Thesis Ref No._____

SEROLOGICAL INVESTIGATION OF FOOT AND MOUTH DISEASE IN CATTLE AND PIGS IN SELECTED COMMERCIAL FARMS AND MOLECULAR CHARACTERIZATION FROM ACTIVE OUTBREAK CASES

MVSc. THESIS

BY

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JUNE, 2019

BISHOFTU, ETHIOPIA

SEROLOGICAL INVESTIGATION OF FOOT AND MOUTH DISEASE IN CATTLE AND PIGS IN SELECTED COMMERCIAL FARMS AND MOLECULAR CHARACTERIZATION FROM ACTIVE OUTBREAK CASES

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

By

Nato Hundessa Mutal

JUNE, 2019

BISHOFTU, ETHIOPIA

Signature Sheet

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As MVSc research advisors, we here by certify that we have read and evaluated this Thesis prepared under our guidance by Nato Hundessa, entitled: "**SEROLOGICAL INVESTIGATION OF FOOT AND MOUTH DISEASE IN CATTLE AND PIGS IN SELECTED COMMERCIAL FARMS AND MOLECULAR CHARACTERIZATION FROM ACTIVE OUTBREAK CASES"**; we recommend that it can be accepted as fulfilling the thesis requirement for the Degree of Master of Veterinary Science in Veterinary Epidemiology.

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STATEMENT OF THE AUTHOR

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Date of Submission: June, 2019

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion Test
BEPT	Bovine Epithelial Tissue
BHK	Baby Hamster Kidney Cell
CFT	Complement Fixation Test
CPE	Cytopathic Effect
Ct-value	Cycle Threshold Value
EA	East Africa
ELISA	Enzyme Linked Immunosorbant Assay
FAO	Food and Agriculture Organization of the United Nation
FMD	Foot-and-Mouth Disease
IFN	Interferones
IRES	Internal Ribosome Entry Site
ISGs	IFN-a/b-stimulated genes
LPBE	Liquid-phase blocking ELISA
MAbs	Monoclonal Antibodies
NAHDIC	National Animal Health Diagnostic and Investigation Center
NF-kB	Nuclear Transcription Factor
NSP	Non Structural Protein
OIE	Office International des Epizooties
OD	Optical Density
OP	Oesophageal Pharyngeal
ORF	Open Reading Frame
OR	Odds Ratio
PKR	Protein Kinase R
RF	Replicative Form
RNA	Ribonucleic Acid
RNase L	Ribonuclease L
rpm	Revolution Per Minute

RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAT	South African Territory
SNNPRs	Southern Nations, Nationalities and Peoples Regional State
SPCE	Solid-phase competition ELISA
TNF	Tumor Necrosis Factor
UTR	Untranslated Region
VNT	Virus Neutralization Test
VPg	Viral Protein Genome linked
VP1	Viral Protein 1
WRL	World Reference Laboratory

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ABSTRACT

A cross sectional study was conducted from October 2018 to May 2019 to investigate the epidemiology of foot-and-mouth disease. Multistage sampling was implemented to determine sero-prevalence and asses the risk factors of foot and mouth disease. Additionally, active outbreaks were investigated to identify the virus using molecular tools. Questionnaire survey was also carried out and revealed that various factors affect the occurance of foot-and-mouth disease. The result of 3ABC ELISA showed an overall prevalence of 24.39% (269/1103) in cattle and 2.11% (9/426) in pigs. Multivariable logistic regression analysis showed statistically significant differences among districts: cattle from Bahir Dar Zuria, Ada'a, Sodo Zuria and Gonder Zuria had less odds of being sero-positive with odds ratio of 0.56 (CI= 0.34-0.92), 0.23 (CI= 0.09-0.57), 0.22 (CI= 0.11-0.42) and 0.15 (CI= 0.09-0.27), respectively compared to cattle from Alage district. Pigs from Bishoftu were less likely to test postive than pigs from Addis Ababa with an odds ratio of 0.04 (95% CI= 0.01-0.34). Female and older cattle greater than 3 years of age have higher odds of being sero-postive than their male and younger counterparts, respectively. The disease virus genome was detected in 66.7% (46/69) of active outbreak samples by rRT-PCR. Further, 9 samples were characterized by serotyping and sequencing of virus isolates identified 6 serotype A African topotype of G-IV and 3 serotype O of EA-3(2) and EA-4(1) topotypes. The viruses isolated in this study were clustered with other African isolates. Thus, control strategy should be designed in such a way that implementation of appropriate biosecurity measures, regular serosurveilance and frequent outbreak investigation in function of effective vaccine design and vaccination.

Key words: *Cattle, Commercial farms, Ethiopia, FMDV, Molecular characterizarion, Pigs, Risk factors, Sero-pevalence, Serotype.*

1. INTRODUCTION

Ethiopia is a resourceful country bestowed with the highest livestock density in Africa with estimated 54 million heads of cattle population (CSA, 2013). The livestock sector forms the backbone of rural economies and has a significant role in socioeconomic activities of the country and contributes much to the national economy. Despite having the largest livestock population in Africa, the country is not benefiting as a result of wide spread endemic and transboundary animal diseases (TADs) (Beyene *et al*., 2015; Mishamo, 2016; Wagari, 2016). Following an uprising globalization, the transboundary animal diseases distributed everywhere in the world causes a serious threat or risks to the world animal agriculture and food security and endangers the international trade (Zepeda, 1998). During the most recent decades, the world has been confronted with devastating socio-economic impact to farmers and livestock production sectors due to major outbreaks of TADs, such as foot-and-mouth disease (FMD) (Balkhy and Memish, 2003).

Foot-and-mouth disease is one of the most devastating and economically important OIE notified highly contagious vesicular viral transboundary animal disease affecting multiple species of susceptible wild and domestic cloven hoofed animals leading to trade restrictions on animals and their products and continues to be a major concern for world livestock industry (Perry and Rich, 2007). Foot-and-mouth disease virus (FMDV), the etiologic agent, is classified within *Aphthovirus* of the family *Picornaviridae*. It is an antigenically variable virus, reflected in seven immunologically distinct serotypes with varying global distributions, namely, A, O, C, Asian 1, and three strains predominantly circulating in Sub-Saharan Africa, South African Territory (SAT)-1,2 and 3. Several of these serotypes circulate currently or periodically in the Middle East and North Africa. Multiple subtypes evolved due to the high mutational rate of the virus each with a wide spectrum of antigenic diversity. These serotypes do not provide any cross immunity among each other after immunization or infection so that distinct serotypes do not trigger cross-immunity against each other; for this reason, there is no universal vaccine that can confer protection against all serotypes and subtypes (Knight-Jones *et al*., 2016).

Depending on host and virus characteristics, FMD exhibits a broad range of clinical presentations resulting in significant morbidity. In general, the disease is clinically characterized by an acute febrile reaction and formation of vesicular lesions in the mouth, feet, nares and teats as well as inappetency, lameness, severe mastitis and abortion 4 to 72 hours post-infection. The disease has debilitating effects causing serious production losses in adults and high mortality rate in young animals thus affecting farmers' livelihoods, compromise food security, rural income generation and the national economy through undermining the livestock sector potential by impairing international livestock trade of live animals and animal products (Arzt *et al*., 2011a; Donaldson, 2004).

FMD is widely distributed and is endemic in the developing world, in particular in large areas of Africa, Asia and South America and has shown an extraordinary ability to cross international boundaries and cause epidemics in previously free areas (Knowles *et al*., 2001). In most of the sub Saharan African countries, serotypes O, A, SAT-1 and SAT-2 are still in circulation. The distribution of FMDV serotypes in endemic regions is not uniform thus complicating the epidemiology and control of the disease in the region. Despite a historically world-wide distribution, serotype C has not been encountered in any outbreak since 2004 (Knight-Jones *et al*., 2016). According to Vosloo et al. (2002) serotype SAT 3 has been recorded only in Uganda (Vosloo *et al*. 2002). The spread of FMD due to the lack of veterinary infrastructure; human resources, movement controls, and appropriate vaccines consequently exposes many developing countries to severe economic crisis (Perry and Rich, 2007).

Several studies in most parts of Ethiopia ranked FMD among the top five economically most important viral disease of cattle (Admassu, 2004; Shiferaw *et al*., 2010). The disease was first recorded in Ethiopia in 1957 (Gulima, 2011); ever since, it remain endemic in the country causing several outbreaks each year (Ayelet *et al*., 2012). Different and multiple surveillance studies and outbreak investigations revealed the endemicity of the disease in country with a reported sero-prevalences of 9% - 26% at the animal level and up to 48% at the herd level in cattle (Megersa *et al*., 2009; Bayissa *et al*., 2011; Mekonen *et al*., 2011; Bogale, 2005; Gelaye *et al*., 2005; Rufael *et al*., 2008; Hailu

et al., 2010), 4% to 11% in small ruminants (Sahel, 2004; Beyene *et al*., 2015) and 30% in artiodactyl wild mammals (Sahel, 2004). Sulayeman et al. (2018) also indicated 24% of sero-prevalence in central Ethiopia. Four of the seven serotypes namely O, A, SAT 1 and 2 are reported in recent times being circulated in all regions of the country (Ayelet *et al.*, 2009); indicating that serotype O (72%) and A (19.5%) are dominant responsible for substantial economic losses (Gelaye *et al*., 2005; Ayelet *et al*., 2009; Nigussie *et al*., 2011). SAT viruses are limited in the central and southern half of the country where 70% of the country's cattle population is found (Ayelet *et al*., 2009).

In Ethiopian context, traditional livestock management with uncontrolled movement of animals, FMD spread is attributed to moving infected cattle. In general, extensive movement of livestock, the high rate of contact among animals in communal grazing areas, watering points and at commercial markets could be considered as major risk factors for the transmission and dissemination of the virus. For disease free countries, introduction of FMD is mainly attributed to live animal import, livestock products, and feed of animal origin (Beyene *et al*., 2015). Several risk factors were identified for spread of FMD in Ethiopia which include production system, age of animals, contact with wildlife and season of the year (Megersa *et al*., 2009). The significant association of herd size and difference in geographical locations with sero-positivity of FMD was also justified (Jenbere *et al*., 2011).

The disease remains a major economic concern for the livestock industry in many developing countries and a continued threat to countries that are disease free due to its potential negative impact on trade in agricultural products (Bruner and Gillespie, 1973). This fact together with the ability of the virus to infect even in small doses, the fast replication of virus, wide hose range, the multitude of transmission routes, including spread by the wind, makes FMD the major constraint to international trade in livestock. The situation is complicated by the fact that after the acute stage, FMDV may cause a prolonged, asymptomatic but persistent infection in ruminants. The persistence and continuously evolving situation of the disease may be a result of inadequate epidemiological understanding of the disease and ineffectiveness of the control measures

that are being applied. Thus, for initiating and implementing effective control and prevention measures, it is a prerequisite to clarify many epidemiological factors, typically, evaluation of the prevalence of FMD and identification of some risk factors, understanding of the geographic distribution of the disease and serotypes of FMD virus through effective and regular surveillances. On top of this, agent characterization from active outbreaks also tells the biology of circulating strains which intern helps to design and develop appropriate vaccine. Though much has been done on the disease epidemiology, still there exists lack of extensive recent information on the agent characterization, sero epidemiology and associated risk factors to date.

Therefore, this study was designed with key purposes of:

- Estimating the sero-prevalence of FMD in selected commercial farms of cattle and pigs
- Assessing the potential risk factors for the disease prevalence
- Molecular characterization of the FMDV field isolates collected from active outbreaks.

2. LITERATURE REVIEW

2.1. The Disease Definition

Foot-and-mouth disease (FMD) is a disease caused by an RNA *Aphtovirus*, belonging to the *Picornaviridae* family. It is one of the most devastating and highly contagious diseases of susceptible cloven-hoof wild and domesticated ruminant animals in which cattle and pigs are affected most severely. The disease is also known to cause enormous economic losses both nationally and internationally by reducing livestock productivity and imposing a restriction to trade of live animals and animal sources. Infected animals present fever and blister-like sores in the mouth, on the teats and between the hooves. Transmission occurs through aerosol and direct contact between infected and susceptible animals. According to OIE statistics, the disease is currently present in animals in many areas of the world (Knowles *et al*., 2016; Wang *et al*., 2015).

2.2. The FMD Virus Characteristics

The virion appears as a non enveloped small size (approximately 30 nm diameter), roughly spherical, hollow protein capsid of icosahedral symmetry (Carrillo *et al*., 2005; Knowles *et al*., 2016; Wang *et al*., 2015) that contains a single-stranded, positive-sense genomic RNA molecule of approximately 8500 nucleotides (nt) classified within the *Aphthovirus* genus as a member of the family *Picornaviridae* (Longjam *et al*., 2011; Nsamba, 2015). The virus has the ability to survive in chilled or frozen bone marrow and lymph nodes for lengthy period, resists alcohol and disinfectants but sensitive to P^H below 6.5 or above 11(Sahle *et al*., 2004). The virus can survive regular pasteurization, drying and may persist for days or weeks in organic matter under moist and cool conditions. Droplet aerosol particle size of 0.5 - 0.7 μm is optimal for prolonged survival of the virus in the atmosphere, while smaller aerosols dry out (Mwanandota, 2013).

2.3. Genetic and Antigenic Variation

The genetic diversity or antigenic variation of FMDV is a consequence of the high mutation on the gene encoding the capsid protein due to error-prone RNA polymerase lacking proof reading activity during RNA replication at the rate of 10^{-3} to 10^{-5} per nucleotide site per genome replication resulting an evolvement of new subtypes which are immunological distinct variants that can re-infect individuals that have been previously infected by related viruses (Knowles and Samuel, 2003). This high error rate leads to differences of its viral replicated genomes from the original parental genome of 0.1 to 10 base positions, and populations that consist of genetically related but non-identical viruses known as quasispecies (Grubman and Baxt, 2004; Haydon *et al*., 2001).

Due to antigenic variation over the time, emergence of field variant is increasing. Either the infected or vaccinated species of host may undergo immunologic pressure to generate antigenic variants. Presence of variable antigenic type in different geographical area and even the concurrence of different antigenic type in same geographical area is always a need to select a vaccine strain based on the antigenic type prior to start a control and eradication program (Rudreshappa *et al*., 2012). Currently, FMDV isolates are grouped in to seven different serotypes namely, O, A, C, Asia-1 and SAT 1-3 based on the antigenicity of the capsid coating proteins, each with a diversity of topotypes, genetic lineages and strains. Some lineages have different properties that may contribute to sporadic spread beyond their recognized endemic areas. Due to change in capsid protein, it led to lack of cross-protection between variants within serotypes, particularly evident within the serotype A (Chakraborty *et al*., 2014; Ding *et al*., 2013).

2.4. Virus Genome Organization and Viral Proteins

The viral particle consists of single-stranded positive-polarity RNA genome of approximately 8500 nucleotides long enclosed within a protein capsid assembled of 60 copies of each of three major structural polypeptide termed VP1, VP2, VP3, and a smaller polypeptide termed VP4, encoded by 1D, 1B, 1C and 1A genomic regions,

respectively. Among which VP4 is internal whereas others are exposed on virion surface. The genome includes a single long open reading frame (ORF), flanked by two initiation sites of untranslated regions (UTRs) which is polyadenylated (poly-A tail) at 3' end and carries a small covalently linked viral protein, VPg at 5' end of viral RNA. Subsequently, the coding region containing polyproteins, L, P1, P2 and P3 are posttranslationally cleaved by viral proteases and processed into several structural proteins and several nonstructural (NS) proteins. (Grubman and Baxt, 2004; Mason *et al*., 2003).

Capsid polypeptide precursors encoded by the P1 region generate mature capsid proteins (VP1, VP2, VP3 and VP4). After the encapsidation of the virion RNA, mature virion is produced accompanied by the cleavage of VP0 to VP2 and VP4. The capsid proteins, VP1-3, are exposed on the capsid surface contribute to the antigenic properties of the virus that elicit response to vaccination or infection (Jackson *et al*., 1997). VP1 is the most immunogenic protein having its G-H loop protruded from the surface involved in the interaction between the virus and the integrin family of receptors in the host and the C-terminus forming large part (54%) of virus surface (O'Donnell *et al*., 2005). The P2 and P3 regions encode for NS viral proteins 2A, 2B and 2C; and 3A, 3B, $3C^{pro}$ and $3D^{pol}$ respectively, that are involved in viral RNA replication and protein processing in which, 3C is a viral protease and 3D an RNA-dependent RNA polymerase (Belsham, 2005). The overall genomic organization of the FMDV is illustrated in Figure 1 below.

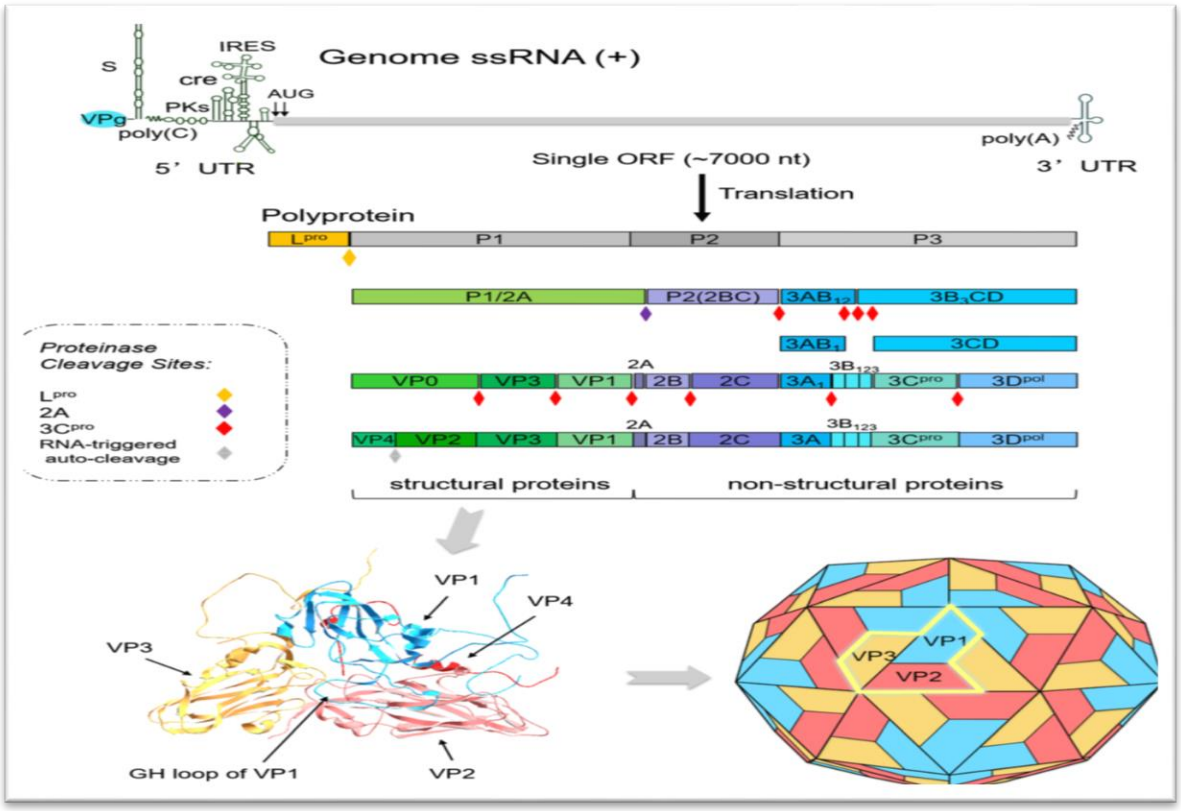

Figure 11: Schematic diagram of FMDV genome organization

Source: (Gao *et al*., 2016)

2.5. Entry and Replication Cycle

The FMDV genome contains untranslated RNA found upstream (5′ UTR) and downstream (3′ UTR) of the ORF which involve in viral replication and virulence (Garcia-Nunez *et al*., 2014). The structured region in the 3′-UTR is essential for FMDV infectivity and replication. The first step in the virus RNA replication is the synthesis of a minus-strand RNA molecule. Following the initiation reaction, elongation of the minus strand begins, catalyzed by $3D^{pol}$. For this to occur, the initiation complex must translocate to the 3′ end of the plus-strand template (Paul, 2002). After formation of the replicative form (RF), new plus-strand synthesis can begin resulting in the formation of a partially double stranded RNA molecule, replicative intermediate (Agol *et al*., 1999). For plus-strand synthesis to proceed, the RF must be unwound. The virus 2C protein both has

ATPase activity (Pfister *et al.*, 2000; Rodriguez and Carrasco 1993) and contains helicase motifs (Klein *et al*., 2000).

Accordingly, 2C and a cellular protein (p38) bind to the minus-strand 3` stem-loop (Banerjee *et al*., 1997), and act to destabilize the replicative form molecule. RNA synthesis occurs within a membranous replication complex, which is derived from membranes of the endoplasmic reticulum and Golgi and contains viral NS proteins encoded by both the P2 (2B, 2BC, and 2C) and P3 (3A and its precursors, $3C^{pro}$, and $3D^{pol}$) regions (Egger *et al*., 2002; Gosert *et al*., 2000).

The final steps in the replication cycle are the encapsidation of the plus-strand viral RNA and maturation cleavage of VP0 to VP2 and VP4 to form the mature virion. In broad terms, the $3C^{pro}$ cleavage products of the P1 region are assembled into a protomer structure containing one copy of each of the proteins VP0, VP1, and VP3. Five protomers can assemble into a pentamer, and 12 pentamers assemble into the final capsid structure. Following encapsidation of the RNA, the maturation cleavage reaction (VP0 to VP2 and VP4) takes place. Maturation cleavage is required for the generation of infectious virus. Finally, the virion particles with complete assembly are released from the infected host cells (Knipe *et al*., 1997) (Figure 2).

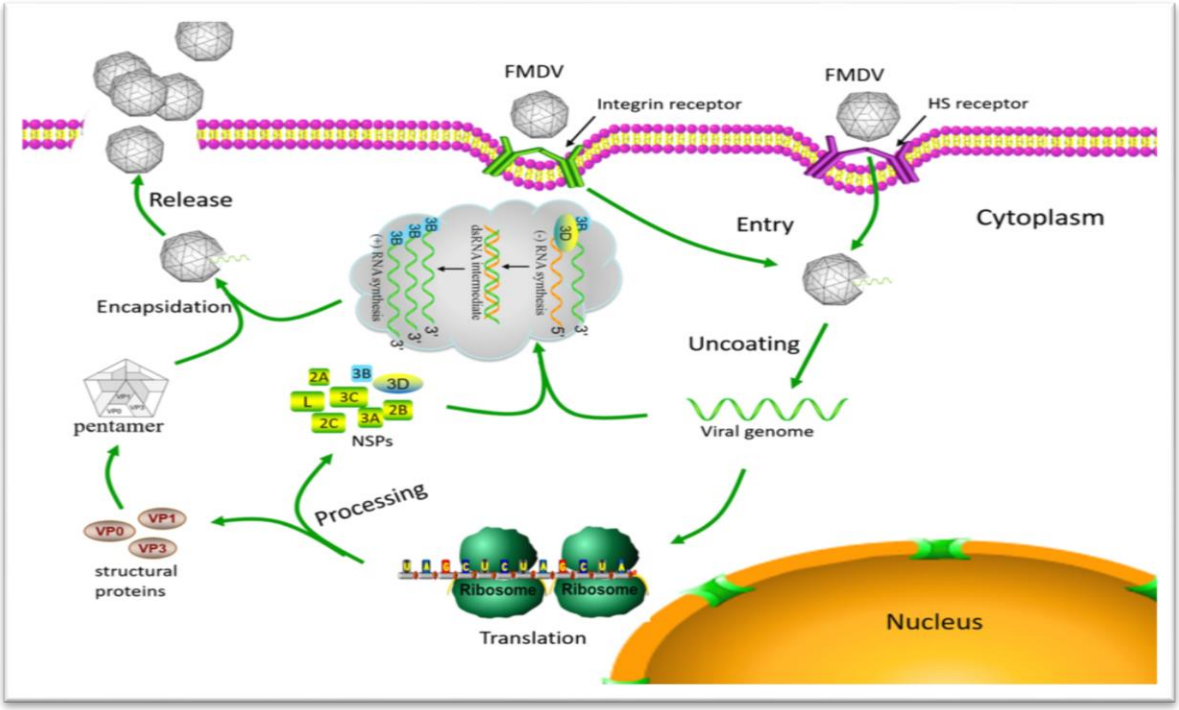

Figure 2: Replication cycle of FMDV

Source: (Gao *et al*., 2016)

2.6. FMD Host-Pathogen Interaction

The NS proteins of FMDV, leader (L), 3C and 2C, have proteinase activity and are involved in processing the viral polyprotein. The L and 3C proteinases could cleave various host proteins (Ziegler *et al*., 1995; Belsham *et al*., 2000). FMDV mRNA is translated by a cap independent mechanism that utilizes an internal ribosome entry site (IRES) and does not require intact eIF4G, host translation initiation factor; the virus takes over the host cell protein synthesis machinery to produce virus progeny (Belsham and Brangwyn, 1990; Kuhn *et al*., 1990).

FMDV leader protein, Lpro, remains the most thoroughly investi-gated determinant of virulence (Arzt *et al*., 2011a). Virus constructs lacking the Lpro sequence (leaderless FMDV) have been shown to be avirulent in cattle and pigs (Brown *et al*., 1996; Chinsangaram *et al*., 1998; Uddowla *et al*., 2012). Cattle exposed to leaderless FMDV by

aerosol donot develop viremia or clinical signs of FMD and have minimal quantities of viral RNA (vRNA) (Brown *et al*., 1996; Uddowla *et al*., 2012).

Viral replication is inhibited by pretreatment with IFN-a/b and IFN-a/b-stimulated genes (ISGs), double-stranded RNA dependent protein kinase (PKR) and RNase L, are involved in the inhibition of FMDV replication (Chinsangaram *et al*., 2001; de los Santos *et al*., 2006). These suggest that L^{pro} blocks the innate immune response to virus infection in primary cells and susceptible animals by inhibition of host mRNA translation, including IFN-a/b mRNAs. Upon infection, a molecular sensor nuclear transcription factor (NF-kB) is released and translocates to the nucleus, and in conjunction with other factors activates transcription of IFNb (de los Santos *et al*., 2007).

The existence of viral protein $3C^{pro}$ is associated with cleavage of the histone H3 at its amino terminus at early times after FMDV infection (Falk *et al*., 1990; Tesar and Marquardt, 1990). Besides, the deleted portion of histone H3 corresponds to the presumed domain implicated in the regulation of transcriptionally active chromatin, this viral activity may be reflected as the inhibition of host RNA synthesis (Falk *et al*., 1990). Viral 2C protein has also been speculated to act as an important regulator; integrating multiple cell signaling during FMDV infection, including apoptosis, immune response, and autophagy. This protein reduces the cellular killing effect against viruses and promotes virus survival and proliferation, thereby facilitating viral proliferation and release of virus particles (Geo *et al*, 2016).

FMDV is capable to bind and become internalized in skin dendritic cells, no evidence of viral RNA replication or production of viral proteins or virus progeny could be detected. Furthermore, FMDV infection had no effect on the phagocytic activity or the expression of co-stimulatory molecules on the surface of these cells (Bautista *et al*., 2005). Although innate immunity is not able to stop initial viral replication, adaptive immunity could be stimulated and potentially clear virus from the organism (Nfon *et al*., 2008). In fact, upon FMDV infection, the release of apoptogenic cytokines such as tumor necrosis factor (TNF-a) would trigger this type of cell death in the infected host suggesting that this

inflammatory cytokine could play a role in the host defense mechanism against FMDV infection (Ku *et al*., 2005) (Figure 3).

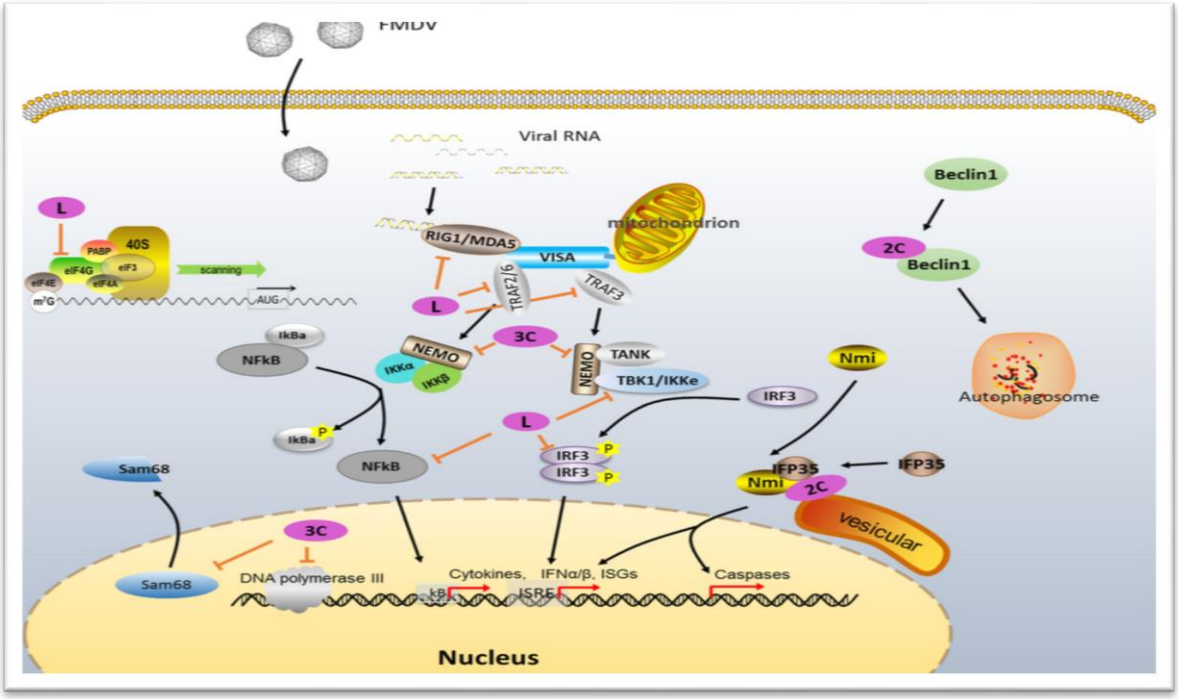

Figure 3: Cellular regulation mechanism of FMDV inside host cell
Source: (Gao *et al*., 2016)

2.7. Epidemiology of the Disease

2.7.1. Global distribution

In recent times, FMD has been endemic in many countries of the world particularly in Asia, Africa and few parts of South America; and currently parts of Europe, North America, Central America and Australia are free of the disease (Knowles *et al*., 2012). Previously, FMD was prevalent all over the world. As of May 2012, only 65 countries in the world had adopted strict control and eradication measures that resulted in its lower prevalence and had achieved freedom from FMD without vaccination (OIE, 2013). (Chakraborty *et al*., 2014). Ten countries possess FMD free zones. Australia, New

Zealand, Western Europe, North America, majority of South America and most Island countries in pacific are free of the disease (Depa *et al*., 2012). Serotypes O, A, C and Asia-1 are continuously circulating in many FMD endemic countries in Asia and Europe. Serotype A and O have the widest distribution, occurring in Africa, Asia and South America. However, Asia has its own unique serotype, Asia-1. Serotype C now appears extremely rare. In Africa all the different serotypes of the virus are present except Asia-1 (Saeed *et al*., 2015; Sangula *et al*., 2011). The global distribution of foot-and-mouth is shown in Figure 4 below.

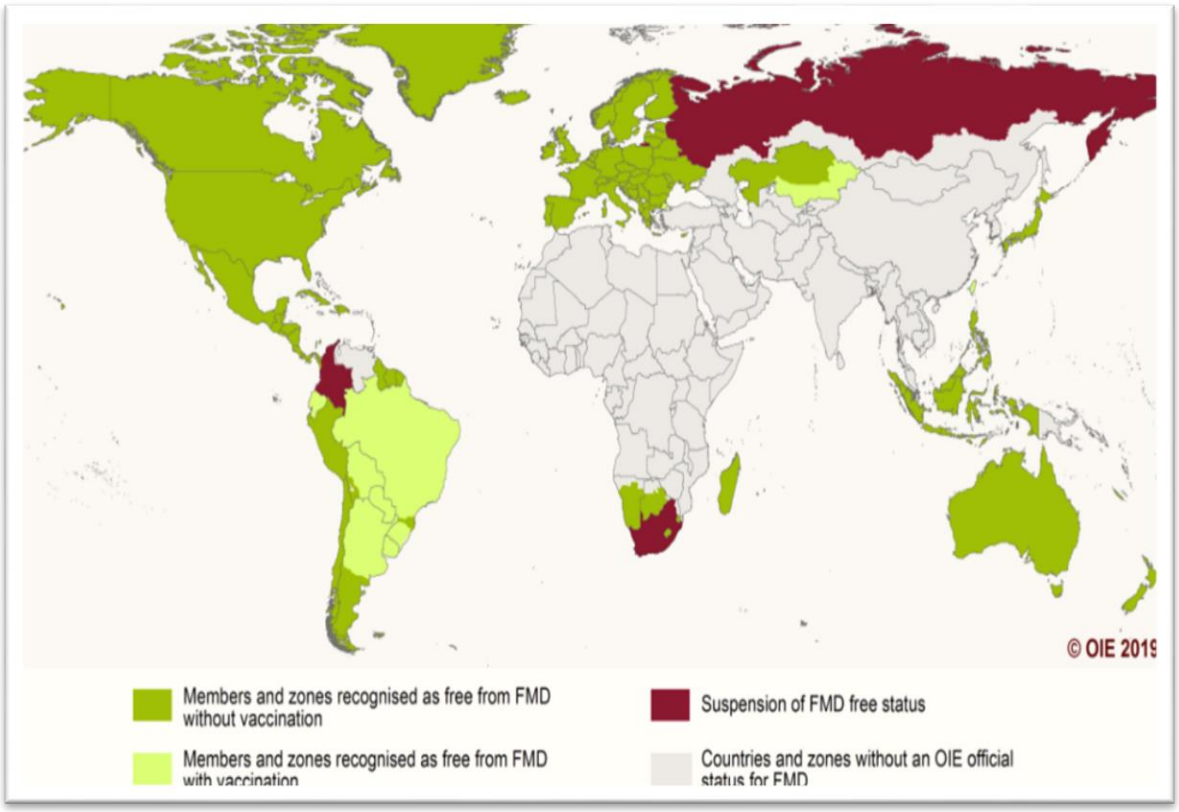

Figure 4: World Foot-and-mouth disease status

Source: (OIE, 2019)

2.7.2. Distribution of FMDV regional pools

The global FMDV population can be roughly sub-divided into seven regional pools based on genetic and antigenic analysis. Countries within each pool share similar FMD virus serotypes. South-East Asia extended into Eastern Asia represented by Pool 1, pool 2 represents Southern Asia and Pool 3 covers Middle East and Euro-Asia. In these three pools serotype O, A and Asia 1 are circulating virus. On the other hand, Pools 4, 5 and 6, cover Eastern, Western and central, and Southern Africa, respectively. In pool 4 serotype O, A and SAT-1, 2 and 3 are circulating. In pool 5, serotype O, A, SAT-1 and 2 and in pool 6, only the SATs are tends to circulate. Pool 7 covers South America and has only serotype A and O circulating (Di Nardo *et al*., 2012; Logan, 2017). Distribution of FMDV pools is designated in the Figure 5 below.

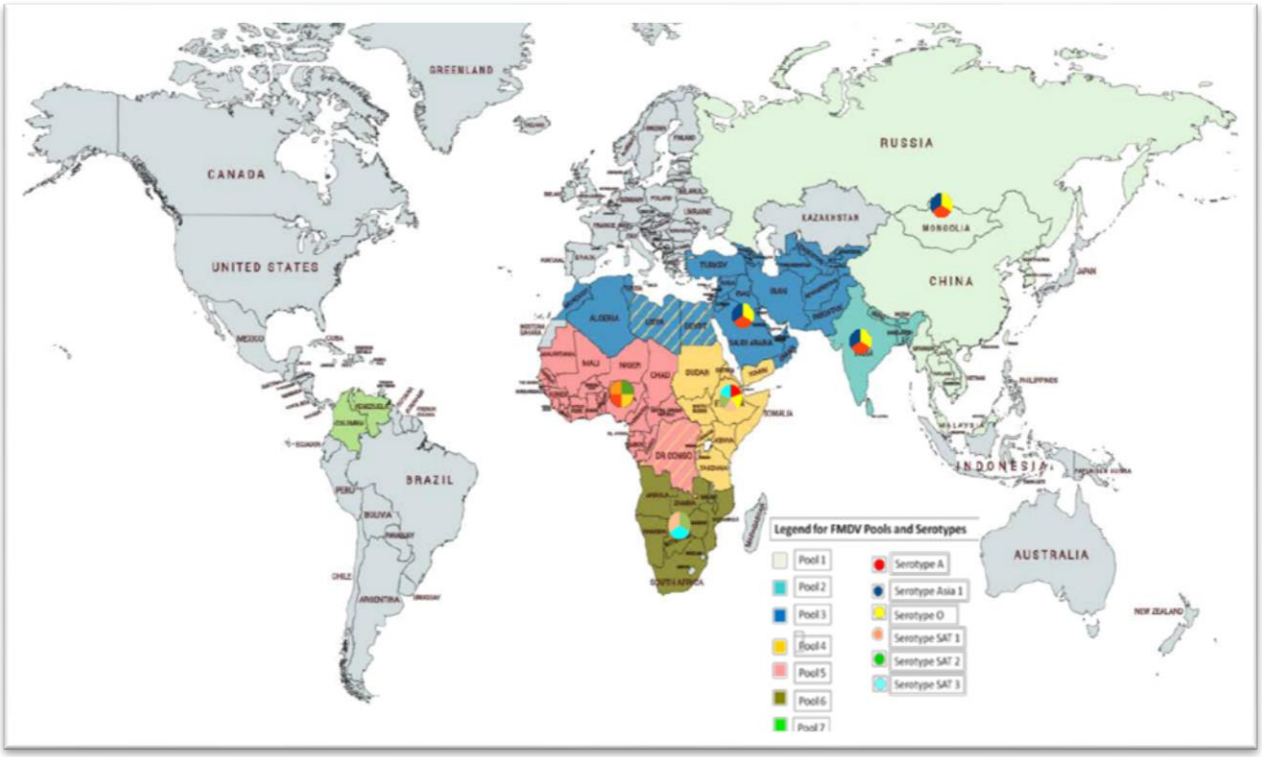

Figure 5: Foot-and-mouth disease virus pools world distribution, 2013-2017
Source: (FAO, 2018)

2.7.3. Susceptible species

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

2.7.4. Carrier state of animals

Animals with antibodies against FMDV NSPs are considered as potential carrier of infectious virus indicating previous exposure of replicating virus. Following the acute phase of FMDV infection, a long asymptomatic persistent phase of infection, animals being virus positive for at least 28 days post-infection (dpi), could be induced in cattle, sheep and goats and that live virus could be isolated from oesophageal pharyngeal (OP) fluids of such animals (Grubman and Baxt, 2004; Cox *et al*., 2005). Pigs, however, cleared the infection in 3-4 weeks and so do not become carriers but considered as key amplifiers of the virus since they excrete large amounts of virus in their expiration (Sellers and Gloster, 2008). The maximum duration of the carrier state that has been reported in cattle is 3.5 years; in sheep, 9 months; in goat, 4 months; in African buffalo, 5 years; and in water buffalo, it is unknown. Consequently, the FMD carrier state is a factor that highly influences national policies directing FMD countermeasures in areas in which the disease is not endemic (Bastos *et al*., 2000; Mwanandota, 2013). Although, precise mechanisms involved in development and resolution of the FMDV carrier state in cattle have not yet been fully described, few studies on the disease pathogenesis in cattle reported that, virus persists either through sustained low level of replication within pharyngeal epithelium (Zhang and Kitching, 2001; Arzt *et al*., 2011b) or retention of

intact but quiescent viral particles trapped by follicular dendritic cells (FDCs) within germinal centers of lymphoid follicles (Juleff *et al*., 2008).

2.7.5. Source of infection and mode of transmission

Foot-and-mouth disease is highly infectious. The FMDV can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, milk, urine, feces and semen. Transmission and spread of FMD is predominantly affected by contact between infected and susceptible animals to the excretions and secretions of acutely infected animals or contaminated food products. Animals suffering from sub-clinical infections may disseminate the disease through direct contact with susceptible livestock or indirectly by the transportation of the virus on inanimate objects such as vehicles and contaminated equipments to susceptible animals (Sumption *et al*., 2008).

Airborne dissemination of infectious aerosols is also often implicated. Under certain circumstances, the virus travels over extensive distances, over water >250 km and no more than 10 km over land. The virus can spread from pigs, which can expire up to 3,000 times more virus than cattle, to more susceptible cattle hosts via aerosol (Donaldson *et al*., 1987; Alexandersen *et al*., 2003). Hence, transmission can occur via aerosol spread, contact or oral routes (Tomasula and Konstance, 2004) and (Sutmoller and Casas, 2002). Air borne transmission via respiratory route is the major route of transmission in cattle. The spread of the virus from sheep and goats to those susceptible species is vague. Contaminated materials (fomites) may introduce virus into the skin or mucous membranes and infection spreads (Barnett and Cox, 1999).

2.7.6. Incubation and latency period

FMDV can be shed by breath, saliva, feces, urine, milk, and semen for up to 4 days before clinical signs appear. Depending on the route of transmission, virulence of the virus, the age and species of animals the length of incubation period varies. In susceptible animals it can range from two to eight days, but can be up to twenty-one days post

infection with the virus. Depending on the infected animal species incubation period of FMD is; 3 to 5 days in cattle, 3 to 8 days in sheep, and 2 to 14 days in pigs. In some cases, the incubation period may be long up to two weeks. Infected animals can spread the virus one to two days prior to the onset of clinical signs and for seven to ten days after the presentation of clinical signs (Alexandersen *et al*., 2003; Kitching, 2002; Kitching and Hughes 2002).

Pigs have a shorter latent and incubation period than cattle, but pigs can produce approximately 3,000 times more airborne FMDV particles than do cattle or sheep; thus, swine can considerably boost the disease. Nevertheless, the aerosol production of infectious FMDV by pigs differs strain by strain (Alexandersen and Donaldson, 2002; Kitching and Alexandersen, 2002).

2.7.7. Morbidity and case fatality rate from FMD

Morbidity from FMD varies with the animal's species, breed and pre-existing immunity, as well as the dose of virus and other factors. The morbidity rate can approach 100% in susceptible naive cattle or pigs, but relatively low morbidity rate is seen in sheep flock that the disease disappears after infecting a low percentage of the animals. The pattern of the disease is influenced by the epidemiological situation (Radostits *et al*., 2007). In adult animals, mortality due to FMD is negligible but death can occur in up to 50% of young animals due to cardiac involvement and complications such as secondary infection, exposure or malnutrition (MacLachlan and Dubovi, 2011). In lambs and suckling pigs, mortality can range from 20-75% in most extreme cases depending on the age of the animals. Mortality is higher in animals under 4 weeks of age infected with FMD and decrease rapidly as animals get older. During outbreaks in endemic and developed countries, most deaths are due to a slaughter policy that usually involves all susceptible animals and herds in contact with or within a certain radius of infected herds (Quinn *et al*., 2002).

2.7.8. FMD virus serotypes and subtypes

Due to a lack of error-correction mechanisms during genome replication, RNA viruses and particularly FMDV, have high mutation rates within the capsid coding region which is responsible for its significant antigenic variation (Knowles and Samuel, 2003). Traditionally, FMDV had been classified according to the serological criteria by which they were classified on the basis of the lack of cross protection after infection or vaccination (Bachrach, 1968). The FMDV isolates can be grouped into seven distinct serotypes and are given the names of their areas of distribution. The Euroasiatic serotypes are A, O, C and Asia-1 and the South African territories serotypes SAT-1, SAT-2 and SAT-3 (Knowles *et al.,* 2007).

Viruses showing partial cross protection were assigned to the same serotype but to different subtype. Over 80 subtypes have been described using genetic and immunological tests. The availability of complete genome sequences have allowed the generation of phylogenetic trees that strongly correlate with the serotypical classification and have replaced the division into subtypes (Sahle *et al*., 2004).

2.8. Pathogenesis and Clinical Signs

In the cattle infected via the respiratory tract, the virus initially replicates in non-cornified epithelial cells of the pharynx (Alexandersen *et al*., 2003) from where it proceeds towards the epithelium of the mucosa associated lymphoid tissue of the nasopharynx (Arzt *et al*., 2010; Pacheco *et al*., 2010), mucous membrane of oral cavity and invades the basal layer of the stratified epithelium of the tongue and produce primary lesions. Vesicles are formed due to virus multiplication in the stratum spinosum layer where cytolysis takes place and hence giving rise to small cavities in the epithelial layer. Virus also invades the lymphatics and enter into the blood stream resulting in spread of virus to other organs and tissues such as epithelium of mouth, dental pad, coronary band, interdigital space of hoof, mammary gland, teats (cattle) and snout where the secondary lesions develop (Alexandersen and Mowat, 2005; Arzt *et al*., 2009; Yang *et al*., 2011).

Clinical signs become evident following an incubation period. The clinical signs of the disease ranges from mild to severe based on strain of the virus, the exposure dose, the age and breed of the animal, the host species and its degree of immunity (Admassu *et al*., 2015). The disease is characterized by an acute febrile reaction (up to 40^{o}c) and formation of vesicles on the dental pad, tongue, muzzle or snout, the hooves, the teats and other site of the skin which rapture within 3 days to leave shallow erosions that heal rapidly. In cattle, most obvious and typical signs include inappetence, drooling of saliva due to oral lesions. On the hooves of cattle and pigs, vesicles are found in the interdigital space, at the bulb of the heel, and along the coronary band. In sheep and goats the signs may be severe but are generally much more subtle than in pigs and cattle. In mild cases the lesions are superficial and transient, and heal rapidly (Alexandersen *et al*., 2003; Nsamba, 2015).

2.9. Diagnosis

The accurate diagnosis of FMDV infection is of utmost importance for the control and eradication of the disease in endemic regions. The diagnosis of FMD, due to the rapidity of spread, should be completed with prompt, sensitive and specific techniques in specifically-designated laboratories having specific arrangement to safely handle exotic disease organisms. Initial diagnosis is based upon clinical signs (Remond *et al*., 2002; Senawi, 2012; OIE, 2012). However, clinically it's similar to other vesicular diseases which are hard to differentiate. Therefore, confirmed laboratory diagnosis of any suspected FMD case to ascertain serotype/subtype of the causal virus is a necessity to enable proper control of the disease as a supportive measure to the stamping out policy in FMD-free areas (Longjam *et al*., 2011). Based on the detection of clinical signs, virus, or antibodies against the virus, diagnosis of FMD can be categorized in to 3 types (Senawi, 2012). Laboratory diagnostic technique for FMD is achieved by a combination of serological tests, virus isolation and nucleic acid recognition methods (Sutmoller *et al*., 2003; OIE, 2008). Samples containing vesicular fluid, epithelium, blood in anticoagulant, serum and esophageal/pharyngeal fluids collected with a probang are taken for laboratory diagnosis (Quinn *et al*., 2005).

2.9.1. Clinical examination for FMD diagnosis

Identifications of FMD by clinical signs by means of close physical examination of infected animals is the best way of diagnosis in areas where laboratory equipments are not available. However, the host species, environment and the virus strain determine the clinical outcome. Following an incubation period, usually between 2 and 8 days, clinical signs become evident (Syed and Graham, 2013); Saravanan *et al*., 2012). Affected animals become lame due to foot lesions, anorectic and febrile, produce profuse drooling of foamy saliva and production of vesicles preferentially in the mouth and feet. Vesicular, erosive and ulcerative gross lesions appear on mouth, feet, teat ends, mammary gland and ruminal epithelium (Quinn, 2002). Most of the affected animals eventually recovered and may become carrier of FMDV in which virus can be recovered after 28 days of infection. (Saravanan *et al*., 2012).

2.9.2. Antibodies detection by serological tests

To detect specific antibody response to FMDV infection serological tests are essential in determining the freedom from infection, for certification of animals for import/export, and in examining vaccine efficacy. Previous or current infections can be diagnosed by detection of antibodies to viral structural proteins (SP) via virus neutralization test (VNT, Gold standard test), Liquid-phase blocking ELISA (LPBE) and Solid-phase competition ELISA (SPCE). Complement fixation test (CFT) has been used extensively for distinguishing different serotypes of FMD virus (Deb *et al*., 2013; Singh *et al*., 2008; Verma *et al*., 2009).. Antibody levels to NSP can be measured via agar gel immunodiffusion test (AGID) and 3ABC NSP ELISA test. Development of ELISA tests against 3ABC (NSP) has greatly enhanced the serosurveillance of FMD as it detects exposure to live virus for all the seven serotypes of virus even in vaccinated herds (Bronsvoort *et al*., 2006a).

2.9.3. Molecular technique for Nucleic acid recognition

The amplification of specific sequences of the viral genome fragments by polymerase chain reaction (PCR) technique can be used for detection of viral RNA. Reverse transcription (RT)-PCR is practical for typing FMDV isolates as it offers the advantages of fast, sensitive and reliable diagnosis where specific universal primers are designed to distinguish all seven serotypes (Mohapatra *et al*., 2006). Reverse Tanscribtion (RT) when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes. However, conventional RT-PCR does not have optimal results in terms of specificity and sensitivity (Alexandersen *et al*., 2003). In epidemiological studies of FMD virus, nucleotide sequencing of the VP1 gene has been extensively used to determine the relationships between the field isolates (Mwanandota, 2013).

2.9.4. Antigen detection through virus isolation

The presence of viral antigens in high concentration can be detected by an antigen ELISA. However, too low virus concentration is difficult to be detected by ELISA so that has to be propagated in susceptible cell cultures to isolate live virus. Primary cell cultures (such as bovine thyroid cells and porcine or ovine kidney cells) or cell lines (such as BHK-21 or IBRS2) are considered as commonly suitable for FMDV isolation. To demonstrate the presence of virus, field samples suspected to contain FMD virus are inoculated into cell cultures (primary pig kidney cells), incubated at 37°C and examined for appearance of cytopathic effect (CPE), 24 to 48 hours post infection. However, some FMDVs fail to grow in a specific cell type, the absence of apparent growth does not guarantee absence of the virus The results may be confirmed by virus neutralization (VNT) assay and typing of the virus by Enzyme linked immunosorbant assay (ELISA) (OIE, 2012).

2.10. Treatment

No specific treatment exists for FMD (Quinn *et al*., 2002), other than supportive care and preventing secondary bacterial infection is recommended where slaughter policy is not enforced (Radostits *et al*., 2007). Most animals recover from infection, albeit with permanently reduced production yield based on the infection state of the disease, though mortality can be high in the young. Slaughtering of infected animals is usually practiced depending on the country's economy (Hirsh and Zee, 2002). The use of antiseptics and disinfectants such as lemon juice or other acidic agents have made kown potency to kill the virus and encourage wound healing (Lubroth, 2002).

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2.11. Prevention and Control

FMD is endemic to most of sub-Saharan Africa, except in a few countries in southern Africa. Control policies and prevention strategies adopted by a country vary depending on its epidemiologic conditions (Beyi, 2012), FMD status and the risks of incursions of the disease (Ahl *et al*., 1991). Reducing the susceptibility of bovine populations through systematic mass vaccination campaigns, separation of infected wildlife from susceptible livestock, together with the control of livestock movement and responses to outbreaks are some of the control measures. In countries where mass slaughter is not possible, strict quarantining and movement restriction should be enforced. In endemic areas, vaccination, movement restriction of animals and quarantine are implemented for controlling the disease (Asseged, 2005; OIE, 2000); the cleaning and disinfection of affected areas, farm equipments and vehicles also practiced. A systematic mass and ring vaccination around the outbreak locations including restriction of animal and animal product movement have been applied in these countries as major control and eradication programs. Fencing of wildlife reserves to prevent contact with domestic livestock have helped limit the spread of virus in certain areas (Tomassen *et al.*, 2002; Kobayashi *et al.*, 2007).

Current control and prevention policies in FMD free countries are based on strict import and quarantine regulations, stamping out by slaughter of all affected and in-contact susceptible animals (Depa *et al*., 2012); and strict restrictions on movement of animals and vehicles around infected premises. After slaughter, the carcasses are either burned or buried and the buildings are thoroughly washed and disinfected with mild acid or alkali and fumigated (Aiello, 1995).

2.12. Economic Losses and Consequences due to FMD `

Foot-and-mouth disease ranks highly among the most economically devastating animal diseases in the world. Significant economic losses are associated with high morbidity (100%) and variable rate of mortality (1-100%). Mortality rate in young animals may reach up to 100%. Economic losses can be attributed to both direct and indirect costs. Direct economic effects including loss of productivity in terms of meat and milk, loss of weight, reduced draught power, delayed conception, abortion and death of young animals (James and Rushton, 2002). The indirect losses are attributed to the restrictions in trade of animals and animal products due to its transboundary nature of transmission (Rushton, 2009) (Figure 6). In endemic countries, the impact of FMD on household, national and international economy posed due to its negative influence to whole livestock industry remarked damaging consequences resulting in disruption of international trade of animals and animal products (Mersie *et al*., 1992).

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

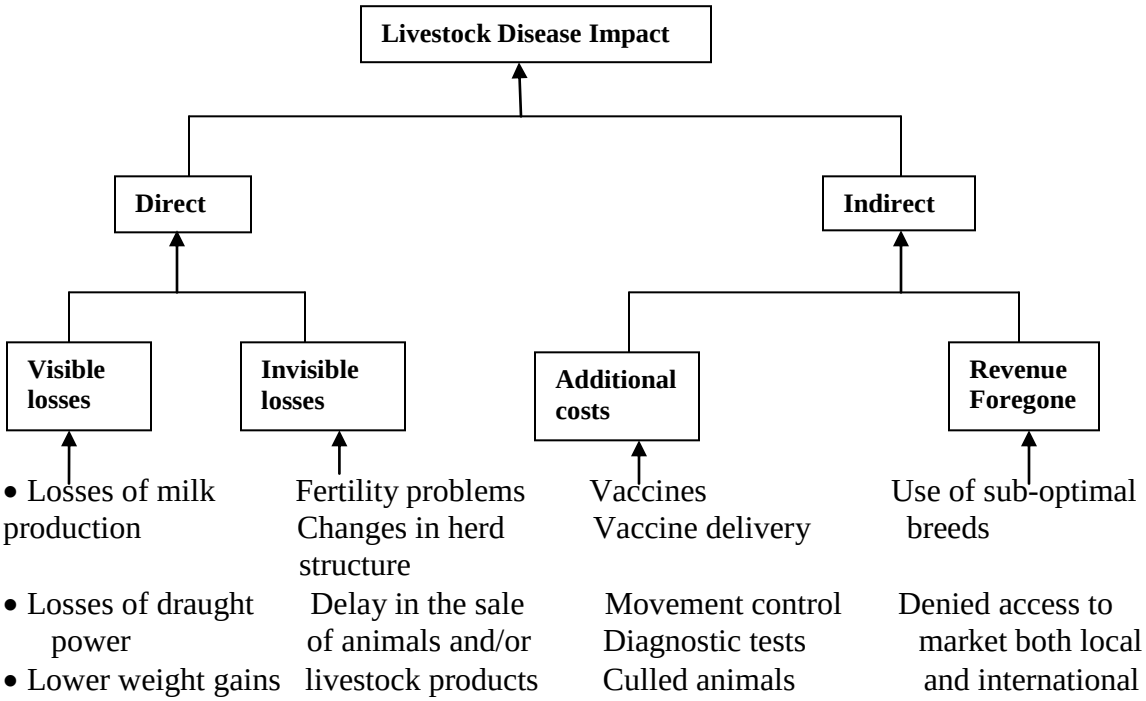

Figure 6: The impacts of foot-and-mouth disease

Source: (Rushton, 2009).

2.13. The Disease Situation and Its Distribution in Ethiopia

2.13.1. The status of FMD in Ethiopia

Foot-and-mouth disease is endemic and known to have wider distribution in Ethiopia, mainly occurring after rainy season while animals are confronted with the stress of harvesting and thrashing. Outbreaks of clinical disease are being encountered and reported frequently (Solomon, 1980). It's occurrence is rising that in 1999 almost 10% of cattle were under risk of infection and in 2000 and 2001 a total of 27 and 88 disease outbreaks were reported (Gelaye *et al*., 2005). Four of the seven FMD virus serotypes (A O, C and SAT2) were reported to be responsible for FMD disease outbreaks (Roeder *et al*., 1994; Gelaye *et al*., 2005). So far, studies conducted did not cover all corners of the country. However, this vesicular disease is widely distributed in Ethiopia and its prevalence varies from place to place. Recent investigation conducted showed that FMD

is posing a major threat in different areas of the country thereby causing substantial economic losses through animal morbidity, mortality and trade restriction (Abdela, 2017).

2.13.2. Serotypes and topotype

In addition to its wider distribution in Ethiopia, serotypes O, A, C, SAT1 and SAT2 were recorded as responsible causes of FMD outbreaks for the last decades. These serotypes of FMDV are maintained in the country (Sahle *et al*., 2004; Gelaye *et al*., 2005; Ayelet *et al*., 2008; Legesse, 2008; Ayelet *et al*., 2009; Negusssie *et al*., 2011). Recently, Jemberu et al. (2015) identified as serotypes O, A, SAT 2 and SAT 1 were the causal serotypes of the outbreaks during the year 2007–2012 being circulated in all regions of the country whereas serotype C has not been reported in Ethiopia since 1983 (Ayelet *et al*., 2009) Serotype O (72%) and A (19.5%) are dominant serotypes in the country (Jemberu *et al*., 2015; Ayelet *et al*., 2009). The FMDV serotypes isolated in Ethiopia are shown in Figure 7 and Table 1 summarizes the identified serotypes and topotypes in Ethopia.

Table 1: FMDV serotype and their topotype identified in Ethiopia

Serotype identified	Topotypes identified	References
O	EA-3	(WRLFMD, 2013)
	EA-4	(WRLFMD, 2013; Balinda *et al*., 2010)
	ME-SA	(Samuel *et al*., 2001)
C	Africa (II)	(Sumption *et al*., 2007)
A	Africa	(WRLFMD, 2013; Sangula, 2010)
SAT-1	IX	(Ayelet *et al*., 2009)
SAT-2	IV	(WRLFMD, 2013)
	XIII	(WRLFMD, 2013)
	XIV	(WRLFMD, 2013; Ayelet *et al*., 2009)

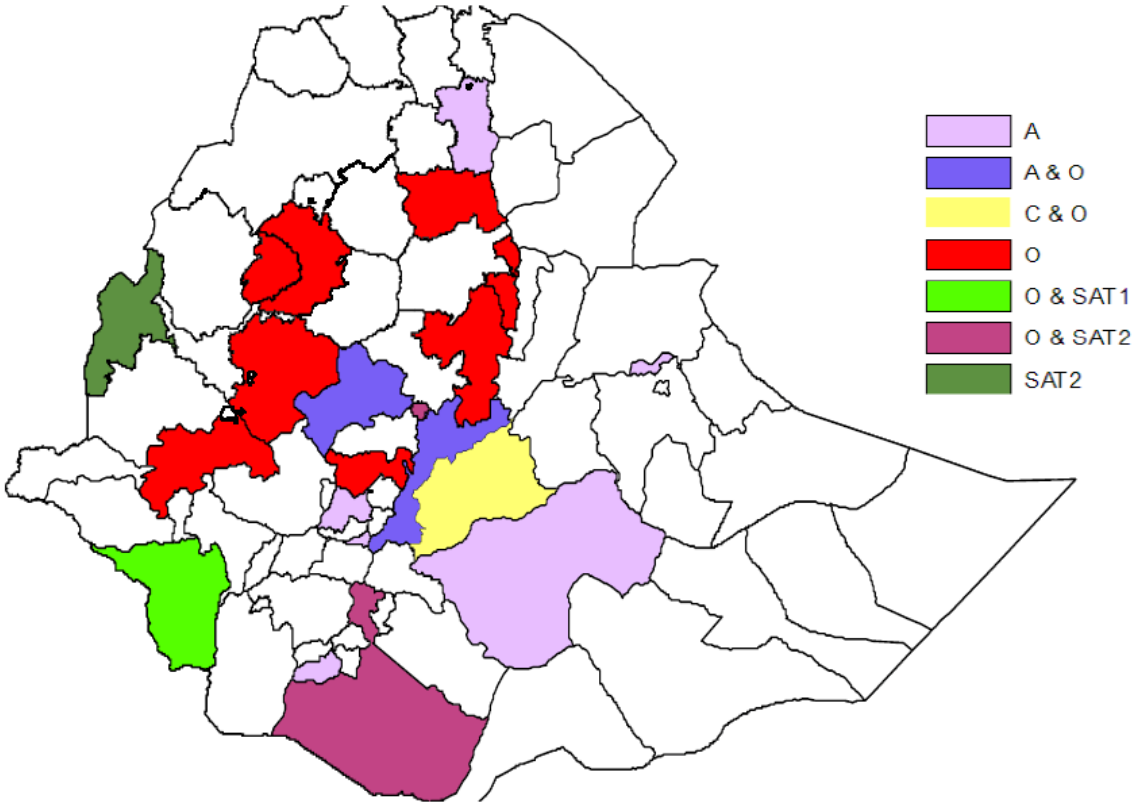

Figure 7: Serotypes of FMDV identified in Ethiopia from 1981-2008
Source: (Gelagay *et al*., 2008)

2.13.3. Sero prevalence

Serological surveys undertaken so far in the country reported sero prevalences of 9% - 26% at the animal level and up to 48% at the herd level in cattle indifferent parts of the country (Megersa *et al*., 2009; Bayissa *et al*., 2011; Mekonen *et al*., 2011 Bogale, 2005; Gelaye *et al*., 2005; Rufael *et al*., 2008; Hailu *et al*., 2010; Sulayeman *et al*., 2018), 4% to 11% in small ruminants (Sahel, 2004; Beyene *et al*., 2015) and 30% in artiodactyl wild mammals (Sahel, 2004).

2.13.4. Risk factors for FMD in Ethiopia

The existence and consequences of the potential risk factors for the occurrence of FMD and spread of the virus in Ethiopia have been described (Rufael *et al*., 2008). The factors that could possibly be associated with the occurrence of FMD are; age (younger animals), sex and breed of the animal, herd size (Jenbere *et al*., 2011), production or farming system (mixed species raising), seasonal influence (dry season) and previous disease (Sarker *et al*., 2011); geographic location and altitude (low lands), contact with wildlife and farm managements especially biosecurity systems (Megersa *et al*., 2009), lack of control of animal movement, lack of effective vaccine, absence of systematic diseases surveillance and absence of reliable epidemiological data were identified for spread of the disease in Ethiopia (Sahle *et al*., 2004). There is also a possibility of transboundary disease transmission from neighboring countries (Gelaye *et al*., 2009). In general, farm management, feed source, animal trades, husbandry, and geographical factors can be predisposing risk factors for the disease existence and transmission or spread (Sahle, 2004).

2.13.5. Economic losses due to FMD

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

FMD infection in the livestock causes significant drop in milk yield (minimum 25%), reduction in meat and wool production, crippled agricultural draught power, and abortion in pregnant animals, poor semen quality in bulls, and increased mortality in calves. Trade barrier for export of FMD infected livestock and their products and massive expenditure spent by Government(s) on FMD control and treatment of ailing animals also cause a great economic loss to the country (Sileshi *et al*., 2006, Ayelet *et al*., 2009).

2.13.6. Control and prevention strategies

The prolonged convalescence, short term immunity with no interserotype cross protection, and establishment of carrier status complicates the control and eradication of this devastating disease. In Ethiopia, actions for controlling FMD includes involvement of quarantine, isolation of infected animals, restriction of animal movement, proper disposal of infected carcass, vaccination programs, and other methods which are feasible to the country's economy (Tassew, 2011). Currently, due to absence of country-wide vaccination program intended to control FMD, a ring vaccination is carried out around an infected area. The National Veterinary Institute is producing an inactivated vaccine considering the wide prevalence of serotypes O and A, (Tadesse, 2003), that the cocktail of vaccine produced contains O, A, SAT-2.

3. MATERIALS AND METHODS

3.1. Study Areas and Study Animal Population

The study was conducted to estimate the sero-prevalence of FMD, identify its risk factors and characterize the FMD virus to its genetic level using molecular methods. It was carried out from October 2018 to May 2019 in the districts of Zones of three Regional states. These districts were Munessa, Alage, Ada'a and Ziway from East Shewa Zone of Oromia Region; Bahir Dar Zuria from West Gojam Zone and Gonder Zuria from North Gonder Zone of Amhara Region; and Sodo Zuria from Welayita Zone of Southern Nations, Nationalities and People's (SNNP) Regional states. Besides, pigs raised under commercial settings were sampled from Ada'a and Alage districts of Oromia Regional state; and from Addis Ababa city Administration. Following a report, areas that recently experienced an outbreak of FMD in Oromia, SNNPR, Amhara and Tigray Regions were also investigated. Various districts from Oromia Regional state such as Ale, Ada'a, Dugda, Guduru, Fitche, Mukaturi and Kuyu district; from SNNP Regional state including Geweta, Shashogo and Worka district; from Amhara Regional state Gonder Zuria and Kewet; and Asegede Tsimbela district in Tigray Regional state were the areas that an outbreak was reported and investigated under the study. The study sites and their mean annual temperature, altitude and distance from Addis Ababa are described in Table 2; and map of study areas is shown in Figure 8 and 9 below.

Table 2: Study sites and their mean annual temperature, altitude and rain fall

Region	District	Distance from Addis Ababa	Mean annual temperature	Altitude	Mean annual rain fall
	Munessa	175 km SE	5-28^{0}C	2430 m a.s.l	2000-4000 mm
	Addis Ababa	-	10.6-22.8ºC	2300 m a.s.l	1180 mm
	Ale	357 km SW	16-24ºC	1500–2000 m a.s.l	2000 mm
	Alage	217 km SW	11-29°C	1600 m a.s.l	700-900 mm
	Ada'a	37.9 km SE	18.7°C	1920 m a.s.l	892 mm
Oromia	Dugda	134 km SE	15-28^{0}C	1836 m a.s.l	700- 800 mm
	Kuyu	155 km N	17.5-20^{0}C	2757-1390 m a.s.l	833-1326 mm
	Ziway	160 km S	18.5°C	1643 m a.s.l	979 mm
	Guduru	314 km W	14.9-27°C	2296 m a.s.l	1000-2400 mm
	Fitche	115 km N	10-25°C	2500-2800 m a.s.l	
	Gaweta	460 km SE	15-22^{0}C	501-3000 m.a.s.l	1400- 2200 mm
	Worka	430 km S	13-26°C	517-4207 m a.s.l	2400-600 mm
SNNPR	Sodo Zuria	390 km S	21.3°C	1500-3200 m a.s.l	1484 mm
	Shashogo	360 km S	11-37^{0}C	1800-2300 m a.s.l	900-1250 mm
Amhara	Bahir Dar Zuria	564 km NW	19.6°C	1700-2300 m a.s.l	1035 mm
	Gonder Zuria	750 km NW	15-21°C	2133 m a.s.l	1150 mm
	Kewet	225 km NE	16.5- 31°C	1380 m a.s.l	1007 mm
Tigray	Aseged Tsimbela	1335 km N	20-35^0 C	800-2300 m a.s.l	500-750 mm

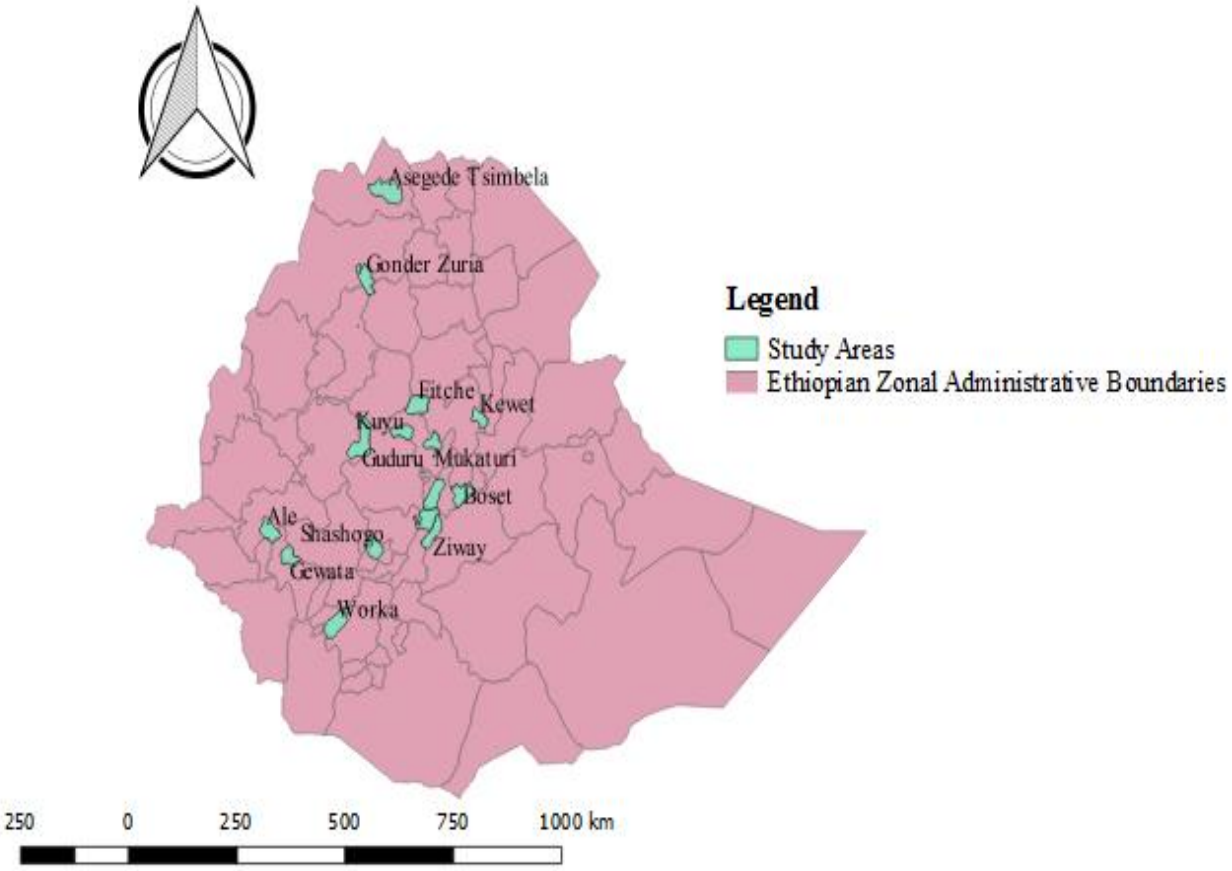

Figure 8: Map of study areas for FMD outbreak investigation

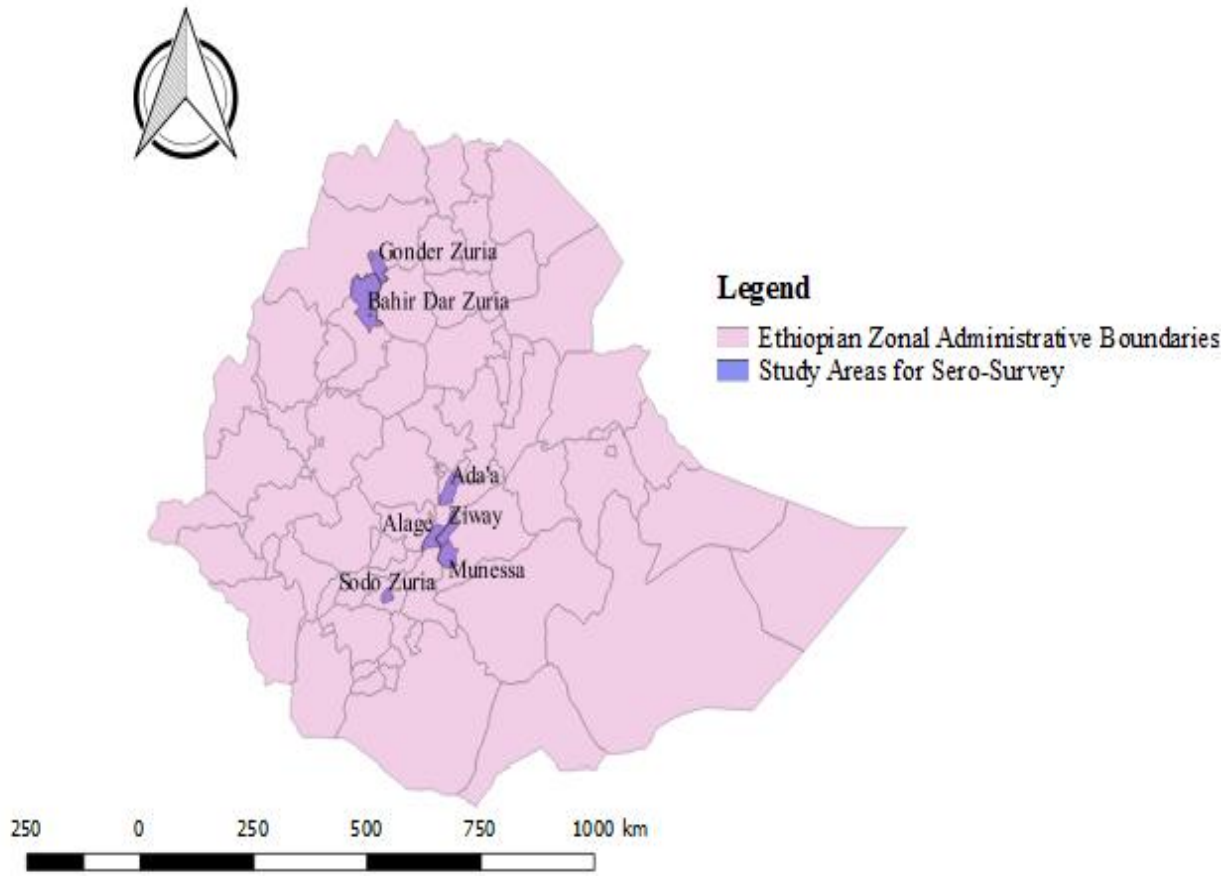

Figure 9: Map of study areas for sero-survey

3.2. Study Design

A cross-sectional survey was conducted. Potential risk factors that could be associated with FMD in the study areas were assessed using semi-structured questionnaire administered through interviewing comprising of important information which includes geographic information, FMD outbreak information, movement of people and vehicles, animal husbandry systems, animal movement information and FMD control strategy in the area. In this study, cattle were grouped into three age categories: calf (6 months-1 yr), young (1-3 yrs), adult (>3 yrs); and pigs as young ($\geq$6 month-1 year) and adult (> 1 year).

3.3. Sample Size Determination

The total number of animals needed for this study was computed using Epitools described by Humphry *et al*. (2004) depicted as:

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

Where; n= required sample size; d= desired level of precision; Pexp= expected prevalence;

To this end these parameters were used: 95% confidence interval, desired precision of 5%, expected prevalence of 14.5% in cattle and 50% in swine. Hence, a minimum of 191 cattle and 384 pigs were required although 1103 cattle and 426 pigs were sampled. In addition to the serum samples, questionnaire survey was done by involving 150 individuals that comprises of farmers and animal health professionals working in the livestock industries.

3.4. Sampling Technique

Multistage (six stages) cluster sampling technique had been applied for estimation of sero-prevalence of the disease. Hence, Regional states were selected purposively as first unit. Then, Zones as second, districts as third, peasants associations as fourth, herds/commercial farms as fifth and individual animal as sixth sample units. Regions, Zones, districts, peasant associations and farms were selected purposively. From the districts, specific peasant associations and commercial farms were again selected based on their accessibility and convenience (availability) whereas, each individual animal were randomly selected. Samples were selected within these areas based on the abundance of livestock population, existence of dairy and swine farms; and their accessibility and geographical locations as well as owners' willingness. Each individual animal had been selected for sampling regardless of sex, breed, body condition and production type.

Following a report of the disease outbreak an investigation was carried out purposively in and around specific outbreak areas. Thorough physical examination was conducted on clinically sick animals to record clinical signs and disease conditions. Those animals that had obvious clinical signs and symptoms suggestive of FMD were considered to be sampled.

3.5. Study Methodology

3.5.1. Questionnaire survey

Questionnaire survey was done by participating 150 individuals including farmers and animal health professionals working in the livestock industries. In this research, the questionnaire was expected to be the supporting source of the data gathering tools. It was designed to both close and open ended question by English language and was translated for those respondents aiming for the clarity as means of interview. Then, the questionnaire was distributed to the individuals to gather relevant data to the problem under study.

3.5.2. Outbreak Investigation

Outbreak reports were received from the regional laboratories through communications and an attempt was made to reach at those outbreak areas on time of active outbreak. Soon after arrival at the specific outbreak sites during disease outbreak investigation, the animals were thoroughly examined for evidence of salivation and lameness. Salivating and/or limping animals were restrained for sampling. The mouth cavities of salivating animals were opened and examined for evidence of intact and/or ruptured vesicles, erosions and ulcers on the tongue, dental pad and mucosa of the oral cavity. The hooves of lame animals were thoroughly washed with water and then carefully examined for similar lesions particularly on the coronary bands and inter-digital spaces.

Fifty bovine epithelial tissue (BEPT) and 19 retro-pharyngeal probang samples were collected from the FMD suspected cattle. Epithelial samples were collected from unruptured or recently ruptured vesicles and lesions on the buccal mucosa and tongue of cattle. The area over the vesicles was washed with phosphate-buffered saline (PBS) to remove all gross contamination such as feed. Using sterile scissors and forceps, a piece of epithelial sample at least 2cm x 2cm was collected without any attached subcutaneous fat or muscle. The samples were then placed in sterile screw capped test tubes containing transport medium composed of equal amounts of glycerol and 0.04 M phosphate buffer, pH 7.2–7.6, with added antibiotics (Penicillin, neomycin sulphate, polymyxin B sulphate or mycostatin). The samples were then labeled, kept in an ice box, transported and submitted to National Animal Health Diagnostics and Investigation Center (NAHDIC) virology laboratory. Sampling was done following procedures described by OIE (2009).

RNA Extraction

An epithelial tissue suspension was prepared by grinding the sample in sterile pestle and mortal with sterile sand. The suspension was centrifuged at 3000 revolution per minute (rpm) for 10 minutes and the supernatant was taken for the molecular work. Total viral RNA was extracted from the digested tissue supernatants and OP sample using the QIAamp® Viral RNA extraction kit (Qiagen Inc, Hilden, Germany) following the mini NucleoSpin column protocol according to the manufacturer's instructions. The extracted RNA was reverse transcribed to detect the presence of FMD viral genetic material using the Onestep rRT-PCR according to the manufacturer's instructions (Kafeero *et al*., 2016). The rRT-PCR was used to amplify genome fragments of FMD virus in diagnostic materials including epithelium and probang samples (Amarel *et al*., 1993).

Briefly, 140 µl of the original epithelial tissues and probang sample suspension was added to 560µl buffer AVL plus carrier RNA in the mirco centrifuge and vortexed for 15 seconds to mix and then incubated at room temperature (25^0c) for 10 minutes. The tube was briefly centrifuged to remove drops from the inside of the lid. Then, 560µl of ethanol (96%) was added to the sample and mixed by pulse vortexing for 15 seconds followed by

brief centrifuging to remove drops from the inside lid. Then, 630µl of the solution were applied to the QIAamp Mini column in a 2ml collection tube and centrifuged at 6000g (8000rpm) for 1 minute. The filtrate was discarded and the column was placed in a fresh 2ml collection tube. Then, 500µl of buffer AW2 were added to the column then centrifuged at 20,000 ×g (14,000 rpm) for 3 minutes and the filtrate was discarded. Then 65µl of Buffer AVE was added to the column equilibrated at room temperature for one minute then centrifuged at 6000g (800rpm) for 1 minute. Finally, after extraction, RNA was eluted in a final volume of 60 µl and stored at -80°C. Then after, the RNA sample was used for detection and molecular characterization of FMDV.

Detection of viral RNA by rRT- PCR

The presence of viral RNA was screened by using real time RT-PCR method targeting the universal 3D regions of FMD virus specific primers: 3D forward (5′- ACT GGG TTT TAC AAA CCT GTGA-3’) and 3D reverse (5′ -GCG AGT CCT GCC ACG GA -3′); and the TaqMan 3D probe (6-FAM 5’ – TCC TTT GCA CGC CGT GGG AC – 3’ TAMRA) (Callahan *et al*., 2002). A master mix assay was used with the final volume per reaction as 20.0 µL, plus 5.0 µL from each template RNA. Briefly, for each sample PCR reaction mix was prepared by considering a reaction mix per one reaction as follows: 2× reaction mix at 12.5 µl, nuclease free water at 1.5 µl, 3D forward primer at 2.0 µl, 3D reverse primer at 2.0 µl, 3D TaqMan probe at 1.5 µl and Superscript III RT/Platinum TaqMix at 0.5 µl. Asia1 was used as positive control and RNase free water was used as negative control. The PCR thermal regime for amplification of VP1 domain was as follows: 30 minutes at 60 °C (reverse transcriptase step), 10 minutes at 95 °C (inactivation reverse transcriptase or activation DNA polymerase), 15seconds at 95 °C (denaturation) and 1 minute at 60 °C (annealing and elongation), with the two last steps performed for 50 cycles.

Measurement fluorescence was taken at the end of second step at stage 3. Cycle threshold or crossing point (Ct) for each sample was then determined. The cycle threshold (Ct-value) corresponds to the number of cycles required for a given sample to reach the

threshold above which it is considered positive. Using a positive cut-off Ct-value of 32.0 (Shaw *et al*., 2007), FMDV genome was detected by rRT-PCR and results interpretation was as follows: samples with Ct < 32 were classified as positive, samples with 32 < Ct < 40 were ambiguous and marked for retesting and samples with no Ct (undetected) were classified as negative. The cycle threshold value (Ct value) was fixed automatically from the pre-loaded machine software. Nine of selected FMDV positive samples were further analysed for virus isolation, serotyping and VP1 sequencing at World Reference Laboratory (WRL) for FMD, Pirbright, United Kingdom (UK).

Detection of FMDV by antigen ELISA

The detection and typing of FMD viruses from epithelial tissue suspensions was done by using IZSLER® (Brescia, Italy), antigen capturing ELISA test kit for typing of FMDV. The assay is an indirect sandwich ELISA that performs with selected combinations of anti-FMDV monoclonal antibodies (MAbs), used as coated and conjugated antibodies. The assay was performed per the manufacturer's recommended protocol.

The cell-culture isolates were subjected to the test assay and serotyped. Briefly, samples were diluted 1/2 in diluents buffer. Then, 50 µl volume of each sample was distributed in 12 wells of a row, two replicates for each type-specific catching MAb and for the pan-FMDV-MAb. After that, 50 µl of the diluents buffer was added in all wells of G and H rows. The plate was incubated at room temperature (temperature range: 18-22°C). After 1 hour incubation period, the plate was emptied to remove all the remaining residual fluid. Next, 200 µl volume of washing solution was added and incubated for 3 minutes at room temperature (18-22°C). The plate was emptied and the circle of washing was repeated 3 times. After this washing step, 50 µl volume of appropriately diluted conjugate A was added into columns from 1 to 8 and conjugate B from 9 to 12. The plate was incubated for 1 hour at room temperature. After incubation, four cycles of washing were repeated as mentioned above leaving the last one for 5 minutes. After which 50 µl of the substrate-chromogen solution was added to all wells. The plate was covered and left at room temperature (18-22°C) in dark for 20 minutes. The reaction was later stopped by the addition

of a stop solution and the plates were read on a spectrophotometer ELISA plate reader (Thermo Scientific, USA) at 450 nm wavelength. Results were interpreted according to the protocol criteria for test validity and interpretation based on the manufacturer's instruction. Interpretation of the results is depicted in Appendice 5.

Sequencing and Phylogenetic analysis

Nine selected epithelial tissue suspensions were shipped in dry ice to be submitted to WRL for FMD, Pirbright, UK, for further serotyping, topotyping, sequencing and phylogenetic analysis. Tissues were labeled using the following format: three-letter country code/isolate number/year (e.g., ETH/14/2019). The three letter country codes were designated as outlined by the WRL for FMD. One sequence from each FMD serotype viruses were used for online blast search to retrieve closely related sequences from Genbank using Molecular Evolutionary Genetic Analysis software (MEGA, V 6.0) (Tamura *et al.,* 2013). The VP1 nucleotide sequences were aligned by using Clustal W algorithm program imbedded in the MEGA 6.0 software. The alignment sequences were used to construct phylogenetic tree analysis using midpoint-rooted neighbor-joining tree and Kimura 2-parameter nucleotide substitution model using the program MEGA 6.0. Midpoint-rooted neighbour-joining trees were then constructed. The robustness of the tree topology was assessed with 500 bootstrap replicates as implemented in the program.

3.5.3. Sero-prevalence Study

Blood samples were aseptically collected using 10 mL plain vacutainer tubes from apparently healthy cattle and pigs, respectively through jugular venipuncture and ear vein puncture of randomly selected animals. The tubes were then labeled. The blood samples were allowed to stand overnight at room temperature to allow serum separation. The sera were transported from the collection site to NAHDIC laboratory using an ice-box and were then kept at -20°C until analysis. Sampling was done following procedures described in OIE, (2009).

Serological Analysis

Serum samples were screened for antibodies against the highly conserved NSP of the FMDV using FMD non-structural protein ELISA (3ABC-ELISA) to identify FMD sero-positive and negative animals. A commercially available test kit (PrioCHECK® FMDV NSP, Netherlands) was used. Test plates of the kit contain FMDV NSP captured by the coated 3ABC specific mAb. The assay was performedaccording to the manufacturer's instructions/protocol. Briefly, 80 µl of the ELISA buffer and 20 µl of the test sera were dispensed to the 3ABC antigen-coated test plates. Negative, weak positive and strong positive control sera were added to designated wells on each test plate. The plate was then sealed by plate sealer, gently shook to evenly dispense then incubated at room temperature for 18 hour. The plates were then emptied by washing off 6 times with 200 µl of washing solution, and 100 µl of diluted conjugate was dispensed to all wells. The test plates were sealed and incubated for 20 minutes at 22°C. The plates were then washed 6 times with 200 µl of the washing solution, and 100 µl of the chromogen (tetra-methyl benzidine) substrate was dispensed to all wells of the plates and incubated for 20 minutes at 22°C. Following incubation, the color development was stopped by dispensing 100 µl of stop solution to all the wells and mixed gently. Color development of each well was measured optically at wavelength of 450 nm and readings were taken on a spectrophotometer using micro plate reader, and the optical density 450 (OD450) values of all samples were expressed as percentage inhibition (PI) of the control and the test sera which calculated according to the formula. The results were analyzed and interpreted using Percentage Inhibition (PI) value of each sample calculated as:

$$\text{Percentage Inhibition (PI)} = 100 - \left(\frac{OD_{450}\ \text{test samples}}{OD_{450}\ \max} \right) * 100$$

Specifically,

PI = <50% (negative), antibodies against the NS protein of FMDV are absent in the test sample.

PI = ≥50% (positive), antibodies against the NS protein of FMDV are present in the test sample.

3.6. Data Analysis

Descriptive and analytical statistics were carried out using STATA Version 12 (Hamilton, 2012). The occurrence of association between dependent variable (sero-positivity) and independent variables was analyzed by logistic regression analysis and odds ratio was employed to assess the level effects of risk factors on FMD. In all analysis, confidence level was held at 95 % and $P<0.05$ was set for significance.

One sequence from each FMD serotype viruses were used for online blast search to retrieve closely related sequences from Genbank using Molecular Evolutionary Genetic Analysis software (MEGA, V 6.0) (Tamura *et al.*, 2013). The VP1 nucleotide sequences were aligned by using Clustal W algorithm program imbedded in the MEGA 6.0 software. The alignment sequences were used to construct phylogenetic tree analysis using midpoint-rooted neighbor-joining tree and Kimura 2-parameter nucleotide substitution model using the program MEGA 6.0. Midpoint-rooted neighbour-joining trees were then constructed. The robustness of the tree topology was assessed with 500 bootstrap replicates as implemented in the program.

4. RESULTS

4.1. Questionnaire Survey

Semi-structured questionnaire was administered to 150 livestock keepers and farm attendants in the study districts to obtain information on the status of FMD across their living areas, factors affecting its occurrence and control methods. Among the participants, the majority (72%) replied frequent occurrences of FMD outbreak on their farms, whereas 28% responded with no outbreak occurred in their farm (Figure 10).

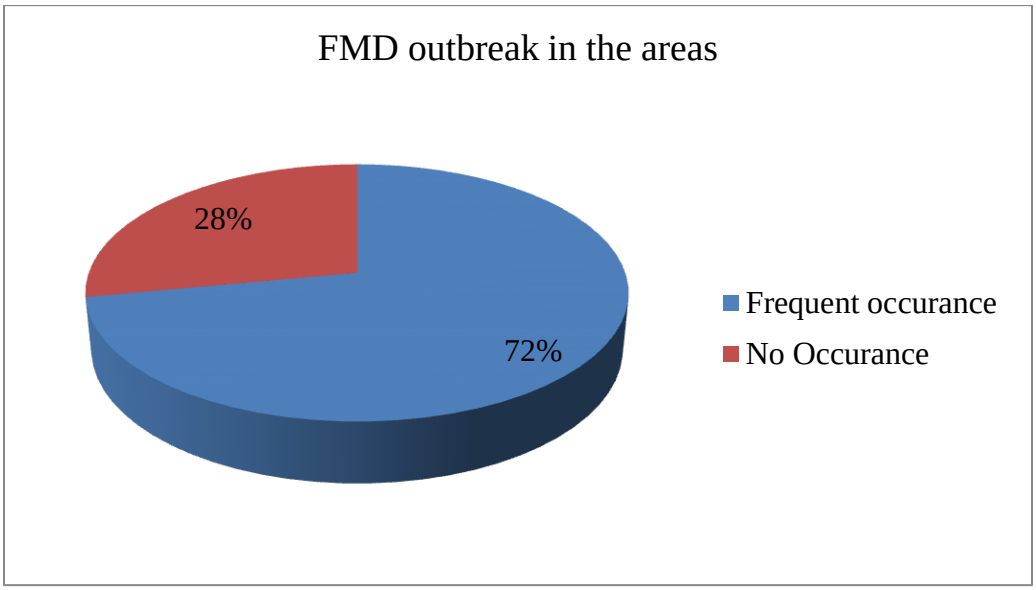

Figure 10: Status of FMD in the areas according to the respondants

Only few (12%) of the farm owners keep their cattle in intensive/ restricted farming system whereas the majority 57.3% and 30.7% keep their cattle in extensive and semi intensive system, respectively. Eighty percent and 61% of the respondents, who rear their animals in extensive and semi intensive system, complained a repeated outbreak of FMD on their farms, respectively. Among those who indicated occurrence of FMD outbreak, majority (30.7%) keep cattle together with small ruminants. In regard to geographic

areas, out of 109 respondents living in the lowland areas, 75% and among 40 respondents living in highlands only 65% responded with repeated FMD outbreaks in their farms and surroundings (Figure 11).

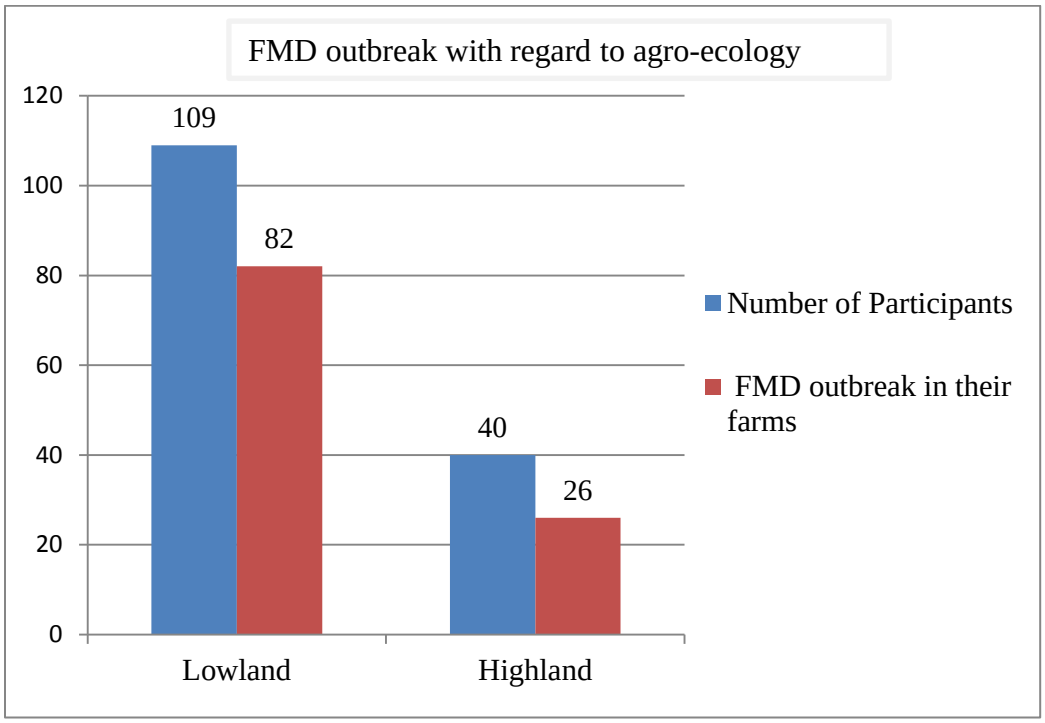

Figure 11: Occurrence of FMD outbreaks according to respondents in different agro-ecologies

Among 150 participants, 64 (43%) replied that their farm is in close proximity with other commercial or/and semi intensive farms and their farm faced multiple outbreaks of FMD. Majority (62.7%) of the respondent complained with death of their animals due to FMD infection. Fifty eight percent of the participants stated that they report to the veterinary offices whenever their animal/s confront with the disease.

Majority of the respondents gave reference factors for the occurrence of the disease in their farms and surroundings. These includes river flow across their area (38.6%), flooding (32.6%), livestock market nearby (36.6%), share pasture with small ruminants (55.3%), allowing animals in wildlife grazing areas (46%), communal water points (5%),

movement of animals in and out of the farm (53%), share pasture with other neighbor animals (48.6%) and movement of personnel, vehicles, workers inside their farm (52.6%).According to the respondents, 63.3% replied that they follow and treat their animals as to manage the spread of the disease in their farms while the other 26.7%, 5.3% and 2% of respondents follow vaccination program, movement restriction; and use vaccination, treatment and movement restriction approaches, respectively to control and prevent disease incidence and/or spread. In the contrary, 2.7% of respondents use no any intervention to control the disease (Figure 12). Among the respondents who vaccinate their animals (n=43), only 26 (60.5%) complained a probable vaccine failure.

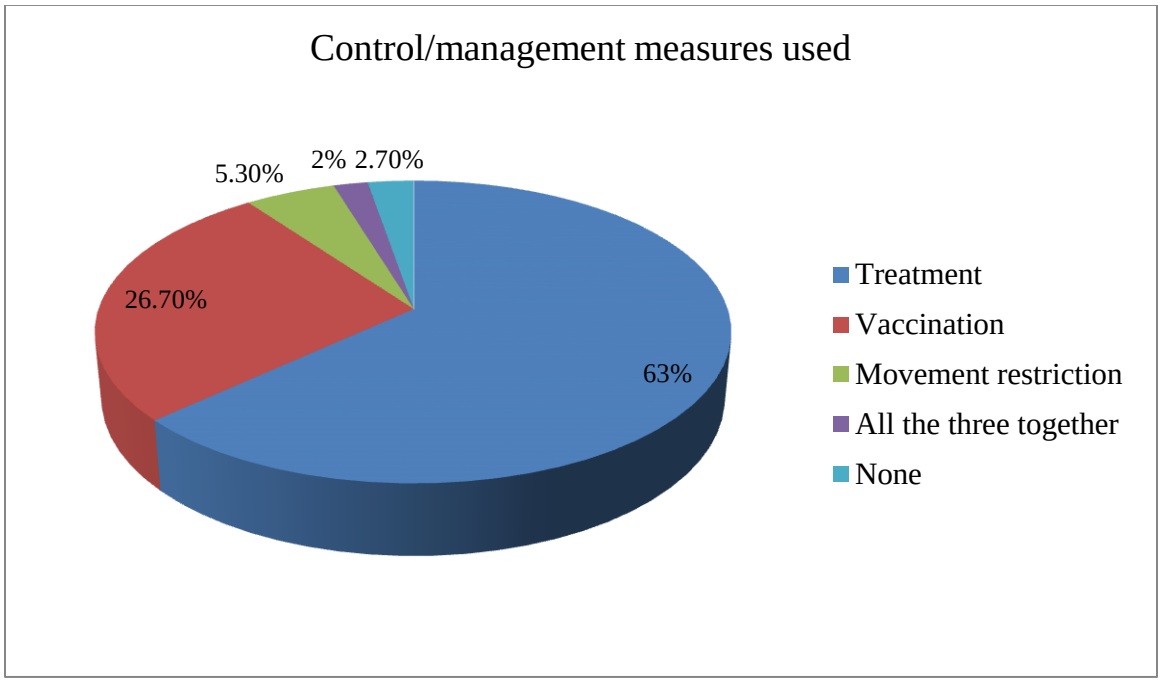

Figure 12: Control/management measures being used by the respondents

4.2. Sero-prevalence of FMD in Cattle

From the total of 1,103 cattle sera examined, 269 (24.39%; CI: 21.87-27.03) of them were identified positive for antibodies against FMDV NSP. The highest FMD sero-prevalence (56.00%) was recorded in Munessa, followed by Alage (46.61%), Bahir Dar Zuria (34.60%), Ada'a (31.03%), Sodo Zuria (16.30%), Ziway (15.51%) and Gonder

Zuria (13.84%) districts. Among the sex groups, higher prevalence (27.84%) was observed in female animals. Similarly, amongst age categories, adult animals were marked with the highest level of sero-positivity (33.77%) for FMD infection. Based on breed specific prevalence, crossbreeds showed the highest level of FMD sero-positivity (28.53%) (Table 3).

The effects of the various factors that supposed to affect the occurrence of FMD were computed using logistic regression analysis (Table 3). The results showed that cattle in Bahir Dar Zuria district had lower odds of being infected with FMD (OR= 0.56; 95 % CI= 0.34-0.92) than those cattle in Alage. Similarly, cattle reared in Ada'a (OR= 0.23; 95 % CI= 0.09-0.57), Sodo Zuria (OR=0.22; 95 % CI= 0.11-0.42) and in Gonder Zuria (OR= 0.15; 95 % CI= 0.09-0.27) had significantly lower odds of being infected compared to those reared in Alage. Calves and young cattle had lower odds of infection (OR= 0.16; 95 % CI= 0.06-0.41) and (OR= 0.49; 95 % CI= 0.34-0.75), respectively than adult cattle. There was also a statistically significant difference in prevalence of FMD between cross and Jersy breed; and between vaccinated and non-vaccinated cattle.

Table 3: Results of multivariable logistic regression analysis on the occurrence of FMD in cattle as a function of various risk factors

Risk factors	**Number Tested**	**Number positive**	**Sero - prevalence (%)**	**OR (95% CI)**	**P-value**
Origin					
Alage	118	55	46.61	Ref	Ref
Munessa	25	14	56.00	2.7 (0.6-11.9)	0.182
Ada'a	87	27	31.03	0.6 (0.3-0.9)	0.001
Bahir Dar Zuria	211	73	34.60	0.2 (0.1-0.5)	0.023
Gonder Zuria	224	31	13.84	0.2 (0.1-0.3)	0.001
Sodo Zuria	135	22	16.30	0.2 (0.1-0.4)	0.001
Ziway	303	47	15.51	0.2 (0.1-1.3)	0.100
Sex					

Female	704	196	27.84	Ref	Ref
Male	399	73	18.30	0.6 (0.3-1.1)	0.096
Age					
Calves	81	5	6.17	0.2 (0.1-0.4)	0.001
Young	566	110	19.43	0.5 (0.3-0.7)	0.001
Adults	456	154	33.77	Ref	Ref
Breed					
Cross	729	208	28.53	Ref	Ref
Local	335	59	17.61	0.4 (0.1-1.7)	0.231
Jersey	39	2	5.13	0.05(0.01-0.30)	0.001
Vaccination status					
Vaccinated	378	67	17.72	Ref	Ref
Non vaccinated	725	202	27.86	4.3 (1.4-11.1)	0.004

Ref – Reference

4.3. Sero-prevalence of FMD in Pigs

Out of the 426 pigs sera samples, 9 (2.11%) were identified positive for FMDV NSP antibodies. According to the result, it was indicated that 8.64%, 1.59% and 0.35% prevalence was observed in Addis Ababa, Alage and Bishoftu, respectively. The observed difference in prevalence between Addis Ababa and Bishoftu was statistically significant ($P<0.05$) in which pigs in Bishoftu are less likely to test positive than pigs in Addis Ababa (OR= 0.04; 95 % CI= 0.004-0.336). Similarly, adult pigs showed higher sero-positivity (3.35%) for FMD infection. It was also revealed that higher sero-prevalence (2.53%) was found in male pigs. Additionally, the highest level of sero-positivity for FMDV was recorded in Duroc pigs (2.97%) (Table 4). However, the variables under investigation (age, sex and breed) were not significant predictors of FMDV seropositivity in pigs ($P> 0.05$).

Table 4: Results of analysis of risk factors of occurrence of FMD in pigs

Risk factors	Total tested samples	Sero-positives	Sero-prevalence (%)	OR (95% CI)	P-value
Districts					
Addis Ababa	81	7	8.64	Ref	Ref
Alage	63	1	1.59	0.18(0.02-1.50)	0.112
Bishoftu	282	1	0.35	0.04 (0.01-0.34)	0.003
Sex					
Female	268	5	1.87	Ref	Ref
Male	158	4	2.53	0.95 (0.23-3.89)	0.944
Age					
Young	247	3	1.21	0.41(0.10-1.74)	0.229
Adult	179	6	3.35	Ref	Ref
Breed					
Large white	254	4	1.57	0.54 (0.09-3.3)	0.506
Duroc	101	3	2.97	0.96 (0.14-6.46)	0.962
Cross	71	2	2.82	Ref	Ref

Ref – Reference

4.4. Molecular Detection and Characterization of FMDV

4.4.1. Field clinical examination of FMD outbreak and virus genome detection

During the active disease search and outbreak investigation a total of 69 cattle clinically affected with FMD were observed and tissue samples and retro-pharyngeal probang samples were subjected to molecular analysis. The results showed that 46 (66.7%) of them were tested positive, with Ct-values ranging from 14 to 32 as shown in the real time PCR amplification graph in Appendice 6. Most virus positive samples were from Ale, Kewet, Worka and Aseged Tsimbela where all samples tested positive. The results of rRT-PCR on samples collected and tested are given in Table 5.

Table 5: Results of reverse tanscriptase analysis on field samples from cattle

District	Number tested	Number Positive
Geweta	20	2
Kuyu	17	15
Gonder Zuria	7	6
Ale	4	4
Dugda	3	3
Ada'a	1	1
Kewet	2	2
Worka	2	2
Aseged Tsimbela	4	4
Guduru	4	3
Shashogo	2	1
Fitche	1	1
Mukaturi	1	1
Boset	1	1
Total	69	46

PCR Positive if Ct-value less or equal to 32

PCR Negative if Ct-value greater than to 32

4.4.2. Results of serotyping of FMDV by antigen capture ELISA

A total of 9 virus isolates from field samples were selected and further analysed for molecular typing of FMDV at WRLFMD, Pirbright, UK, of which 6 were identified as serotype A and 3 identified as serotype O. As depicted in Table 6 serotype A was isolated from samples collected from Guduru, Fitche, Kuyu and Mukaturi where as serotype O was detected in 3 samples from Shashogo, Ale and Gewata.

Table 6: Results of serotyping of FMD viruses isolated from field samples collected from cattle

Origin of outbreaks			Number and type of samples	Identified Serotype
Region	**Zone**	**District**		
	Horuguduru	Guduru	3 BEPT	A
		Fitche	1 BEPT	A
Oromia	North Shewa	Kuyu	1 BEPT	A
		Mukaturi	1 BEPT	A
	Illubabore	Ale	1 BEPT	O
SNNPR	Keffa	Gewata	1 BEPT	O
	Hadiya	Shashogo	1 BEPT	O

BEPT- Bovine epithelial tissue

4.4.3. Phylogenetic analysis

Phylogenetic analysis of FMDV serotype O

The complete VP1 sequence was aligned and compared with archieved data from across Africa to reconstruct phylogenetic relationships of the isolates with reference viruses from the gene bank. Of the three, serotype O examined the first isolate obtained from Konda kebele, Gewata district, Keffa Zone of SNNP Region from a local breed cow (O/ETH/72/2018) was compared based on 639 nucleotide sequence of the VP1. Upon analysis, it was found to be 98.26% similar with the Egyptian isolates of Behira 2/EGY/2017, Alexandria 1/EGY/2017 and Giza 1/EGY/2017 suggesting the existence of a close genetic relationship between the current isolates and that of the Egyptian isolates. The second isolate that was from of Gore, Ale district, Ilubabore Zone of Oromia Region from a local breed cow (O/ETH/73/2018) was also compared based on 639 nucleotide sequence of the VP1. Upon analysis it was shown to have 98.10 % similarity with the Egyptian isolate of Fayoum 3/EGY/2017 and Fayoum 1/EGY/2017 and 98.26%

similarity with Behira 2/EGY/2017. The third isolate, which was from SNNP Region of Hadiya Zone, Shashogo district, Mololocho kebele from a local breed cow (O/ETH/14/2019) was seen to have 92.21% similarity with Kenyan isolate (KEN/100/2010), 87.89% to 88.89% similarity with Ugandan isolate. This particular isolate was seen to have 88.36% with UGA /9/98, 87.89% similarity with U/97/99, UGA /17/98 and UGA /10/98.

The genetic relationship among the three isolates was seen by multiple sequence alignment. The isolate of Konda, Gewata district, Keffa Zone of SNNP Region (O/ETH/72/2018) was 99.84 % similar with the isolate from Gore, Ale district, Ilubabore Zone of Oromia region (O/ETH/73/2018) where as it was found that 77.77% similarity with the isolate of Hadiya Zone, Shashogo district, Molocho kebele (O/ETH/14/2019). The isolate from Gore, Ale district, Ilubabore Zone of Oromia region (O/ETH/73/2018) was found 77.93% similar with the isolate of Hadiya Zone, Shashogo district, Mololocho kebele (O/ETH/14/2019). The phylogenetic tree for serotype O is shown in Figure 13.

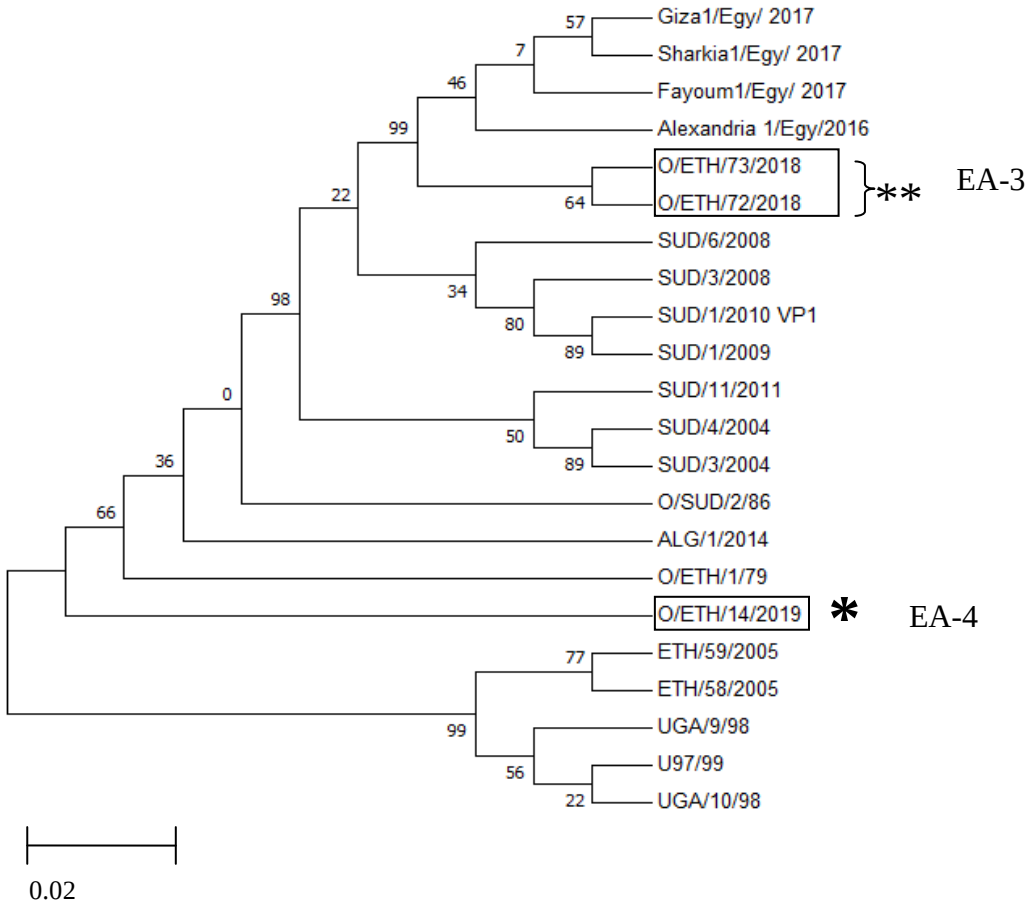

Figure 13: Mid-point-rooted neighbor-joining phylogenetic tree of serotype O FMDV local isolates in comparison with serotype O isolates from Ethiopia and other referenced counties. New isolates obtained from outbreak cases from Ale and Gewata districts of Oromia Region are indicated by (**) and new isolate from Shashogo district of SNNP Region is indicated by (*).

Phylogenetic analysis of FMDV serotype A

The isolate of Guyaguse, Kuyu district, North Showa Zone of Oromia Region from a local breed cow (A/ETH/85/2018) was compared based on 639 nucleotide sequence of the VP1. It was found that 92.95% and 92.79% similarity to the isolates of Eritrea (ERI/1/2006) and Sudan (SUD/3/2006), respectively. Another isolate from the same Region and Zone of Fitche town (A/ETH/86/2018) was found to have 93.42% similarity with ERI/1/2006, SUD/3/2006 and SUD/1/2006 isolates, 88.11% similarity with

ERI/3/97; and 87.77% similarity with ERI/3/98. This shows that this particular isolate have genetic relationship with the Eritrean and Sudanese isolates. The third isolate from the same region and Zone (A/ETH/87/2018) was found to have 92.95 % similarity with Eritrean and Sudanese isolates of ERI/1/2006 and SUD/3/2006. While ERI/3/97 and ERI/3/98 showed to have 87.79% and 87.46% similarity, respectively. Another isolate from Oromia region, Horogduru Zone, Guduru district from a local breed cow of three kebeles: Kombolcha kebele (A/ETH/16/2019), Keneni kebele(A/ETH/18/2019), Kombolcha kebele (A/ETH/19/2019) was found to have similarity with the Eritrea (ERI/1/2006) and Sudan (SUD/1/2006) isolates with 93.42% to 92.73% sequence similarity.

The genetic relationship among the six isolates was seen by multiple sequence alignment. The isolates from Guduru district, Horoguduru Zone of Oromia Region from a local breed cow, Kombolcha kebele (A/ETH/16/2019) was 99.37% similar with Keneni kebele (A/ETH/18/2019), 98.90% similar with another isolates of Kombolcha kebele (A/ETH/19/2019). This isolate (A/ETH/16/2019) was found to have 98.27, 98.12% and 98.43% similarity with (A/ETH/85/2018), (A/ETH/86/2018), (A/ETH/87/2018) sequences of Guyagusee, Fitche and Mukaturi of Oromia Region, respectively. Whereas it was found that the remaining isolates were found to have 98.27% to 99.84 % sequence similarity among each other. The phylogenetic tree for serotype O is shown in Figure 14.

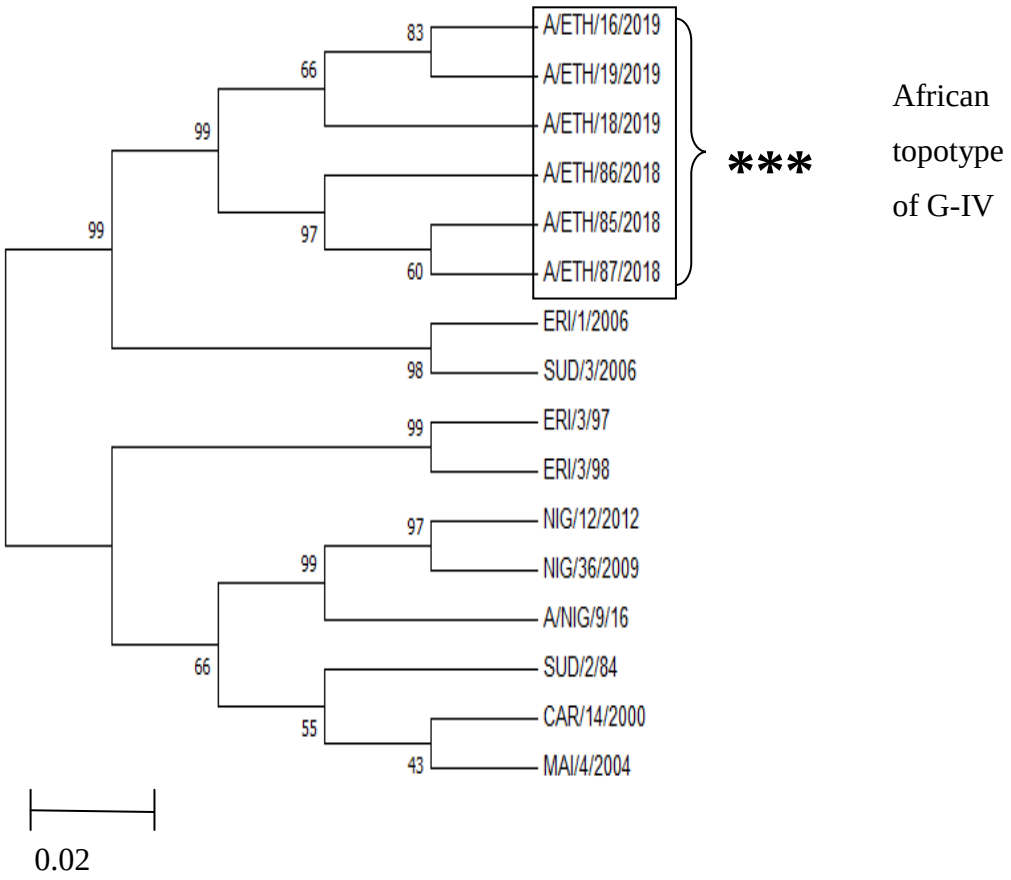

Figure 14: Mid-point-rooted neighbor-joining phylogenetic tree of serotype A FMDV isolates in comparison with Serotype A isolates from Ethiopia and other reference countries. New isolates from Guduru, Kuyu, Fitche, and Muketuri of Oromia Region is indicated by (***).

5. DISCUSSION

Ever since its first recognition in Ethiopia in 1957 (Gulima, 2011), FMD is considered to remain endemic with wide occurrences provoking consequent outbreaks every year (Ayelet *et al*., 2012). The situation is complicated by the fact that the virus causes an asymptomatic persistence infection in ruminants and continuously evolving. Despite its occurrence, still there is an inadequate epidemiological understanding and clarification of the disease. Thus, the frequent disease reporting requires monitoring and an update data of the current FMD epidemiological status in the country. This study could certainly have the value to update data on FMD where the epidemiological status of the disease is poorly understood. In this study an attempt was made to estimate the sero-prevalence of FMD in apparently healthy both cattle and pig animals and identify risk factors in addition to characterizing the virus at its molecular level.

The present study revealed an ascertained sero-positivity for FMD in all study areas with an overall sero-prevalence of 24.39% in cattle, indicating the spread of FMD virus throughout the study areas. The presence of antibodies against the FMDV NSP in the sera of cattle was an indication of previous exposure to natural infection (OIE, 2009). Similar studies conducted at different time and places documented different prevalence. The current finding was relatively comparable to earlier reported prevalence of 26.5%, 24.6 % and 21.4% documented by Rufael et al. (2008), Mekonen et al. (2011) and Desissa et al. (2014), respectively. A recent study by Sulayeman et al. (2018) in Ethiopia and Al-Ajeeli et al. (2018) in Eastern Iraq also reported closely comparable prevalence of 24.2% and 25.3%, respectively. The observed prevalence in the current study could be attributed to geographical location of the farms, failure to periodically vaccinating animals, improper management practices, herding of farm animals in close proximity to wild life areas and contact of animals with livestock of other neighborhoods where they can get infections from disease affected animals could be possible reasons for the high prevalence value.

On the contrary, sero-prevalence documented in this survey showed much higher value when compared to the previous reports of 4.8%, 8.18%, 9.5%, 10.5%, 12.08%, 11.6% and 14.05% by Gelana et al. (2016), Molla et al. (2010), Megersa et al. (2009), Ayelet et al. (2012), Gelaye et al. (2009), Yahya et al. (2013) and Mohamoud et al. (2011), respectively. On the other hand, higher prevalence than the present study was reported with prevalence of 42.7% in Borena, Ethiopia (Melkamsew, 2018), 52.5% in Kenya (Kibore *et al*., 2013), 61% in Uganda (Mwiine *et al*., 2010) and 72.62% in Nigeria (Lazarus *et al*., 2012) registered much higher sero-prevalence in cattle. These differences could be related to variation in agro ecology, geo-spatial distribution of the disease and variations in the production/herding systems among the areas of the study.

The study showed the level of prevalence of FMD has significant variations between districts, mainly relating Sodo Zuria district (16.3%) considered as low prevalence as compared to Alage (46.61%). This difference among districts could be explained by the fact that the variation in location of farms in the disticts, areas with active outbreak and variation in agroecology of the areas could result a significant difference in prevalence among districts.

The present study revealed significantly higher prevalence in adult animals. This finding is supported by previous reports of Rufael et al. (2008), Megersa et al. (2009), Molla et al. (2010), Negussie et al. (2011), Yahya et al. (2013) and Kibore et al. (2013) which stated that sero-positivity increases with age. The high sero-prevalence in adults in present study might be attributed to a possibility of previous infection without being vaccinated/ immunized and/or failure to quarantine infected adult cattle separately. Another possible reason could be that adults are driven freely in grazing and watering points where infection increases by contact to other infected animal. These conditions favor the means for dissemination and thereby transmission of the disease to adult cattle. The low sero-prevalence in the calves and young may be due to their husbandary system that kept them in separation from adults so that exposure to infection would be minimized.

Similar observations was reported by Bayissa et al. (2011); Bronsvoort et al. (2006b) and Molla et al. (2010) who explained the exposure to FMDV is decreased when animals are kept around the homestead or in separated areas. Megersa et al. (2009) also stated rearing young in separation from adults attributes to low sero-positivity in young cattle since there would be low contact rate to infected or convalescent adult animals. Even if there is high prevalence in adult animals there was sero-positive reactors in less than 3 years of age; this could indicate improper management practices, high level of intensification and poor hygienic practices might be attributed an infection among calves and young cattle.

In this study, breed was seen as one of the risk factors and breed specific sero-prevalence was found to be higher in crossbreed animals (28.53%) although the difference in prevalence between breeds was not statistical significant. This is in agreement with the finding of Misgana et al. (2013) who stated as breed was not associated with sero-positivity though highest prevalence with crossbreeds. In contrast, Knowles and Samuel (2003) and Sarker et al. (2011) reported that breed was associated with FMD outbreaks, with local breeds being the most affected. This might be due to poor management practices given to the local as compared to other breeds. However, the finding of high prevalence in crossbreeds in the present study could be due to the fact that crossbreed cattle are highly susceptible to endemic diseases than indigenous or local breeds.

The present study revealed 2.11% prevalence evidence of FMD NSP antibodies in pigs which is an indication of considerable disease dissemination in the study areas. This is in consistent with many other reports such as a similar study in sub-Saharan Africa (Tanzania, Kenya, Uganda and Cameroon) by Fernandez-Pacheco et al. (2012) who reported 2% sero-prevalence in pigs. The present study is also consistent with previous reports of Lazarus et al. (2015) who reported 1.11% prevalence in North East Nigeria, Ehizibolo et al. (2016) reported 1.5% in Nigeria. However, the observed result was much lower than reports of Fakai et al. (2015) from Nigeria, Wang et al. (2016) and Wang (2010) from China who recorded 18%, 19.73% and 15.2%, respectively. The finding of the present study also disagrees with recent study by Wu et al. (2018) who has reported the sero-prevalence of 6.79% for antibodies of FMDV in pigs in China.

However, this finding highly contradict with a report by Lazarus et al. (2012) who stated none of Nigerian commercial pigs kept under intensive production system were sero-reactive for FMDV. This variation in prevalence could be due to the difference in level of farm intensification, biosecurity levels being used, geographical areas and location of the pig farms. This idea is supported by Wu et al. (2018) explaining the distinction between geographic areas at varied time points supposed to bring prevalence variation. Because of the fact that pigs don't shed the virus to remain as carrier, the sero-positivity of pigs in the present study might be due to an ongoing infection or active outbreak in the pig rearing areas, poor control efforts against the disease including non vaccination and poor biosecurity measures.

Although, not significant, prevalence was higher in adult pigs above one year of age than young. Fakai et al. (2015) also reported age of pigs was not associated with sero-positivity, however, higher prevalence in young pigs. This could be explained by the fact that the large number of young pigs sampled for this study could be the reason for high prevalence in young pigs.

Risk factors for FMD were assessed through a questionnaire survey and it revealed that the disease was endemic in the study areas causing frequent outbreaks and resulting in deaths of young cattle animals. According to the questionnaire result, communal grazing places and watering points, intermingling of different animals in close contact, herding animals close to river flow and flooded areas, presence of nearby market places and failure to vaccinate animals could be the possible factors that contributed to the high disease transmission and occurrence. This observation was in agreement with Sahle et al. (2004) who stated that factors such as continuous contact with susceptible animals, intermingling of different herds, and sharing of grazing and watering points with the high number of susceptible animals contribute to the occurrence of FMD outbreak. Ekboir (1999) also suggested that uncontrolled movement and contact between infected and susceptible animals are the most considerable means for dissemination and transmission of FMD.

In the present study, detection of FMDV genomes was conducted from field isolated samples from certain outbreak areas. For an accurate, sensitive and rapid detection of FMDV, rRT-PCR was used targeting the conserved 3D region of the samples viral genome recognition and amplification regardless of their serotypes. Laor et al. (1992) and Meyer et al. (1991) have been described the application of RT-PCR for the rapid and successful diagnosis of FMDV in suspected clinical materials. Most of the outbreaks occurred between October to March; environmental conditions during the months favor the disease spread (Hussain *et al*., 2008; Jamal *et al*., 2010). Epithelial tissues and oro pharyngeal fluids of affected animals were routinely used for diagnosis and typing of FMDV (OIE, 2012).

Based on the RT-PCR result, 46 samples (66.7%) were positive for FMD viral RNA. The remaining samples detected as negative indicating absence of viral genome. The possible reason for the absence of detectable viral genome in some samples probably indicate that sampling was done at late phase of infection, low virus load in the tissues, low quantity/amount of quality of tissue that could hampered the amplification by RT-PCR. Moreover, it may be due to degradation of RNA genome that the bacterial RNAses or other degradative enzymes may have degraded the viral genome during sample storage or transportation. These reasons could be the possible causes to bring in either weak or no detectable signals by RT-PCR.

The present study described the genetic characterization of two FMDV serotypes which were responsible for the outbreaks in the study areas in 2018/19. The study described that the molecular typing of FMDV has confirmed the circulation of serotype A and O in the areas being investigated.The two FMDV serotypes detected in the course of this study along with previous reports in Ethiopia establish the facts that these are the most prevalent serotypes circulating among cattle in the country (Ayelet *et al*., 2009; Gelaye *et al*., 2005; Negussie *et al*., 2011).

This finding agreed with report of Sahle (2004) with surveys in the Omo National Park and Bale mountain area of Ethiopia and Dejene (2004) in dairy farms in and around

Addis Ababa detected FMD serotypes O and A. This is also in agreement with Gelaye et al. (2005) who isolated Serotypes O and A isolated from cattle during outbreak investigations between 1982 and 2000. Similarly, a survey by Ayelet et al. (2009) during 1981–2007 in regions of Ethiopia reported the isolation of serotypes O and A from samples collected. A particular study in 2005 also reported the isolation of serotypes O and A in Ethiopia (FAO, 2007).

In this study, most of the outbreaks were caused by serotype A, followed by serotype O unlike the previous reports of Ayelet et al. (2009); Gelaye et al. (2005); Ayelet et al. (2009) and Negusssie et al. (2011) documented that serotype O was a dominant FMD virus serotype circulating in Ethiopia. The finding of the present study could be due to small number of samples from few areas were subjected to typing. So that according to this study it may not be a true reflection to say serotype A was dominantly causing the outbreaks since not all samples from the outbreak reported areas were investigated and typed.

Phylogenetic analysis conducted using VP1 region showed that serotype O isolates fell into topotype of East Africa (EA) 3 and 4, while serotype A showed that African topotype (G-IV). This finding confirm and supported the previously published reports of Ayelet et al. (2009) who documented the existence and circulation of EA-3, EA-4 and African topotype of genotype IV in Ethiopia. Recently, Lloyd-Jones et al. (2017) also reported EA-3 and EA-4 as the predominant topotypes with continued circulation that caused several outbreaks each year.

6. CONCLUSION AND RECOMMENDATIONS

In the present study, the result of 3ABC ELISA showed an overall prevalence of 24.39% in cattle and 2.11% in pigs. Additionally, factors such as close proximity with other commercial farms, presence of river flow, flooding, nearby livestock market, sharing communal grazing and water points; and animals, personnel and vehicles movement in and out of the farm were found to contribute for occurance of FMD outbreak. Antibiotic treatement was the chief management measure applied in the study areas; with very few practicing vaccination together with movement restriction. The disease was seen as prevalent in cattle and pigs. The detection of antibodies in pigs sera suggested FMD viral replication that signify evidence of active infection or ongoing viral exposure despite absence of obvious clinical lesion. However, in case of cattle, antibodies against FMDV implied either exposure to active or previous viral infection. The circulating strain topotypes were characterized and found nucleotide sequence similarity among themselves, previous Ethiopian isolates and neighbouring countries, Sudan, Eritrean and Egypt, topotypes. Generally, the current study ascertained and explained the continuity of FMD introduction and the endemic situation of the disease with widely distributed and identified circulating serotypes in the study areas.

Hence, in reference to the conclusion remarks, recommendations are pointed out:

- Farmers, and livestock owners should be trained about the disease, husbandary practices and to report the FMD outbreak
- Genetic and antigenic characterization of virus shoud be done regularly inorder to identify newer candidate vaccine strain and develop an improved and effective vaccine combinations
- Successful control and prevention strategies such as vaccination and biosecurity; and eradication preparations should be implemented throughout the country.
- Effective surveillance systems for timely information about occurrence, spread and causative serotypes are mandatory for efficient control.
- Various research and epidemiological studies are needed to investigate existence of an association between wildlife-pigs-cattle interaction

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8. APPENDICES

APPENDICE 1: Serum Sample Collection Sheet for Sero-Survey

Sample code	District	Sex	Age	Species	Breed	Production type	Contact with wild animals	Vaccination status

APPENDICE 2: Tissue Sample Collection Format During Outbreak Investigation

Sample code	Outbreak site	Species	Sex	Age	Sample Type

APPENDICE 3: Plate Layout of FMDV 3ABC-Ab ELISA Test

OD	1	2	3	4	5	6	7	8	9	10	11	12
A	C-	3										
B	C-	4										
C	C+	5										
D	C+	6										
E	C++	7										
F	C++	8										
G	1	9										
H	2	10										

APPENDICE 4: Plate Layout for Antigen Detection Sandwich ELISA Test

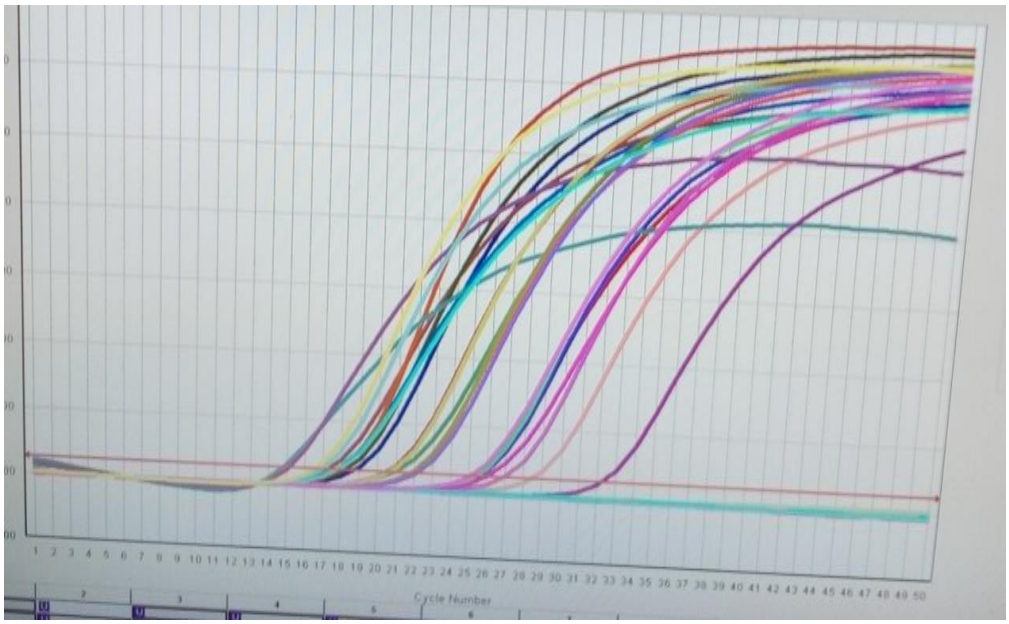
Catching MAbs	Type O		Type A				Pan-O, A, C, Asia 1		Type SAT 1		Type SAT 2	
	MAb 3B11		MAb 4D12		MAb 4F6		MAb 1F10		Pool 2 MAbs		Pool 2 MAbs	
	1	2	3	4	5	6	7	8	9	10	11	12
Sample1 A												
Sample2 B												
Sample3 C												
Sample4 D												
Sample5 E												
Sample6 F												
Pos. Ctr G												
Neg. Ctr H												

MAbs – Monoclonal antibodies

APPENDICE 5: 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 pan-FMDV 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
FMDV positive (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

APPENDICE 6: The Real Time PCR Amplification Graph



APPENDICE 7: Multiple Sequence Alignment (Clustal W)

Serotype O

```
O/ETH/72/2018    ACCACCTCCCCGGGCGAATCGGCTGACCCTGTGACTGCCACCGTTGAGAACTACGGCGGC
O/ETH/73/2018    ACCACCTCCCCGGGCGAATCGGCTGACCCTGTGACTGCCACCGTTGAGAACTACGGCGGC
O/ETH/14/2019    ACCACCTCCCCAGGTGAATCAGCCGACCCCGTGACCGCCACTGTTGAGAACTATGGTGGC
                 ********** ** ***** ** ***** ***** ***** ********** ** ***

O/ETH/72/2018    GTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCATTCTCGACAGATTTGTG
O/ETH/73/2018    GTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCATTCTCGACAGATTTGTG
O/ETH/14/2019    GAGACACAGGTCCAGAGGCGTCAACACACCGACGTCTCGTTCATCCTCGACAGGTTTGTG
                 * ******** ****** ** ******** ** *** ******* ******** ******

O/ETH/72/2018    AAGGTAACACCACAACCCCAAACAAACGTGTTGGACCTGATGCAGACCCCCCCACACACG
O/ETH/73/2018    AAGGTAACACCACAACCCCAAACAAACGTGTTGGACCTGATGCAGACCCCCCCACACACG
O/ETH/14/2019    AAGGTAACACCAAGAGAGGACCTAAATGTTTTGGACCTGATGCAGATTCCTGCCCACACA
                 ********** *  *  *** ** ********** ** * *****
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O/ETH/72/2018   CTGGTCGGGGCACTCCTCCGCTCTGCTACCTACTACTTTGCAGACCTTGAAGTGGCAGTG
O/ETH/73/2018   CTGGTCGGGGCACTCCTCCGCTCTGCTACCTACTACTTTGCAGACCTTGAAGTGGCAGTG
O/ETH/14/2019   CTGGTGGGGGCGCTCCTCCGTACTGCCACCTACTACTTCGCTGATCTGGAGGTGGCGGTC
                ***** ***** ******** **** ********** ** ** ** ** ***** **

O/ETH/72/2018   AAGCACGAGGGGAACCTCACGTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAAC
O/ETH/73/2018   AAGCACGAGGGGAACCTCACGTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAAC
O/ETH/14/2019   AAGCACGAAGGGAACCTCACATGGGTCCCGAACGGAGCGCCCGTGTCAGCACTGGAAAAC
                ******** ********** ******** ***** ***** *  **** ***** ***

O/ETH/72/2018   ACAACCAACCCAACAGCCTACCACAAGGCACCACTTACTCGGCTTGCCCTACCATACACG
O/ETH/73/2018   ACAACCAACCCAACAGCCTACCACAAGGCACCACTTACTCGGCTTGCCCTACCATACACG
O/ETH/14/2019   ACCACCAACCCAACAGCTTACCACAAAGCACCACTCACCCGTCTTGCTCTGCCCTACACA
                ** ************** ******** ******** ** ** ***** ** ** *****

O/ETH/72/2018   GCACCACACCGCGTGCTCGCAACTGTCTACAACGGGAGTTGTAAGTACGGTGAGGCTCGG
O/ETH/73/2018   GCACCACACCGCGTGCTCGCAACTGTCTACAACGGGAGTTGTAAGTACGGTGAGGCTCGG
O/ETH/14/2019   GCGCCCCACCGCGTTTTGGCAACCGTTTACAACGGAAACTGCAAATACGGAGAGACACCA
                ** ** ********  * ****** ** ******** *  ** ** ***** *** * *

O/ETH/72/2018   GAGACCAACGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGCCCGAACGCTGCCC
O/ETH/73/2018   GAGACCAACGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGCCCGAACGCTGCCC
O/ETH/14/2019   GTGGTCAATGCGAGGGGTGATCTTCAAGTGTTGGCCCAGAAGGCAGCTAGGACGCTGCCC
                * *  *** * *** ***** ** ***** ****************  * *********

O/ETH/72/2018   ACTTCTTTCAACTACGGTGCCATCAAAGCCACTCGGGTGACAGAATTGCTCTACCGCATG
O/ETH/73/2018   ACTTCTTTCAACTACGGTGCCATCAAAGCCACCCGGGTGACAGAATTGCTCTACCGCATG
O/ETH/14/2019   ACCTCCTTCAACTACGGTGCCATCAAGGCTACCCGGGTGACTGAGCTGCTCTACCGCATG
                ** ** ******************** ** ** ******** ** **************

O/ETH/72/2018   AAGAGGGCTGAGACGTACTGCCCTCGCCCGCTGTTGGCAGTCCACCCGACTGAAGCTAGA
O/ETH/73/2018   AAGAGGGCTGAGACGTACTGCCCTCGCCCGCTGTTGGCAGTCCACCCGACTGAAGCTAGA
O/ETH/14/2019   AAGAGGGCTGAGACGTACTGTCCCAGACCCCTCCTAGCTATTCACCCAAGCGAGGCCAGA
                ******************** ** * ** ** * ** * ****** * ** ** ***
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O/ETH/72/2018    CACAAGCAGAAGATAGTGGCACCTGTGAAACAACTCCTG
O/ETH/73/2018    CACAAGCAGAAGATAGTGGCACCTGTGAAACAACTCCTG
O/ETH/14/2019    CACAAACAGAAGATTGTGGCACCCGTGAAACAGCTTTTG
                 ***** ******** ******** ******** ** **
```

Serotype A

```
A/ETH/16/2019    ACCACTGCAACGGGGGAATCTGCAGACCCTGTCACCACTACTGTGGAGAACTACGGTGGT
A/ETH/19/2019    ACCACTGCAACGGGGGAATCTGCAGACCCTGTCACCACTACTGTGGAGAACTACGGTGGT
A/ETH/18/2019    ACCACTGCAACGGGGGAATCTGCAGACCCTGTCACCACTACTGTGGAGAACTACGGTGGT
A/ETH/85/2018    ACCACTGCAACGGGGGAATCTGCAGACCCTGTCACCACTACTGTGGAGAACTACGGTGGT
A/ETH/86/2018    ACCACTGCAACGGGGGAATCTGCAGACCCTGTCACCACTACTGTGGAGAACTACGGTGGT
A/ETH/87/2018    ACCACTGCAACGGGGGAATCTGCAGACCCTGTCACCACTACTGTGGAGAACTACGGTGGT
                 ************************************************************

A/ETH/16/2019    GAGACACAAGTCCAGAGGCGACACCACACAGATGTCGGCTTCATAATGGACAGATTTGTG
A/ETH/19/2019    GAGACACAAGTCCAGAGGCGACACCACACAGATGTCGGCTTCATAATGGACAGATTTGTG
A/ETH/18/2019    GAGACACAAGTCCAGAGGCGACACCACACAGATGTCGGCTTCATAATGGACAGATTTGTG
A/ETH/85/2018    GAGACACAAGTCCAGAGGCGACACCACACAGATGTCGGCTTCATAATGGACAGATTTGTG
A/ETH/86/2018    GAGACACAAGTCCAGAGGCGACACCACACAGATGTCGGCTTCATAATGGACAGATTTGTG
A/ETH/87/2018    GAGACACAAGTCCAGAGGCGACACCACACAGATGTCGGCTTCATAATGGACAGATTTGTG
                 ************************************************************

A/ETH/16/2019    AAAGTGAACAGTTCTACTCCCATCCATGTCATAGACCTCATGCAAACCCACCAACACGGG
A/ETH/19/2019    AAAGTGAACAGTTCTACTCCCATCCATGTCATAGACCTCATGCAAACCCACCAACACGGG
A/ETH/18/2019    AAAGTGAACAGTTCTACTCCCATCCATGTCATAGACCTCATGCAAACCCACCAACACGGG
A/ETH/85/2018    AAGGTGAACAGTTCTACTCCCATCCATGTCATAGACCTCATGCAAACCCACCAACACGGG
A/ETH/86/2018    AAAGTGAACAGTTCTACTCCCATCCATGTCATAGACCTCATGCAAACCCACCAACACGGG
A/ETH/87/2018    AAAGTGAACAGTTCTACTCCCATCCATGTCATAGACCTCATGCAAACCCACCAACACGGG
                 ** ***************************************************

A/ETH/16/2019    TTAGTAGGTGCACTGTTGCGCTCGGCCACCTACTACTTCTCTGACCTGGAGGTTGTGGTA
A/ETH/19/2019    TTAGTAGGTGCGCTGTTGCGCTCGGCCACCTACTACTTCTCTGACCTGGAGGTTGTGGTA
A/ETH/18/2019    TTAGTAGGTGCGCTGTTGCGCTCGGCCACCTACTACTTCTCTGACCTGGAGGTTGTGGTA
A/ETH/85/2018    TTAGTAGGTGCGCTGTTGCGCTCGGCCACCTACTACTTCTCTGACCTGGAGGTTGTGGTA
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A/ETH/86/2018    TTAGTAGGTGCGCTGTTGCGCTCGGCCACCTACTACTTCTCTGACCTGGAGGTTGTGGTA
A/ETH/87/2018    TTAGTAGGTGCGCTGTTGCGCTCGGCCACCTACTACTTCTCTGACCTGGAGGTTGTGGTA
                 ********** **********************************************

A/ETH/16/2019    AGGCACCAGGGAAATCTGACTTGGGTGCCCAACGGCGCCCCGGAGGCAGCCCTTTCAAAC
A/ETH/19/2019    AGGCACCAGGGAAATCTGACTTGGGTGCCCAACGGCGCCCCGGAGGCAGCCCTTTCAAAC
A/ETH/18/2019    AAGCACCAGGGAAATCTGACTTGGGTGCCCAACGGCGCCCCGGAGGCAGCCCTTTCAAAC
A/ETH/85/2018    AGGCACCAGGGAAACCTGACTTGGGTGCCCAACGGTGCCCCGGAGGCAGCCCTTTCAAAC
A/ETH/86/2018    AGGCACCAGGGAAACCTGACTTGGGTGCCCAACGGCGCCCCGGAGGCAGCCCTTTCAAAC
A/ETH/87/2018    AGGCACCAGGGAAACCTGACTTGGGTGCCCAACGGCGCCCCGGAGGCAGCCCTTTCAAAC
                 * ********** ****************** **********************

A/ETH/16/2019    ACGAGCAACCCCACAGCATACCACAAGGCACCGTTCACCAGGCTGGCCCTCCCCTACACT
A/ETH/19/2019    ACGAGCAACCCCACAGCATACCACAAGGCACCGTTCACCAGGCTGGCCCTCCCCTACACT
A/ETH/18/2019    ACGAGCAACCCCACAGCATACCACAAGGCACCGTTCACCAGGCTGGCCCTCCCCTACACT
A/ETH/85/2018    GCGGGCAACCCCACAGCATACCACAAGGCACCGTTCACCAGGCTGGCCCTCCCCTACACT
A/ETH/86/2018    ACGGGCAACCCCACAGCATACCACAAGGCACCGTTCACCAGGCTGGCCCTCCCCTACACT
A/ETH/87/2018    ACGGGCAACCCCACAGCATACCACAAGGCACCGTTCACCAGGCTGGCCCTCCCCTACACT
                 ** ******************************************************

A/ETH/16/2019    GCACCACACCGCGTGTTGGCAACAGTGTACAACGGGACGAACAAGTACTCAACAGGTGCT
A/ETH/19/2019    GCACCACACCGCGTGTTGGCAACAGTGTACAACGGGACGAACAAGTACTCAACAGGTGCT
A/ETH/18/2019    GCACCGCACCGCGTGTTGGCAACAGTGTACAACGGGACGAACAAGTACTCAACAGGTGCT
A/ETH/85/2018    GCACCACACCGCGTGTTGGCAACAGTGTACAACGGGACGAACAAGTACTCAACAGGTGAT
A/ETH/86/2018    GCACCACACCGCGTGTTGGCAACAGTGTACAACGGGACGAACAAGTACTCAACAGGTGAT
A/ETH/87/2018    GCACCACACCGCGTGTTGGCAACAGTGTACAACGGGACGAACAAGTACTCAACAGGTGAT
                 ***** ************************************************** *

A/ETH/16/2019    TCACCCAGACGAGGAGATTTGGCATCCCTCGCGGCGAGGGTTGCTACGCAGCTCCCGTCG
A/ETH/19/2019    TCACCCAGACGAGGAGATTTGGCATCCCTCGCGGCGAGGGTTGCTACGCAGCTCCCGTCG
A/ETH/18/2019    TCACCCAGACGAGGAGATTTGGCATCCCTCGCGGCGAGGGTTGCTACGCAGCTCCCGTCG
A/ETH/85/2018    TCACCCAGACGAGGAGATTTGGCATCCCTCGCGGCGAGGGTAGCTACGCAGCTCCCGTCG
A/ETH/86/2018    TCACCCAGACGAGGAGATTTGGCATCCCTCGCGGCGAGGGTAGCTACGCAGCTCCCGTCG
A/ETH/87/2018    TCACCCAGACGAGGGGATTTGGCATCCCTCGCGGCGAGGGTAGCTACGCAGCTCCCGTCG
                 ************* ************************* *****************
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A/ETH/16/2019   TCCTTCAACTACGGAGCACTTCGCGCCGAGGCCATCCAAGAGATTCTCGTGCGCATGAAA
A/ETH/19/2019   TCCTTCAACTACGGAGCACTTCGCGCCGAGGCCATCCAAGAGATTCTCGTGCGCATGAAA
A/ETH/18/2019   TCCTTCAACTACGGAGCACTTCGCGCCGAGGCCATCCAAGAGATTCTCGTGCGCATGAAA
A/ETH/85/2018   TCCTTCAACTACGGAGCACTTCGCGCCGAGGCCATCCAAGAGATTCTCGTGCGCATGAAA
A/ETH/86/2018   TCCTTCAACTACGGAGCACTTCGCGCCGAGGCCATCCAAGAGATTCTCGTGCGCATGAAA
A/ETH/87/2018   TCCTTCAACTACGGAGCACTTCGCGCCGAGGCCATCCAAGAGATTCTCGTGCGCATGAAA
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A/ETH/16/2019   CGGGCTGAGCTCTACTGCCCTAGACCACTGCTGTCAGTAGAGGTGAGCTCGGCGGACAGG
A/ETH/19/2019   CGGGCTGAGCTCTACTGCCCTAGACCACTGCTGTCAGTAGAGGTGAGCTCGGCGGACAGG
A/ETH/18/2019   CGGGCTGAGCTCTACTGCCCTAGACCACTGCTGTCAGTAGAGGTGAGCTCGGCGGACAGG
A/ETH/85/2018   CGGGCTGAGCTCTACTGCCCCAGACCACTGCTATCAGTAGAGGTGAGCTCGGCGGACAGG
A/ETH/86/2018   CGGGCTGAGCTCTACTGCCCCAGACCACTGCTGTCAGTAGAGGTGAGCTCGGCGGACAGG
A/ETH/87/2018   CGGGCTGAGCTCTACTGCCCCCGACCACTGCTGTCAGTAGAGGTGAGCTCGGCGGACAGG
                ******************* ********** ***************************

A/ETH/16/2019   CACAAGCAGAAGATCATTGCGCCTGCAAAGCAGCTCCTT
A/ETH/19/2019   CACAAGCAGAAGATCATTGCGCCTGCAAAGCAGCTCCTT
A/ETH/18/2019   CACAAGCAGAAGATCATTGCGCCTGCAAAGCAGCTCCTT
A/ETH/85/2018   CACAAGCAGAAGATCATCGCGCCTGCAAAGCAGCTCCTT
A/ETH/86/2018   CACAAGCAGAAGATCATCGCGCCTGCAAAGCAGCTCCTT
A/ETH/87/2018   CACAAGCAGAAAATCATCGCGCCTGCAAAGCAGCTCCTT
                *********** ***** ********************
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APPENDICE 8: Nucleotide Sequence of VP1 Region of FMDV Isolates of the Current Study and reference countries Isolates from the Genebank.

O/ETH/72/2018

ACCACCTCCCCGGGCGAATCGGCTGACCCTGTGACTGCCACCGTTGAGAACT
ACGGCGGCGTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCA
TTCTCGACAGATTTGTGAAGGTAACACCACAACCCCAAACAAACGTGTTGGA
CCTGATGCAGACCCCCCCACACACGCTGGTCGGGGCACTCCTCCGCTCTGCTA
CCTACTACTTTGCAGACCTTGAAGTGGCAGTGAAGCACGAGGGGAACCTCAC

GTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAACACAACCAACCC
AACAGCCTACCACAAGGCACCACTTACTCGGCTTGCCCTACCATACACGGCA
CCACACCGCGTGCTCGCAACTGTCTACAACGGGAGTTGTAAGTACGGTGAGG
CTCGGGAGACCAACGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGC
CCGAACGCTGCCCACTTCTTTCAACTACGGTGCCATCAAAGCCACTCGGGTG
ACAGAATTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGCCCTCGCCCGC
TGTTGGCAGTCCACCCGACTGAAGCTAGACACAAGCAGAAGATAGTGGCACC
TGTGAAACAACTCCTG

O/ETH/73/2018

ACCACCTCCCCGGGCGAATCGGCTGACCCTGTGACTGCCACCGTTGAGAACT
ACGGCGGCGTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCA
TTCTCGACAGATTTGTGAAGGTAACACCACAACCCCAAACAAACGTGTTGGA
CCTGATGCAGACCCCCCCACACACGCTGGTCGGGGCACTCCTCCGCTCTGCTA
CCTACTACTTTGCAGACCTTGAAGTGGCAGTGAAGCACGAGGGGAACCTCAC
GTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAACACAACCAACCC
AACAGCCTACCACAAGGCACCACTTACTCGGCTTGCCCTACCATACACGGCA
CCACACCGCGTGCTCGCAACTGTCTACAACGGGAGTTGTAAGTACGGTGAGG
CTCGGGAGACCAACGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGC
CCGAACGCTGCCCACTTCTTTCAACTACGGTGCCATCAAAGCCACCCGGGTG
ACAGAATTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGCCCTCGCCCGC
TGTTGGCAGTCCACCCGACTGAAGCTAGACACAAGCAGAAGATAGTGGCACC
TGTGAAACAACTCCTG

O/ETH/14/2019

ACCACCTCCCCAGGTGAATCAGCCGACCCCGTGACCGCCACTGTTGAGAACT
ATGGTGGCGAGACACAGGTCCAGAGGCGTCAACACACCGACGTCTCGTTCAT
CCTCGACAGGTTTGTGAAGGTAACACCAAGAGAGGACCTAAATGTTTTGGAC
CTGATGCAGATTCCTGCCCACACACTGGTGGGGGCGCTCCTCCGTACTGCCAC

CTACTACTTCGCTGATCTGGAGGTGGCGGTCAAGCACGAAGGGAACCTCACA
TGGGTCCCGAACGGAGCGCCCGTGTCAGCACTGGAAAACACCACCAACCCAA
CAGCTTACCACAAAGCACCACTCACCCGTCTTGCTCTGCCCTACACAGCGCCC
CACCGCGTTTTGGCAACCGTTTACAACGGAAACTGCAAATACGGAGAGACAC
CAGTGGTCAATGCGAGGGGTGATCTTCAAGTGTTGGCCCAGAAGGCAGCTAG
GACGCTGCCCACCTCCTTCAACTACGGTGCCATCAAGGCTACCCGGGTGACT
GAGCTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGTCCCAGACCCCTCC
TAGCTATTCACCCAAGCGAGGCCAGACACAAACAGAAGATTGTGGCACCCGT
GAAACAGCTTTTG

Giza1/Egy/ 2017

ACCACCTCCCCGGGCGAATCGGCTGACCCCGTGACTGCCACCGTTGAGAACT
ACGGCGGCGTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCA
TTCTCGACAGATTTGTGAAGGTAACACCACAACCCCAAACAAACGTGTTGGA
CCTGATGCAGACCCCCCCACACACGCTGGTCGGGGCGCTCCTCCGCTCTGCTA
CCTACTACTTTGCAGACCTTGAAGTGGCAGTGAAGCACGAGGGGAACCTCAC
GTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAACACAACCAACCC
AACAGCCTACCACAAGGCACCACTCACTCGGCTTGCCCTGCCATACACGGCG
CCACACCGCGTGCTTGCAACTGTCTACAACGGGAGTTGCAAGTACGGTGAGG
CTCGGGAGACCAATGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGC
CCGAACACTGCCCACTTCTTTCAACTACGGTGCCATTAAAGCCACTCGGGTGA
CAGAATTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGCCCTCGCCCGCT
GTTAGCAGTCCACCCGACTGAAGCTAGACACAAGCAGAAGATAGTGGCACCT
GTGAAACAA

Alexandria 1/Egy/2016

ACCACCTCCCCGGGCGAATCGGCTGACCCCGTGACTGCCACCGTTGAGAACT
ACGGTGGCGTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCAT
TCTCGACAGATTTGTGAAGGTAACACCACAACCCCAAACAAACGTGTTGGAC

CTGATGCAGACCCCCCCACACACGCTGGTCGGGGCGCTCCTCCGCTCTGCTAC
CTACTACTTTGCAGACCTTGAAGTGGCAGTGAAGCACGAGGGGAACCTCACG
TGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAACACAACCAACCCA
ACAGCCTACCACAAGGCACCACTTACTCGGCTTGCCCTGCCATACACGGCGC
CACACCGCGTGCTTGCAACTGTCTACAACGGGAGTTGCAAGTACGGTGAGGC
TCGGGAGACCAATGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAGGGCAGCC
CGAACACTGCCCACTTCTTTCAACTACGGTGCCATTAAAGCCACTCGGGTGAC
AGAATTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGCCCTCGCCCGCTG
TTGGCAGTCCACCCGACTGAAGCTAGACACAAGCAGAAGATAGTGGCACCTG
TGAAACAA

Fayoum3/Egy/ 2017

ACCACCTCCCCGGGCGAATCGGCTGACCCCGTGACTGCCACCGTTGAGAACT
ACGGCGGCGTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCA
TTCTCGACAGATTTGTGAAGGTAACACCACAACCCCAAACAAACGTGTTGGA
CCTGATGCAGACCCCCCCACACACGCTGGTCGGGGCGCTCCTTCGCTCTGCTA
CCTACTACTTTGCAGACCTTGAAGTGGCAGTGAAGCACGAGGGGAACCTCAC
GTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAACACAACCAACCC
AACAGCCTACCACAAGGCACCACTTACTCGGCTTGCCCTGCCATACACGGCG
CCACACCGCGTGCTTGCAACTGTCTACAACGGGAGTTGCAAGTACGGTGAGG
CTCGGGAGACCAATGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGC
CCGAACACTGCCCACTTCTTTCAACTACGGTGCCATTAAAGCCACCCGGGTG
ACAGAATTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGCCCTCGCCCGC
TGTTAGCAGTCCACCCGACTGAAGCTAGACACAAGCAGAAGATAGTGGCACC
TGTGAAACAA

Behira2/Egy/ 2017

ACCACCTCCCCGGGCGAATCGGCTGACCCCGTGACTGCCACCGTTGAGAACT
ACGGCGGCGTGACACAGGACCAGAGACGCCAACACACGGATGTCGCGTTCA

TTCTCGACAGATTTGTGAAGGTAACACCACAACCCCAAACAAACGTGTTGGA
CCTGATGCAGACCCCCCCACACACGCTGGTCGGGGCGCTCCTCCGCTCTGCTA
CCTACTACTTTGCAGACCTTGAAGTGGCAGTGAAGCACGAGGGGAACCTCAC
GTGGGTCCCCAACGGGGCGCCAGAAACAGCTCTGGACAACACAACCAACCC
AACAGCCTACCACAAGGCACCACTCACTCGGCTTGCCCTGCCATACACGGCG
CCACACCGCGTGCTTGCAACTGTCTACAACGGGAGTTGCAAGTACGGTGAGG
CTCGGGAGACCAATGTGAGAGGTGACCTCCAAGTCTTGGCCCAGAAGGCAGC
CCGAACACTGCCCACTTCTTTCAACTACGGTGCCATTAAAGCCACTCGGGTGA
CAGAATTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGCCCTCGCCCGCT
GTTAGCAGTCCACCCGACTGAAGCTAGACACAAGCAGAAGATAGTGGCACCT
GTGAAACAA

KEN/100/2010 (K72/10)

ACCACCTCCCCAGGTGAATCAGCCGACCCCGTGACCGCCACTGTTGAGAACT
ACGGTGGCGAGACACAGGTCCAGAGGCGTCAACACACGGACGTCTCGTTCAT
CCTTGACAGATTTGTGAAAGTAACGCCAAGAGAAAACCCAATCAATGTCTTG
GACCTGATGCAGACTCCTGCCCACACACTGGTGGGGGCGCTCCTCCGCGCTG
CCACCTACTATTTCGCTGACTTAGAGGTGGCGGTCAAACACGAAGGGAACCT
CACGTGGGTCCCGAACGGAGCGCCCGAGTCAGCACTGGACAACACCACCAA
CCCAACGGCATACCACAAAGCACCACTTACCCGTCTTGCTCTGCCCTACACA
GCGCCCCACCGCGTGTTGGCAACCGTTTACAACGGGAACTGCAAGTACGGAG
ATGCACCAGTGGCTAACGTGAGGGGTGATCTCCAAGTGTTGGCCCAGAAGGC
AGCTAGGACGTTGCCTACCTCCTTCAACTACGGTGCTATCAAGGCCACCCGG
GTGACTGAGCTGCTCTACCGCATGAAGAGGGCCGAGACATACTGTCCCAGAC
CTCTTCTGGCTATTCACCCAAGCGAGGCCAGACACAAACAGAAGATTGTGGC
GCCTGTGAAACAACTCTTG

UGA/9/98

ACCACCTCCCCGGGTGAGTCAGCTGACCCCGTGACCGCCACTGTTGAGAACT
ACGGTGGCGAAACACAGGTCCAGAGGCGCCAACACACAGACGTCTCGTTCAT
TCTTGACAGATTTGTGAAAGTGACACCAAGAGAAAACCAAATTAATGTCTTG
GACTTGATGCAGACCCCTGCTCACACACTGGTGGGAGCGCTCCTCCGCACTG
CTACCTACTACTTCGCTGATTTAGAGGTAGCGGTCAAACATGAGGGGAACCT
CACGTGGGTCCCGAACGGGGCGCCGGAGTCAGCACTGGACAACACCACCAA
CCCAACAGCATACCACAAGGAACCACTCACCCGTCTTGCTTTGCCTTACACG
GCGCCGCACCGCGTGTTGGCAACCGTTTACAACGGGAACTGCAAATACGGCA
CTGCCCCACTGACCAACGTGAGGGGTGATCTTCAAGTATTGGCACAGAAGGC
AGCGAGGACGTTGCCTACCTCCTTCAATTACGGTGCCATCAAGGCCACCCGG
GTGACTGAGCTGCTCTACCGCATGAAGAGGGCTGAAACGTACTGTCCCAGAC
CCCTTCTGGCTGTTCAACCAACTGAGGCCAGACACAAGCAGAAAATTGTGGC
GCCTGTGAAACAACTGTTA

U97/99

ACCACCTCCCCGGGTGAGTCAGCTGACCCCGTGACCGCCACTGTTGAGAACT
ACGGTGGCGAAACACAGGTCCAGAGGCGCCAGCACACAGACGTCTCGTTCAT
TCTTGACAGATTTGTGAAAGTGACACCAAGAGAAAACCAAATTAATGTCTTG
GACTTGATGCAGACCCCTGCCCACACACTGGTGGGAGCGCTCCTCCGCACTG
CTACCTACTACTTCGCTGATTTAGAGGTAGCGGTCAAACATGAGGGGAACCT
CACGTGGGTCCCGAACGGGGCGCCGGAGTCAGCACTGGACAACACCACCAA
CCCGACAGCATATCACAAGGAACCACTCACCCGTCTTGCTCTGCCTTACACG
GCGCCGCACCGCGTGTTGGCAACCGTTTACAACGGGAACTGCAAATACGACA
CTGCCCCACTGACCAACGTGAGGGGTGATCTTCAAGTATTGGCACAGAAGGC
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GTGACTGAGCTGCTCTACCGCATGAAGAGGGCTGAAACGTACTGTCCCAGAC
CCCTTCTGGCTGTTCACCCAACTGAGGCCAGACACAAGCAGAAAATTGTGGC
GCCTGTGAAACAACTGTTA

UGA/17/98

ACCACCTCCCCGGGTGAGTCAGCTGACCCCGTGACCGCCACTGTTGAGAACT
ACGGTGGCGAAACACAGGTCCAGAGGCGCCAGCACACAGACGTCTCGTTCAT
TCTTGACAGATTTGTGAAAGTGGCACCAAGAGAAAACCAAATTAATGTCTTG
GACTTGATGCAGACCCCTGCCCACACACTGGTGGGAGCGCTCCTCCGCACTG
CTACCTACTACTTCGCTGATTTAGAGGTAGCGGTCAAACATGAGGGGAACCT
CACGTGGGTCCCGAACGGGGCGCCGGAGTCAGCACTGGACAACACCACCAA
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CTGCCCCACTGACCAACGTGAGGGGTGATCTTCAAGTATTGGCACAGAAGGC
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GTGACTGAGCTGCTCTACCGCATGAAGAGGGCTGAAACGTACTGTCCCAGAC
CCCTTCTGGCTGTTCACCCAACTGAGGCCAGACACAAGCAGAAAATTGTGGC
GCCTGTGAAACAACTGTTA

UGA/10/98

ACCACCTCCCCGGGTGAGTCAGCTGACCCCGTGACCGCCACTGTTGAGAACT
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TCTTGACAGATTTGTGAAAGTGGCACCAAGAGAAAACCAAATTAATGTCTTG
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CACGTGGGTCCCGAACGGGGCGCCGGAGTCAGCACTGGACAACACCACCAA
CCCGACAGCATATCACAAGGAACCACTCACCCGTCTTGCTCTGCCTTACACG
GCGCCGCACCGCGTGTTGGCAACCGTTTACAACGGGAACTGCAAATACGACA
CTGCCCCACTGACCAACGTGAGGGGTGATCTTCAAGTGTTGGCACAGAAGGC
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GTGACTGAGCTGCTCTACCGCATGAAGAGGGCTGAAACGTACTGTCCCAGAC
CCCTTCTGGCTGTTCACCCAACTGAGGCCAGACACAAGCAGAAAATTGTGGC
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ETH/59/2005

ACCACCTCTTCGGGTGAGTCAGCTGACCCCGTGACTGCCACTGTCGAGAACT
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GACCTGATGCAGACCCCTGCTCACACACTGGTGGGAGCGCTCCTTCGCACTG
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ACGTGGGTCCCGAACGGGGCACCTGAGTCAGCACTGGACAACACCACTAACC
CAACAGCATACCACAAGGAACCACTCACACGTCTTGCTCTGCCCTACACGGC
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GCCCCAGTGGCTAATGTGAGGGGCGACCTTCAAGTTTTGGCCCAGAAGGCAG
CAAGGACGCTGCCTACATCTTTCAACTACGGCGCCATTAAGGCCACCCGGGT
GATTGAGCTGCTCTACCGCATGAAGAGGGCTGAGACGTACTGTCCCAGGCCT
CTTCTGGCCGTCCACCCAAGCGAGGCCAGACACAAGCAGAAAATTGTGGCGC
CTGTGAAGCAACTTTTG

O/ETH/1/79

ACCACCTCCCTGGGCGAGTCGGCTGACCCCGTAACTGCCACCGTTGAGAACT
ACGGTGGCGAGACACAGGTCCAGAGGCGTCAACACACGGACGTCTCGTTCAT
TCTTGACAGATTTGTGAAGGTAACACCAAAAGACCAAATTAATGTGCTGGAC
TTGATGCAGACCCCTGCCCATACACTGGTTGGAGCGCTCCTCCGCACTGCCAC
TTACTACTTCGCTGATTTAGAAGTGGCGGTGAAGCACGAGGGGAACCTCACA
TGGGTCCCCAACGGAGCGCCCGAATCAGCTCTGGACAACACCACCAACCCAA
CAGCATACCACAAGGCACCACTCACCCGACTTGCCTTGCCGTACACAGCACC
ACACCGCGTGTTGGCAACTGTCTACAACGGGGACTGCAAGTATGGTGAGGCC
CCGGTGACTAATGTGAGGGGTGACCTCCAAGTGTTGGCCCAAAAGGCGGTGA
GGACGCTGCCTACCTCCTTCAACTACGGCGCCATCAAGGCCACTCGGGTGAC
TGAGCTGCTTTACCGCATGAAGAGGGCTGAAACATACTGCCCCCGGCCTCTG
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A/ETH/85/2018

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A/ETH/86/2018

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A/ETH/87/2018

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A/ETH/16/2019

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A/ETH/18/2019

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APPENDICE 9: Questionnaire Sheet Format

Part I. Interviewee general information

Explanation: Please check ✓ the appropriate box ☐ and fill-in the blanks.

1. Occupation of the interviewee:

☐ Farmer, ☐ Veterinarian, ☐ student, ☐ herdsmen

2. education status: ☐ No, ☐ primary school, ☐ Secondary school,☐ High school and ☐ Bachelor or higher

3. Number of animals in house hold:

Cattles…… Swine ……Goats……sheep….other…

4. Is there any commercial or semi-intensive farm around the your village/farm/herd?

☐ Yes, ☐ No, ☐I don' know

5. If yes, how far is the farm? ……, approximately……in m

Part II. Geographic location information

6. Please describe your geographic in your village?

lowland☐, highland ☐, flood☐, wetland, ☐

7. Had your village ever flooding at grazing or pasture areas last year?

☐ Yes, ☐ No, ☐I don' know

8. Is there a river or irrigation canal flow into village/farm/herd?

☐ Yes, ☐ No, ☐I don' know

9. Is there any livestock market near or in this village/farm/herd?

☐ Yes, ☐ No, ☐I don' know

Part III. Foot-and-mouth disease outbreaks information

10. Had the farm/ house hold ever experienced FMD outbreaks?

☐Yes ☐ No, , ☐I don' know If yes, cattle ☐ ,sheep,☐ goat, swine☐

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

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

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

☐ 1 outbreak, ☐ 2 outbreak, ☐ >2 outbreak.

13. How many animals died from the last outbreak on your farm/herd?

☐ ……..…Cattle, ☐ ……sheep, ☐ ……..…Swine, ☐…….Goats.

14. Did you inform officers if FMD outbreak occurred in your farm/herd?

☐ Yes, ☐ No

Part IV. Animal movement information

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

☐ Free movement, ☐ semi restricted, ☐ restricted

16. Is there animal movement from your farm (in or out) during FMD outbreaks?

☐ Yes, ☐ No

17. Is there contact or sharing grazing land of your animals with wild ungulates?

☐ Yes ☐ no, If yes which one ☐buffalo, ☐warthog, ☐ kudu, ☐ wild pigs

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

19. Does your animal graize on household-owned land without mixing with the livestock of other households? ☐ Yes, ☐ No

20. What was the main water source for the cattle on this farm?

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

21. Did your cattle share pastures with goats or sheep? ☐ Yes, ☐ No

22. Did the cattle share pastures with swine? ☐ Yes, ☐ No

23. Is there vehicle/utensils/workers (veterinarians, AH workers, feed delivery, milk collector, children) coming in or going out to your farm or herd?

☐ Yes, ☐ No

Part V. FMD control strategy applied in your area

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

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

If vaccination

A. When was the last vaccine administered to the cattle on your farm? ☐ a month ago, ☐ six month ago ,☐ one year ago

B. Did your herd ever experienced out break after the necessary vaccination?

☐ yes ☐ no; if yes, why do you think that happen; ☐ vaccines efficacy ☐ emerging of new virus type ☐ problem of vaccine production.