " in OIE Terrestrial Manual 2022 2022, ch. (*Chapter 3.1.8), pp 1–34*).

OIE, (2012).Foot and Mouth Disease, Manual of Standard for Diagnostic Tests and Vaccines for Territorial Animals (Mammals, Birds and Bees). *OIE Terrestrial Manual, 7th edition, Paris, France.*

Paton, D (2021). Foot-and-mouth disease vaccine development. *Vaccine*, **39**(**3**): 372–379.

Paton, D. J., Sinclair, M., & Rodríguez, R. (2018). *Transboundary and Emerging Diseases*, **65**(**2**):546-553.

Paton, D.J., Sumption, K.J., Charleston, B. (2009): Options for control of foot-and mouth disease: knowledge, capability and policy. *Philos Trans R Soc Lond B Biol Sci.* **364**:2657–2667.

Perry, B. D., and Grace, D. (2009). The impacts of FMD on livelihoods and poverty. *Transboundary and Emerging Diseases*, **56**(**4**):73–82.

Pharo, H. J., & Cobb, S. P. (2011). *The Veterinary Journal*, **189**(**2**): 129-132.

Radostits, O. M., Blood, D. C. and Gay, C. C. (2007).Veterinary Medicine, a Text Book of the Disease of Cattle, Sheep, Goats, Pigs and Horses.8thed.London: *Balliere Tindall. Pp*.1223- 1227.

Ranjan, R., Biswal, J. K., Sharma, G. K. and Pattnaik, B. (2016). A Review on Foot-and-mouth disease: pathology, diagnosis and its management.

Reid SM, Ferris NP, Hutchings GH, Samuel AR, Knowles NJ (2000). Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. *J Virol Methods* **89**:167–176.

Reid, SM, Grierson, S.S, Ferris, N.P., Hutchings, G.H and , Alexandersen, S(2003). Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *J. Virol. Methods*. **107**(2):129–139. doi: 10.1016/S0166-0934(02)00210-0.

Saeed, A., Kanwal, S., Arshad, M., Ali, M., Shaikh, R. S, and Abubakar, M. (2015).Foot-and mouth disease: overview of motives of disease spread and efficacy of available vaccines. *Journal of Animal Science and Technology*, **57**: 10.

Seifu, K, Muluneh, A., Getachew,Y. Jibril,Y.,and Negussie, H. (2023). Epidemiological study and dairy farmers'knowledge, attitudes, and practices on foot and mouth disease in central Ethiopia. *Heliyon*, **9**(5).

Senawi, B. (2012). Foot-and-mouth disease: Epidemiology and control measures. *Addis Ababa University Press.*

Shao, J., Liu, H., Zhou, X., *et al*. (2017). Thermostability of SAT serotypes of FMDV. Virus Research, **227**:14–19.

Shaw, A. E., Reid, S. M., Ebert, K., Hutchings, G. H., Ferris, N. P., & King, D. P. (2007). *Journal of Virological Methods*, **146**(**1**-**2**): 425-430.

Shawky, M., Abd, A. and Fakry M.(2013). Isolation and molecular characterization of foot and mouth disease SAT-2 virus during outbreak 2012 in Egypt. *Journal of Veterinary Advances*.;**3**(2):60–68.

SouleyKouato, B., Elliot, F. M., King, D. P., Hyera, J., Knowles, N. J., Ludi, A. B., and Saegerman, C. (2018). Outbreak investigations and molecular characterization of foot-and-mouth disease viruses circulating in south-west Niger. *Transboundary and emerging diseases*, **65**(1) :146-157.

Stenfeldt, C., Eschbaumer, M., *et al*. (2016). Persistent FMDV infection in cattle. J. *Virology*, **90**(**6**): 3001–3010.

Stenfeldt, C., *et al*. (2020). Maintenance of FMDV in endemic settings. *Transboundary and Emerging Diseases*, **67**(**5**): 2031–2045.

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.

Sumption, K. J., Vallat, B., & Roeder, P. L. (2007). *Revue scientifique et technique (International Office of Epizootics*), **26**(**2**): 465-475.

Tegegne, H. C., Jemal, S., and Ejigu, E. A. (2024). Epidemiological Study of Foot and Mouth Diseases through Serological and Molecular Investigation in Cattle of SelectedDistricts,Jimma Zone, South west Ethiopia. *BioRxiv*, 2024-06.

Tesfaye, A., Sehale, M., Abebe A., Muluneh, A. and Gizaw, D(2016). Sero-prevalence of foot and mouth disease in cattle in Borena Zone, oromia regional state, Ethiopia. *Ethiopian Veterinary Journal*., **20**(1):55–66. doi: 10.4314/evj.v20i1.4.

Tesfaye, Y., Khan F, and Gelaye E, (2020). Molecular characterization of foot-and-mouth disease viruses collected from Northern and Central Ethiopia during the 2018 outbreak, *Veterinary World*, **13**(**3**): 542-548.

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., Khan, F., & Gelaye, E. (2021). Vaccine matching and antigenic variability of foot-and-mouth disease virus serotypes O and A from 2018 Ethiopian isolates. International Microbiology, **25**(**1**): 47–59.

Thomson, G. R., Vosloo, W., & Bastos, A. D. (2003). Virus Research, **91**(**1**):145-161. Underwood, S., & Shapp, P. (2012). *Journal of Veterinary Medicine and Animal Health*, **4**(**5**):67-74.

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

Tindashe.Y.(2018). Molecular characterization of foot and mouth disease viruses in cattle from outbreaks occurred in different parts of Ethiopia from October 2017 to May 2018. (Doctral dissertation, College of veterinary medicine and Agriculture, Addis Ababa university).

Urge, B., Dawo, F., Alemu, Z., Senbeta, B., Aliyi, A.and Muluneh, A(2020). Foot and mouth disease virus infection seroprevalence study in dairy cattle reared by smallholder farmers in Welmera District, Central, Oromiya Ethiopia. *J. Vet. Health Sci*.;**1**(1):05.

Verma, A. K., Kumar, S., & Kumar, A. (2012). *Indian Journal of Animal Sciences*, **82**(**11**): 1405.

Wang Y, *et al*. Inability of FMDV replication in equine kidney epithelial cells is independent of integrin αvβ3 and αvβ6. *Virology*. 2016; **492:251**–8.

Wang, Y., Yang, L., & Li, J. (2015). *Scientific Reports*, **5**: 10217.

WOAH (2022). Terrestrial Manual foot and mouth disease (infection with foot and mouth disease virus). Chapter 3.1. 8 P: 1-34.

WOAH. (2023). Foot and Mouth disease (FMD)."WOrld Organization for Animal Health. https://www.woah.org/en/disease/foot-and-mouth-disease/ (accessed 21 March 2023).

WOAH-FAO FMD Reference Laboratory Network. (2024). WOAH-FAO FMD Ref Lab Report Jul–Sep 2024. World Reference Laboratory for Foot-and-Mouth Disease. https://www.wrlfmd.org

WOAH-FAO FMD Reference Laboratory Network. (2025). WOAH-FAO FMD Ref Lab Report Apr–Jun 2025. World Reference Laboratory for Foot-and-Mouth Disease. https://www.wrlfmd.org

Woldesenbet, A. A., Muluneh, A., Negussie, H.and Jibril, Y. (2023). Foot and Mouth Disease in Adama and Boset districts, East Shewa zone, Ethiopia: Seroprevalence and Virus serotyping. *Ethiopian Veterinary Journal*, **27**(**1**), 143-156.

WRLFMD (World Reference Laboratory for Foot-and-Mouth Disease). (2023). FMDV genome

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 foot-and-mouth disease viruses circulating in south-west Niger. *Transboundary and* isolates in Ethiopia from 2008 to 2018. *Viruses*, **11**(**11**):1076.

Xie, Q., Yang, Y., Zhao, H., *et al*. (2022). Trehalose and Cu^{2+} stabilize FMD vaccine antigen. *Virology Journal*, **19**: 218.

Xu, L., Hurtle, W., Rowland, J. M., Casteran, K. A., Bucko, S. M., Grau, F. R., ... & McIntosh, M. T. (2013). Development of a universal RT-PCR for amplifying and sequencing the leader and capsid-coding region of foot-and-mouth disease virus. *Journal of virological methods,* **189**(**1**): 70-76.

Yalew, A. W. (2019). Frontiers in Veterinary Science, **6**, 60.

Yang, P. C., Chu, R. M., Chung, W. B., & Sung, H. T. (2011). *Veterinary Microbiology*, **64**(**1**): 19-28.

Yirdaw, B. (2024). Outbreak Investigation and Economic Impact of Foot and Mouth Disease in Bovine in Western Amhara Regional State, North Western Ethiopia. *American Journal of Zoology*, **7**(**4**): 65-75.

Zewdie, G., Akalu, M., Tolossa, W., Belay, H.,Deresse, G., Zekarias, M. and Tesfaye, Y. (2023). A review of foot-and-mouth disease in Ethiopia: epidemiological aspects, economic implications, and control strategies. *Virology Journal*, **20**(**1**):299.

8. LIST OF APPENDICES

Appendix 1: FMD outbreak case for of sample collection from field

Region_____Zone_____Districts_____PA_____Total No. Animal at risk, mortality rate,___ morbidity rate _____Sign _____Date_____

S/N	Animal ID/Owner name	Age	sex	breed	Production system	Clinical sign	Sample type
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

Appendix **2:** Preparation **of BHK -21 for virus inoculation**

- First prepare a complete media containing 1x minimal essential media, 10% fetal calf serum, tryptose phosphate broth, 100x antibiotics and antimycotic solution.
- Pre-warm both Trypsin and phosphate saline solution (PBS).
- All the materials used for sub culturing are disinfected by 70% alcohol and placed in the biosafety cabinet.
- Take cell culture flask with confluent monolayer from the incubator to the biosafety cabinet after disinfecting by 70% alcohol
- Decant old media and wash by PBS to remove residual serum (which would inactivate the trypsin).
- Slowly add enough amount of trypsin and incubate for ten minutes.

❖ Add growth medium and pipette vigorously until monolayer become dispersed to a single cell.

❖ Add the final amount of the complete media and dispense to other 3 TC-25 flask and then incubate at humidified incubator with 5% CO_2 at 37 ^{0}C

❖ Then follow the growth of the cell line and for any contamination using inverted light microscope

Appendix 3: Virus isolation **procedure on BHK-21 cell**

❖ The Epithelial tissue and Oral swabs suspension virus samples and 2% DMEM were thawed.

❖ Place disinfected cells, media, and inoculation materials into the biosafety cabinet.

❖ Remove the old medium from the monolayer cells and gently wash the cells with 3 ml of pre-warmed PBS.

❖ Inoculate 1 ml of virus suspension into 25 cm² flasks and gently rock to evenly distribute over the monolayer.

❖ Incubate inoculated culture in incubator at 37 0C for 1hr to allow virus adsorb.

❖ Add 5 ml of 2% DMEM to the inoculated cells and incubate at 37 °C with 5% CO_2 in a humidified incubator.

❖ Monitor the inoculated tissue flask daily for the development of CPE.

Appendix 4: RNA extraction **protocol**

Extraction

Procedure

1. Lysis (AVL)

❖ Prepare Lysis solution Buffer=nx530 µl, n=Number of sample

❖ Prepare Carrier RNA =n x5.6 µl

❖ Mixe the lysis solution with carrier RNA

❖ Dispense 560 µl mix solution into sterile centrifuge tube

❖ Vortex Swabs specimen fluid

❖ Transfer 140 µl of samples into micro centrifuge tube containing Lysis

❖ Vortexed in 1second and incubate at room temperate for 10 min

2. Binding

❖ Add 560 µl of 96% ethanol to centrifuge tube containing Lysine solution , carrier RNA & samples

❖ Vortex 1 sc and bring Q Amp mini spine column

- Centrifuge at 8000 rpm for 1min
- Discard the flood & take out of Q Amp mini spine column
- Centrifuge at 8000 rpm for 1min
- Discard the flood & take out of Q Amp mini spine column keep in new 2ml collection tube
- Add 630 µl from the total solution to Q Amp mini spin column
- Centrifuge at 8000 rpm for 1min
- Discard the flood & transfer column filter to new collection tube

3.Washing

- Add 500 µl wash of Buffer AW-1 in Q Amp mini spine column & centrifuge at 8000 rpm for 1 minute.
- Discard the flood & transfer column filter to new collection tube
- Add 500 µl of Buffer AW-2 & centrifuge at 14,000 rpm for 3 minutes.
- Discard the flood & transfer column filter to new tube
- Centrifuge at 14,000 rpm for 3min to dry

4.Elusion

(AVE)

- Adding 60 µl of Buffer AVE to the column & incubate at room Tm for 1-3 min
- Centrifuge 8000 rpm for 1 minute to eluted RNA from Q Amp mini spine column
- Remove Q Amp mini spine column & the Elute RNA was stored at -80°C.

Appendix 5: Plate layout for antigen detection sandwich ELISA

ANIMAL HEALTH INSTITUTE (AHI), SEBETA
QUALITY MANAGEMENT SYSTEM
PATE LAYOUT FOR FMD ANTIGEN DETECTION AND SEROTYPING ELISA

Type of test: FMD Antigen detection Date 15/02/2026

Plate No: **1**

Purpose: Serotyping

Catching MAbs		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	
Type O	A													Conjugate A (Pan-OA-C Asia1+ some SATs)
Type A (1st MAb)	B													
Type A (2nd MAb)	C													
Type Asia-1	D													
Type C	E													
Pan-O-AC-Asia-1	F													
TypeSAT2	G													Conjugate B (SAT2)
TypeSAT1														

Note-; MAb, Monoclonal antibody; Po.ctrl, Positive control; Ne.ctrl, Negative

Appendix 6: Interpretation of OD values of Sandwich ELISA

Negative for FMDV	OD <0.1 with all catching MAbs, after subtracting the OD of the respective negative control.
FMDV positive type O	OD ≥ 0.1 with the type O MAbs and the pan-FMDV O, A, C,Asia 1 MAb. Some samples may cross react with MAb A 4D12, 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 panFMDV O,A,C,Asia1 MAb
FMDV positive type SAT1	OD ≥ 0.1 with the type SAT1 catching MAbs, after subtracting the OD of the respective negative control
FMDV positive type SAT2	OD ≥ 0.1 with the type SAT2 catching MAbs, after subtracting the OD of the respective negative control
FMDVpositive (untyped)	OD ≥ 0.1 with the pan-FMDV MAb and <0.1 with the type-specific MAbs, after subtracting the OD of the respective negative control

Appendix 7: Master Mix preparation

Components	**Volume added to each reaction PCR plates**
2X reaction mix	12.5µl
Nuclease-free water	1.5µl
Forward primer	2µl
Revers primer	2µl
Taqman probe	1.5µl
Superscript IIIRT/platinum taq mix	0.5µl
Extracted sample/positive/Negative control	5µl
Total	**25µl**

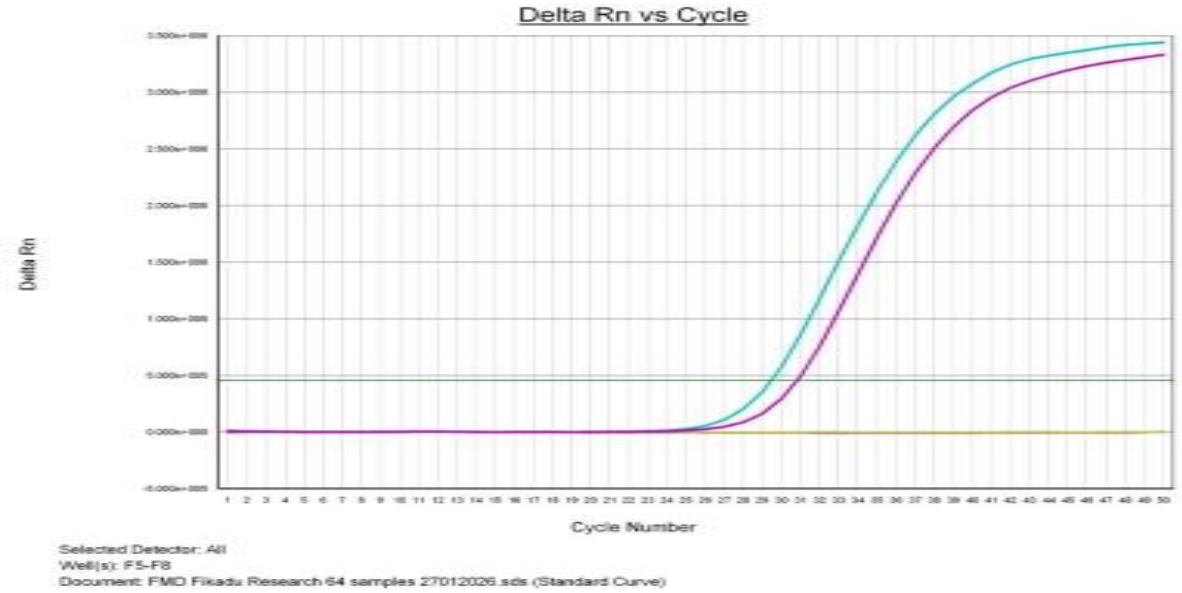
APPENDIX 8: Field examination

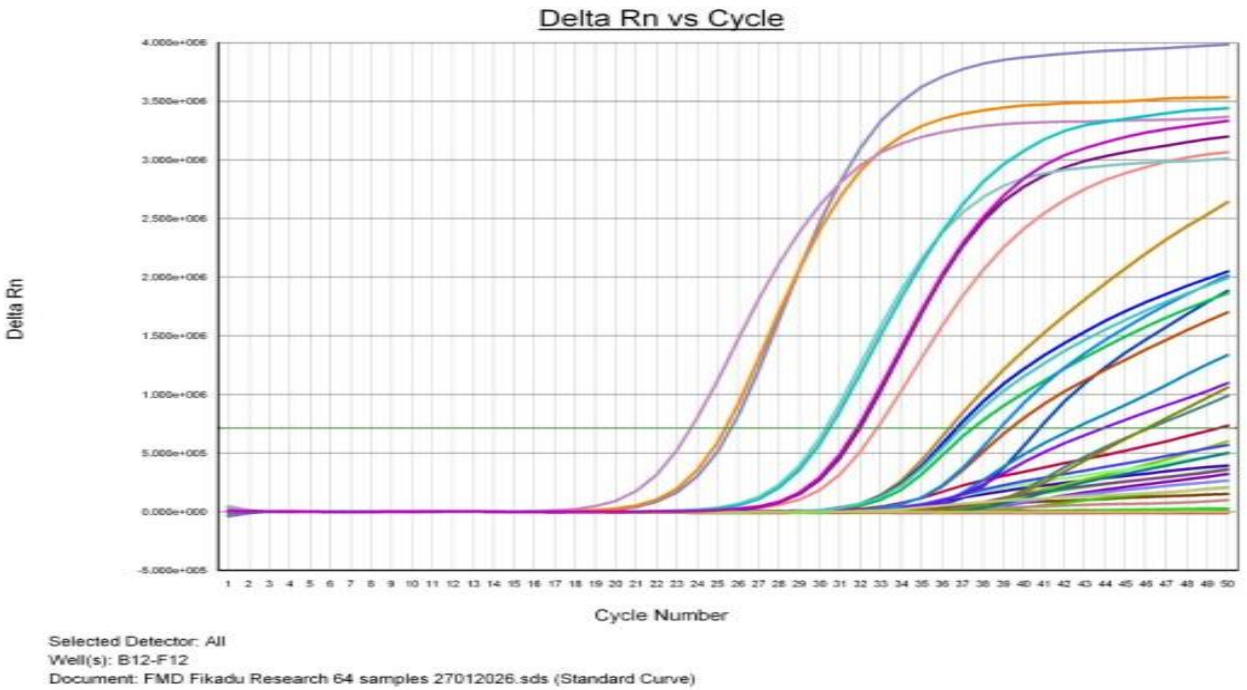




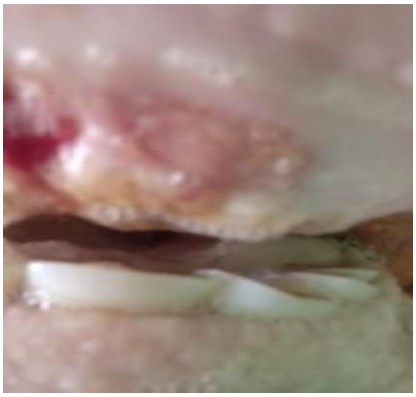


Appendix 9: RT-PCR positive and Negative Ct value of FMDV tested.





Appendix 10: Miscellaneous pictures.







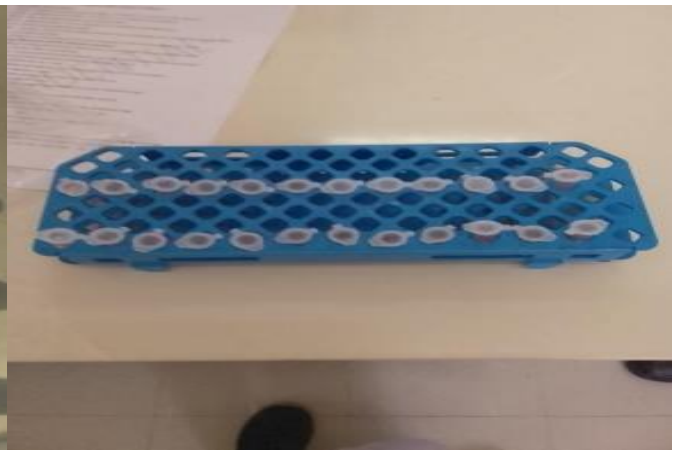








30377
30377










IZSLER Brescia Italy
Store at 2°-8°C

Appendix 11: Ethical Clearance

Waajjira Daayrekteera Qorannoo, Maxxansa, Naamusaa fi Baballisuu

የምርምር፣ ህትመት፣ ሥነ-ምግባርና ሰርጭት ዳይሬክቶሬት

Research, Publication, Ethics and Extension Directorate Office

ቁጥር/Ref:- WU/REC/818
ቀን/Date:- November 25, 2025

To: **Fikadu Itefa**

From: **Research Ethics Review Committee**

Wollega University

Subject: **Issuing Ethical Clearance Certificate**

The Research Ethics Review Committee (RERC) of Wollega University in its meeting held on Date November 25, 2025, (Minutes No.: WU/REC/818) has examined your research proposal entitled with ***"isolation, serotyping and molecular detection of bovineFoot and mouth disease virus from outbreak cases in selected areas of Qellem wallaga,West and East Wallaga zones, Western ethiopia."***. The Proposal is evaluated and approved for implementation.

Hence, this ethical clearance certificate is given for two year of its approval date to conduct the research which is periodically evaluated for implementation.

Chairperson

Name: Girwaye Kemasa Rundasa (PhD)

Signature:

Date: Nov. 25. 2025

Research and Innovation Director

Secretary

Name: Dr Dereje Chala Diriba (PhD)

Signature:

Date: Nov. 25. 2025

Copy

- ✓ Office of Vice President for Research and Technology Transfer
- ✓ School of Veterinary Medicines

WU

ፖ.ሳ.ቁ 395 ነቀምቴ
ስፊክ ቁ. 0578618035
ፋክስ ቁ. 0576617980
Email:- WU@ethionet.et
P.O.Box 395 Nekemte
Tel No. 0578618035
Fax No. 0576617980