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CATTLE FOOT AND MOUTH DISEASE PREVALENCE AND ITS DETEMINANTS IN SELECTED DISTRICTS OF TIGRAY REGION, ETHIOPIA

MSc Thesis

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Bishoftu, Ethiopia

CATTLE FOOT AND MOUTH DISEASE PREVALENCE AND ITS DETEMINANTS IN SELECTED DISTRICTS OF TIGRAY REGION, ETHIOPIA

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

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As member of the examining board of the final MSc open defense, we certify that we have read and evaluated the Thesis entitled **"CATTLE FOOT AND MOUTH DISEASE PREVALENCE AND ITS DETEMINANTS IN SELECTED DISTRICTS OF TIGRAY REGION, ETHIOPIA",** prepared by Azeb G/tensay and recommend that it be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Tropical Veterinary Epidemiology.

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LIST OF ABBREVATIONS

AHAW	Animal Health and Animal Welfare
cDNA	Complementary Deoxyribonucleic Acid
CSA	Central Statistic Agency of Ethiopia
DACA	Drug Administration and Control Authority of Ethiopia
ELISA	Enzyme Linked Immunosorbant Assay
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GPS	Germinetrex Global Positioning System
HRP	Horseradish Peroxidase
HS	Herd size
ICTV	International Committee on Taxonomy of Viruses
IgG	Immunoglobulin G
MOARD	Ministry of Agriculture and Rural Development
NAHDIC	National Animal Health Diagnostic and Investigation Centre
NVI	National Veterinary Institute
OD	Optical Density
OIE	World Animal Health Organization

LIST OF ABBREVATIONS (Continued)

OPF	Oro-Pharyngeal Fluid
PCR	Polymerase Chain Reaction
PI	Percent Inhibition
RGD	Arginine-Glycine-Aspartic acid
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAT	South African Territories
TBoARDAR	Tigray Bureau of Agriculture and Rural Development Annual Report.
TBOFED	Tigray Bureau of Financial and Economic Development
TMB	Tetra-methylbenzidinel
UK	United Kingdom
USD	United State Dollar
VP	Viral Proteins
WRL	World Reference Laboratory

ABSTRACT

A cross-sectional serological study and questionnaire survey were conducted in two districts of Tigray Regional State with the objectives of determine the seroprevalence of FMD and assess potential risk factors associated with FMD occurrence in selected districts of Tigray region. The study was conducted from November 2014 to April 2015. A total of 400 cattle sera sample were collected and tested for antibodies against FMDV using 3ABC ELISA. Out of the total sera 39 (9.8%) were sero-positive and within districts Sheraro 30 (15%) and Seasie Tseada Emba 9(4.5%). Significantly higher prevalence was observed with different Peasant Association in that Adiaser 19.1% whereas lower prevalence was recorded in Saze 2.9%. There was a strong significance difference (p=0.000) in FMD prevalence between lowland 15% and highland 4.5%. Seroprevalence in different age groups showed significant variation 4.5% (8/177), 14.1% (28/198) and 12% (3/25) in age groups ≤ 4, 4-10 and >10 years (P<0.05). The seroprevalence in the different breeds was significantly higher (p= 0.002) in Begait 15% (30/200), compared to local and cross breeds 4.3% (6/139), 4.9% (3/61) respectively. The seroprevalence result between female and male were also recorded with values of 11.9% and 7.1% respectively, with no statistical difference between sexes (p= 0.108). In large herd size there was higher prevalence of FMD 22.7% than small herd size 6.2% with (p= 0.03). In addition, 90% of the respondents reported that there was no vaccination in the study area especially in Sheraro district. All respondents reported their cattle were used common watering point and grazing land. Farmers appears to have good knowledge of the clinical manifestation, seasonality and economic impact of FMD but not aware of the interspecies transmission of the disease between shoats (small ruminants), wildlife and due to contacts at watering and grazing points of risk factors. Further studies are also recommended to characterize the circulating FMDV serotypes in the areas and investigation in wildlife and small ruminant is needed to determine their roles in FMD virus maintenance and transmission.

Keywords: *Cattle; FMD; Prevalence; 3ABC Elisa; Risk factors; Tigray Region; Ethiopia*

1. INTRODUCTION

The total cattle population of Ethiopia is estimated to be about 53.99 million about 25.5 million sheep and the number of goats reported in the country is estimated to be about 24.06 million (CSA, 2013). The highlanders raise livestock and cultivate crops for their livelihood, whereas the lowlanders or pastoralists subsist mainly on livestock and livestock products. Consequently, the government gives due attention to the livestock sector, to take advantage of its contribution to economic growth and to meet the needs of an expanding population (MOARD, 2005). In Tigray region the population of livestock is 4,065,082 cattle, 1,381,622 sheep, 3,191,184 goats, 2,412 horses, 4,690 mules, 692,179 donkeys, 52,541 camels, 5,242,699 poultry and 229,626 beehives (CSA, 2013).

While the livestock sector makes a significant contribution to the national economy, a huge amount of work is required to maximize this contribution. It is accepted that livestock products such as meat, milk, honey, eggs, cheese and butter provide much needed animal protein to improve the nutrition of the people. Livestock also play an important role in providing export commodities, such as live animals, hides and skins, to earn foreign exchange. In addition, livestock provide the draught power to cultivate smallholdings and thresh crops. Livestock also confer security in times of crop failure, as a 'near-cash' capital stock. Moreover, livestock provide farmyard manure, commonly used for both soil fertility and as a source of energy (MOARD, 2005).

Even though, they provide such major benefits, livestock diseases are major constraints for the socio-economic development of resource-poor farmers in the country. Extension in livestock management and disease prevention measures, including zoonoses, is minimal and coverage of the total area by veterinary services is limited (Anonymous, 1998b).

In Ethiopia, many of the known infectious diseases of animals occur commonly and are poorly controlled. Foot and mouth disease (FMD) has a great impact on economic development, causing both direct and indirect losses. FMD is a highly contagious viral disease of cloven-hoofed domestic and wild animal species, characterized by fever, salivation and vesicular eruptions on the feet and mouth (Brooksby, 1982, OIE, 2008). Morbidity can rise to 100% in susceptible animal populations but mortality is low, particularly in adults. Infected animals show a spectrum of responses to FMD, ranging from inapparent infection to severe disease and death (OIE, 2008).

During the last thirty years foot and mouth disease (FMD) has occurred sporadically in the western part of North Africa but has remained endemic in the eastern part of the region (Samuel *et al.*, 1999). The disease is endemic to most countries in sub-Saharan Africa (Vosloo *et al*., 2002) and could not be eradicated from southern and East Africa where infected buffaloes are present. Lack of animal movement control within countries and across international borders for both wildlife and domestic animals aggravates the problem, and gives credence to the fact that FMD will remain a problem on the sub-continent for the foreseeable future (Brückner *et al*., 2002; Thomson *et al*., 2003).

Foot and mouth disease was first recorded in Ethiopia in 1957 when serotypes O and C were found. Five serotypes of the virus: O, A, C, South African Territories (SAT) 1 and SAT 2 were isolated at different times in Ethiopia. SAT 2 was identified for the first time in 1989 from a bovine samples collected from Leben ranch, Borena areas of southern Ethiopia and SAT 1 in 2007 from Mezan Teferi areas. However, SAT 3 has never been reported in Ethiopia (Ayelet *et al*., 2008).

Foot and mouth disease outbreak has been serious challenge every year in Ethiopia. In general the mean number of outbreaks, incidence rate and sero-prevalence of FMD showed that Tigray, central and southern part of Ethiopia are highly FMD affected areas. This disease causes significant financial losses to the nation in general and to the households in particular (Perry *et al*., 1999). This endemic disease is characterized by expanding boundaries and increasing total incidences (Asfaw and Sintaro, 2000).

In Ethiopia Serotypes A, O, C, SAT2, have been identified and characterized by the National Animal health research center at Sebeta and the world reference laboratory for FMD at UK in the years 1969-1994 on samples submitted by Sholla disease investigation laboratory, but from the record of outbreak investigation in cattle by NVI between 1982 - 2000, three serotype O, A and SAT2 FMD were identified. However, Serotype O is the most predominant strain circulating in the country followed by serotype A and SAT2. The presence of multiple serotypes of FMD and lack of cross protection among serotypes and subtypes warrants the need for development of a polyvalent vaccine containing strains that confer broader protection (Gelaye *et al*., 2005; Ayelet *et al*., 2009).

At present FMD is one of the major livestock diseases of socio economic importance in the country. Previously, Foot and Mouth Disease occurred frequently in pastoral herd in the lowland areas of Ethiopia (Haile-yesus, 1988) but in recent years the incidence of this disease has increased and become apparent in the highland areas where 80% of total livestock population is present (Mengistu, 1997). The high incidence of the disease may be associated with extensive movement of livestock and the high rate of contact between animals at marketing and common grazing place; as well as at watering point, (Mersie *et al*., 1992). The role of wild life in the epidemiology of FMD in Ethiopia has not been investigated, but it is accepted that the disease is maintained mostly in a domestic cycle (Sahle *et al*., 2004).

According to Afera *et al*. (2012), study done in selected districts of Tigray region, the overall seroprevalence was 39.2% and SAT2 was detected from outbreak investigated from Maykadra using PCR. In addition to this a study done in and Around Mekelle was reported by Kassaw *et al*. (2013), serotype O isolated from Tigray region O/ETH/59/2011 was also genetically most closely related (92.96-95.31%) with Sudanese Isolates. The work of these authors has focused on identifying the serotype responsible for an outbreak in very limited area in Tigray region particularly in and around Mekelle. Another study has revealed that 95% of the farmers complained that FMD is a major problem for their livestock production (Gezimu *et al*., 2014) revealing the endemicity of the disease in the region. These findings are however, reported in a very limited geographical area in the face of transboundary animal movement, trade, mixed livestock species farming, and wildlife contact in the vast areas of the region. Hence, an in-depth investigation on magnitude, risk factors and economic impact of FMD is lacking in vast areas of the region.

Therefore, the main objectives of this study were;

- To determine the seroprevalence of FMD in Sheraro and Seasie Tseada Emba districts of Tigray region
- To assess potential risk factors associated with FMD occurrence

2. LITERATURE REVIEW

2.1. Disease Definition

Foot and Mouth Disease (FMD) is an extremely contagious viral disease of all cloven hoofed animals/ungulates and pigs, characterized by fever, loss of appetite, salivation, vesicular eruptions in the mouth, on the feet and teats and sudden death of young stock (Thomson, 1994; Quinn *et al*., 2005). It is one of the most important economical notifiable diseases of livestock owing to the infectious and transboundary nature of the disease (OIE, 2004). Significant economic losses are produced by its high morbidity and the international livestock and livestock product export trade restrictions imposed on affected countries (Geering and Lubroth, 2002; Bronsvoort *et al*., 2004; FAO, 2007).

2.2. Etiology

2.2.1. Taxonomy

FMD virus was defined in 1963 by the International Committee on Taxonomy of Viruses (ICTV) as belonging to the genus Aphthovirus, family Picornaviridae. The name, picornaviride is derived from the Latin word ‘Pico’ meaning small and ‘rna’ meaning RNA, which refers to the size and genome type, of the virus while the genus name ‘Aphthovirus’ refers to the vesicular lesions produced in cloven-hoofed animals (OIE, 2004).

2.2.2. Physicochemical properties of the virus

Picorna viruses are small RNA viruses that are enclosed within a non-enveloped protein shell (capsid). The capsid consists of polypeptides, which are devoid of lipo-protein, and hence is stable to lipid solvents like ether and chloroform (Cooper *et al*., 1978). The virus is pH sensitive to both acidic and alkaline conditions. It is more stable between pH 7 and 9 at 4°C and -20°C but all strains are rapidly inactivated below pH 4 and above pH 11 (Domingo *et al*., 2002; Greering and Lubroth, 2002). In milk and milk products, the virion is protected, and can survive at 70°C for 15 seconds and pH 4.6. In meat the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes.

Two percent solutions of NaOH or KOH and 4% Na_2Co_3 are effective disinfectants for FMD contaminated objects, but the virus is resistant to alcohol, phenolic and quaternary ammonium disinfectants (Sahle, 2004). The sizes of droplet aerosol also play an important role in the survival or drying out of the virus; droplet aerosol size of 0.5 - 0.7 µm is optimal for longer survival of the virus in the air, while smaller aerosols dry out. In dry conditions the virus also survives longer in proteins e.g. in epithelial fragments (Donaldson, 1987).

2.2.3. Virus morphology

The FMD viruses are characterized by having a naked nucleocapsids and icosahedral symmetry of protein shell with 22-30 nm in diameter (Cooper *et al*., 1978; Robert and Bruce, 1981). The virion appears to be round particle with smooth surface (Clavijo and Kitching, 2003). The diameter of the virion capsid is ranging from 22-25 nm and the capsid is composed of the four major structural capsid proteins (VP1- 4) and contains 60 copies of each (Cooper *et al*., 1978). One copy of each capsid protein assembles to produce a protomer; in turn, five protomers form a pentamer, and 12 pentamers make the complete capsid (Domingo *et al*., 2002). Viral proteins (VP1-VP3) are exposed on the surface whilst the VP4 protein is located internally and interacts with the viral RNA (Strohmaier and Adam, 1982; Chow *et al*., 1987; Acharya *et al*., 1989).

2.2.4. FMD viral proteins

Structural proteins

The P1 gene product is the precursor of structural capsid proteins 1D, 1B, 1C and 1A and named viral proteins VP1, VP2, VP3, and VP4 (Ryan *et al*., 1989; Belsham, 1993). The intermediate P1 precursor is initially processed with the help of viral protease $3C^{pro}$ to produce the four major structural capsid proteins (VP1-VP4) and the mature virion is produced after the encapsidation of the virion RNA (Cooper *et al*., 1978). The VP1 is an important protein for epidemiological studies of FMD viruses because it is the most antigenic protein involved in cell attachment and carries an immunologically important G-H loop. VP3 is the most conserved surface exposed structural protein among different FMD viruses (Robert and Bruce, 1981; Acharya *et al*., 1990; Barteling, 2002). FMD viruses have a high concentration of histidine residues lining the pentamer interfaces that also play a major role in virus instability at acidic pH (Stanway, 1990). Heat or acid treatment of the virus disrupts the interactions between VP2 and VP3 and the pentameric interfaces resulting in pentamer dissociation with release of the internal capsid protein VP4 and the RNA genome (Strohmaier and Adam, 1982; Chow *et al*., 1987; Acharya *et al*., 1989).

Importance of VP1 Protein in the Antigenicity of FMD virus

The antigenic diversity of FMD viruses has made the diagnosis and control of these viruses very difficult in countries where the disease is endemic. Several studies have shown that VP1 (encoded by 1D) is the most important protein in FMD virus both for its antigenic properties and as the virus-cell attachment site. Two regions of VP1 have been shown to induce antibodies involved in neutralization of viral infectivity (Bittle *et al*., 1982; Pfaff *et al*., 1982; Strohmaier and Adam, 1982). The main immunogenic site exposed on the surface of the virus particle is located at a highly disordered region (G-H loop) of the capsid while the Cterminus antigenic residues are highly ordered (Strohmaier and Adam, 1982; Acharya *et al*., 1989). Since VP1 is the only protein cleaved by trypsin it is believed to be responsible for both immunological and infectivity functions (Bittle *et al*., 1982). Several studies have shown that mutations at the critical amino acid residues in 1D (VP1) can result in antigenic variations in FMD viruses. Four non-overlapping neutralization sites and a fifth conformational site in FMD virus were identified (Crowther *et al*., 1993; Aktas and Samuel, 2000).

Importance of VP1 protein in virus-cell attachment

Several studies have reported that the Arginine-Glycine-Aspartic acid (RGD) sequence within the G-H-loop of the VP1 is involved in attachment of the virus to susceptible cell receptors (Lebermann *et al*., 1991; Leippert *et al*, 1997). The cleavage of VP1 by trypsin highly reduces or abolishes the ability of the FMD virus to bind and infect susceptible cell cultures (Baxt *et al*., 1989).

2.2.5. Antigenic variation

One of the consequences of genetic variation through mutation, selection, and recombination is that new antigenic variants are constantly being generated. Not only is there no cross protection between FMDV serotypes, but vaccination with one antigenic variant of serotype does not necessarily protect an animal when challenged with a different virus of the same serotype (Sangare, 2002). Attempts to characterize the extent of the antigenic variation within the FMD serotype lead to the establishment of the techniques where by viral subtype could be identified. Initially over 60 different subtypes were identified by world Reference laboratory (WRL), but it quickly became apparent that there is a continues spectrum of intratypic antigenic variants, making a difficulty to identify specific subtypes (Asseged, 2005). Changes to the genes encoding capsid proteins can result in antigenic variation and evolvement of new subtypes (Haydon *et al*., 2001). This may give rise to immunological distinct variants that can re-infect individuals that have been previously infected by related viruses. The degree of cross protection among different subtypes of the same serotype thus varies. Since there is continual antigenic drift in enzootic situation this is an important factor to consider when selecting vaccine strains (Grubman and Mason, 2002).

2.2.6. Serotypes and sub types

Currently there are seven serotypes of foot and mouth disease virus (FMDV), namely O, A, C, Southern African Territories (SAT) 1, 2 and 3, and Asia 1, which infect cloven-hoofed animals. Within these serotypes, over 60 subtypes have also been described using biochemical and immunological tests; and new subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used (OIE, 2004). At present, a sequencing of FMD virus is increasingly being used to establish intratypic variations of FMD viruses and classifying viruses in to genotypes and lineages (Sahle, 2004).

2.3. Epidemiology

2.3.1. Global distribution of the disease

Europe has experienced a number of sporadic outbreaks since the cessation of vaccination on the continent during 1990 - 1991 (Samuel and Knowles, 2001; Rweyemamu and Astudillo, 2002). Currently almost all European countries are recognized by the World Animal Health Organization (OIE) as free of FMD without vaccination (FAO, 2006). FMD is endemic in most of Southern Asia, Africa and parts of South America. Most of Europe, North and Central America, Australia, New Zealand and Japan are free (OIE, 2012).

Now a day as the result of globalization, FMD epidemics can change from local and regional spread to wide international spread (Knowles *et al.*, 2005; Cottam *et al.*, 2006). It should be noted that with globalization of trade even areas where FMD is endemic can suffer from introduction of virus strains that are exotic to the region (Brückner *et al.*, 2002). The risk of FMD entry into free areas is low through legal trade of animal and animal products from zones or countries officially recognized as FMD free by the OIE. However, smuggling of animal products is a significant issue and the probable main route of virus introduction into FMD free areas (WRL, 2007).

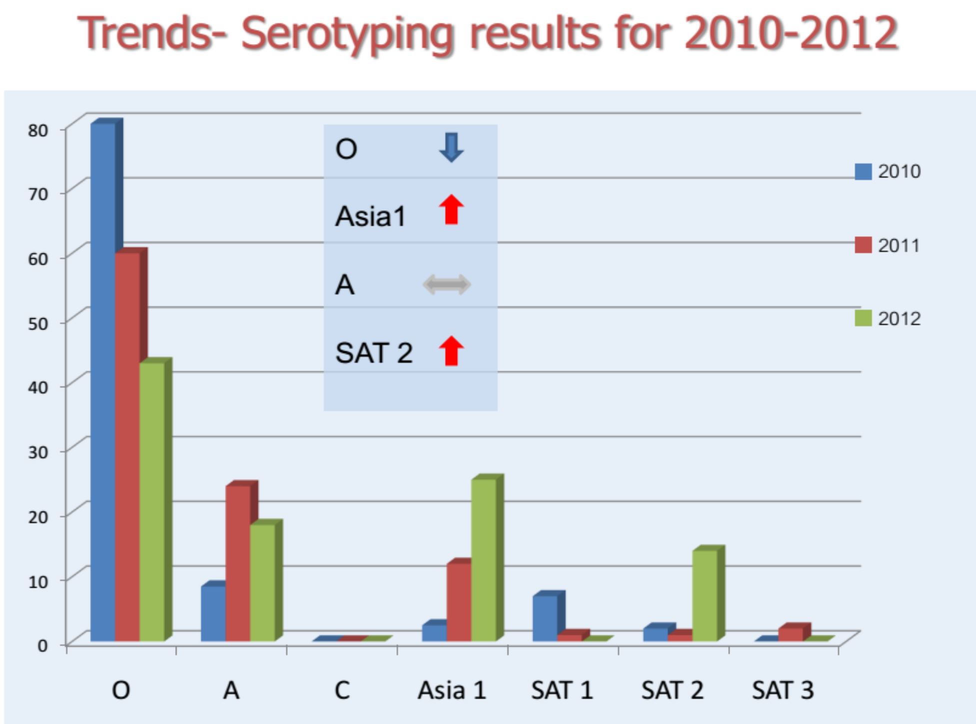

Figure 1: Trends serotyping results for 2010-2012

Source: (OIE, 2012)

2.3.2. The disease situation in sub-Saharan and FMD Serotypes in Africa

The epidemiology of foot and mouth disease (FMD) in sub-Saharan Africa is probably more complicated than in any other regions of the world. Not only six of the seven serotypes prevalent in Africa (only Asia1 has never been recorded), but marked regional differences in the distribution and prevalence of serotypes and intratypic variants occur. Furthermore, wildlife plays a unique and important role in the epidemiology of the disease in Africa although this aspect has been adequately investigated only in Southern Africa (Ferris and Donaldson, 1992; Bastos, 1998; Hargreaves *et al*., 2004; FAO, 2006).

Regardless of the disease endemicity in nearly all countries of sub-Saharan Africa, the majority of outbreaks remains unrecorded and is not notified in a timely fashion due to trade restrictions and pastoral systems where disease surveillance is inadequate and sometimes absent; the transport of sampling material is difficult and expensive, few

African laboratories are able to confirm the diagnosis of FMD (FAO, 2006; Rweyemamu *et al*., 2008).

East African countries are not only having large livestock populations but also the highest concentration of wildlife in the world. Farming is dominated by agro-pastoral and pastoral communities and it is characterized by communal grazing and migrations. This cluster probably contains several major FMD endemic foci and the most complicated FMD situation in the world. There are also wide genetic variations in the virus strains and the role of African buffalo in the maintenance and transmission of FMD serotypes and the role of other potential wildlife reservoirs of the disease that occur in this epidemiological unit have not been systematically studied (Sahle, 2004; Bogale, 2005; FAO, 2006; Rweyemamu *et al*., 2008).

The use of vaccines is sub-optimal in relation to the size of population and most of the FMD susceptible animal populations are found at risk. Countries in southern Africa, contrary to the general trend in Africa, have been largely successful in controlling FMD to ensure access to international markets (Sutmoller *et al*., 2000; Vosloo and Thomson, 2004; Hargreaves *et al*., 2004).

FMD is endemic in sub-Saharan African countries, except for Madagascar. Six serotypes, namely O, A, C, SAT-1, SAT-2 and SAT-3, are endemic in most sub-Saharan African countries with marked differences in the distribution and prevalence of serotypes (Kitching, 1998; Vosloo *et al*., 2002). Serotype A and O are widespread throughout Sub-Saharan Africa, whilst type C appears to have disappeared from the world as a whole (Kitching, 2002a). The three SAT types are also prevalent in southern and eastern Africa, SAT1, and SAT2 circulate in West Africa and are the only serotypes to have made incursions into the Middle East with SAT-3 demonstrating the most restricted (Vosloo *et al*., 2002).

2.3.3. Susceptible hosts

FMD is highly contagious and affects over 70 domestic and wild life species of animals; however, not all FMD viruses have the same host range (Sáiz *et al.*, 2002). As a whole the sensitive species belong to the mammalian order of Artiodactyls. Of the domesticated species, cattle, pigs, sheep, goats and buffalo are susceptible to FMD. Similarly, many species of wild life, such as deer, antelope, wild pigs, Warthogs, elephants, giraffes, camels and llamas, hedgehogs and tapirs may become infected with FMD viruses. Except African buffalo, the importance of other wild life involvement in the epidemiology of FMD is not well studied (Moutou, 2002; OIE, 2004). Human infections have been reported but are extremely rare and mild (Geering and Lubroth, 2002).

The disease is considerably less obvious or sub-clinical in breeds of cattle, sheep and goats indigenous to Africa and Asia, where FMD is endemic; and these animals are believed to have been the source of infection for countries previously considered disease free (Kitching, 2002a; Kitching and Hughes, 2002; Kitching and Alexanderson, 2002).

2.3.4. The role of carrier animals

Carrier, in FMD, is defined as an animal from which FMD virus can be isolated from the oesophageal pharyngeal (OP) area, more than 28 days after infection. Although it is well established that FMD virus persists in buffalo (up to 5 years), cattle (up to 3 years), Sheep (up to 9 months), and goats (between 3-6 month), the mechanisms underlying persistence and the immunological pathway that eventually leads to viral clearance are not well understood (Bastos *et al*.,2000). This may provide a mechanism for the maintenance of the virus in nature and the cause of acute episodes of disease and may contribute to the emergence of new antigenically variant viruses (Domingo *et al*., 1992; Kitching, 1998; Sahle, 2004).

2.3.5. The role of wild life

FMD has been reported in several species of wild life, such as African buffalo (Syncerus caffer), Impala (Aepyceros melampus), Kudu (Tragelaphus strepsiceros) species, Warthog (Phacochoerus aethiopicus), and elephants that has a role in epidemiology of the disease. Buffalo are believed to be the ultimate source of infection for livestock in southern Africa due to their ability to both maintain and transmit the disease. FMDV can persist in an isolated herd of buffalo for up to 24 years, whilst an individual animal can maintain the infection for up to five years. Further, more, buffalo have unequivocally been shown to be a source of infection for cattle under both natural and experimental conditions (Sangare, 2002).

The mechanism facilitating SAT-type virus transmission of virus from buffalo appears to occur readily when; there is close contact between the two species during acute stage of infection and shedding large amounts of virus. Experimental infection of warthog (Phacochoerus aethiopicus) with SAT2 type virus resulted in severe clinical signs of infection, and transmission to in-contact animals. However, these animals do not excrete virus to the level of domestic pigs and are not believed to play an important role in the epidemiology of FMD in Africa (Vosloo *et al*., 2002; Thomson, 1994).

2.3.6. Mode of transmission

FMD virus can replicate and be excreted from respiratory tract of animals leading to airborne excretion of virus during the acute phase of infection, although, FMD virus may occur in all the secretions and excretions of acutely infected animals, including the expired air. Therefore, after an animal becomes infected by any means, the primary mode of spread is via respiratory aerosols from infected animals (requires proper humidity and temperature). When proper humidity and temperature are maintained, FMD virus can be carried up to 250 km across the sea and up to 60 km across the land. The prior condition has been held responsible for the FMD outbreak that occurred in France and then spread to UK in1981 (Kitching, 1992) emphasizing the possible windborne spread of the virus under prevailing environmental conditions. At present, there are Computer models that can predict the most likely wind-borne spread of the virus from infected herds and allow the examination of a variety of control strategies (Sanson *et al*., 1991; Sahle, 2004). Other important means of spread are by direct contact between infected and susceptible animals and indirectly by exposure of susceptible animals to the excretion and secretion of acutely infected animals. A person in contact with infected animals can have sufficient FMD virus in his or her respiratory tract for 24 hours to serve as a source of infection for susceptible animals (Asseged, 2005).

2.4. Pathogenesis

The main route of infection in ruminants is through the inhalation of droplets, but ingestion of infected feed, inoculation with contaminated vaccines, insemination with contaminated semen, and contact with contaminating clothing, veterinary instruments, and so on can all produce infection. In animals infected via the respiratory tract, initial viral replication occurs in the pre pharyngeal area and the lungs followed by viremic spread to other tissues and organs before the onset of clinical disease. FMD virus is then distributed throughout the body, to reach best sites of multiplication sites such as the epithelium of Oro-phrynx, oral cavity, Feet, the udder and heart. Virus probably replicate in the mammary gland of susceptible cow, in the pituitary gland. Viral excretion commences about 24 hours prior to the onset of clinical disease and continues for several days FMD virus. The acute phase of the disease lasts about one week and viremia usually declines gradually coinciding with the appearance of strong humeral responses (Murphy *et al*., 1999). Recovered cattle produce neutralizing antibodies and can resist re-infection by the same subtype of virus for up to one year. It was suggested that heat intolerance was a sequel to FMD and was caused by damage to the endocrine system by (Radostits *et al.,* 1994).

2.5. Clinical signs

When susceptible animals are in contact with clinically infected animals, clinical signs usually develop in 3 to 5 days (Kitching, 2002a), although in natural infection, the incubation period may range from 2-14 days. The severity of clinical signs of the disease varies with the strain of the virus, the exposure dose, the age, and breed of the animal, the host species, and its degree of immunity. The signs can range from a mild or in apparent in sheep and goats to a severe disease occurring in cattle and pigs (OIE, 2004).

In cattle, the initial signs are fever of 103-105^{o}F (39.4-40.6^{o}C), dullness, anorexia, and fall in milk production. These signs are followed by excessive salivation, smacking of the lips, grading of the teeth, drooling, serous nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the tongue, dental pad, gums, soft palate, nostrils, muzzle, interdigital space, coronary band, and teats (Sahle, 2004; Woodbury, 1995). After vesicle formation, drooling may be more marked, and nasal discharge, lameness, or both may increase. Pregnant cows may abort, and young calves may die suddenly without developing any vesicle because of inflammation of the heart (Myocarditis) (Blood *et al*., 1994). Morbidity can approach 100%, but Mortality in adult animals is rare, although in young animals death can occur due to myocarditis and mortality can exceed 50% (Woodbury, 1995). Pregnant cows may abort (Blood *et al*., 1994). The course of an FMD infection is 2 to 3 weeks although infection may delay recovery of mouth, feet and teat lesions, resulting in hoof deformation, mastitis, low milk production, failure to gain weight, and breeding problems. A lactating animal may not recover to pre infection production because of damage to the secretory tissue. A chronic Panting syndrome characterized by dyspnoea, anaemia, hair overgrowth and heat intolerance has been reported as a sequel of cattle recovered from FMD associated with pituitary gland damage (Burrow *et al*., 1981).

In sheep and goats, if the clinical signs occur, it tends to be very mild, and may include dullness, fever; and small vesicles or erosions on the dental pad, lips, gums, and tongue. Mild lameness may be the only sign. In lame animals, there may be vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort and nursing lambs may die without showing any clinical sign (Hughes *et al*., 2002).

2.6. Diagnosis

2.6.1. Field Diagnosis

In cattle, FMD should be considered whenever salivation and lameness occur simultaneously and when a vesicular lesion is seen or suspected. Fever often precedes other clinical signs; therefore, febrile animals should be carefully examined. Early diagnostic lesions may be found before animals start to salivate, have a nasal discharge, or become lame. Clinical diagnosis can present many difficulties due to viral infections of the mucous membrane, which produce similar clinical signs. Differential diagnosis for FMD should include vesicular stomatitis, rinderpest, malignant catharal fever, the bovine herpes 1 infections, swine vesicular disease, vesicular exanthema of swine and bluetongue (Blood *et al*., 1994).

2.6.2. Laboratory Diagnosis

Due to the highly contagious nature and economic importance of FMD, the laboratory diagnosis and serotype identification of the virus should be done in a virus-secure laboratory (OIE, 2004).

Specimens

Appropriate samples for FMD laboratory diagnosis are; Vesicular fluid usually contains the highest quantity of virus. Epitheliums from early vesicles and from recently ruptured vesicles are tissue of choice for virus isolation (OIE, 2004). When epithelium tissue is not available from ruminant animals e.g. in advance or convalescent cases and infection is suspected in the absence of clinical sign, samples of oesophageal-pharyngeal fluids(OP) is collected by means of a probang and used for virus isolation (Asseged, 2005). Other samples such as, blood with anticoagulant, Serum, and lymph nodes, thyroid gland, adrenal gland, kidney, and heart are good sources of specimens from postmortem.

Antibody detection by liquid phase blocking ELISA

The liquid phase blocking ELISA detects and quantifies FMDV antibodies in serum of both infected and vaccinated animals (Hamblin *et al*., 1986a). The test is based upon specific blocking of the FMDV sample. Rabbit antigen-specific antisera for the different serotypes of FMDV are passively adsorbed to polystyrene micro wells. Serial dilution of test serum is allowed to mix with the specific FMDV antigen; the test serum-antigen mixture is then transferred to an ELISA plate coated with FMDV trapping antiserum (rabbit FMD antisera). The presence of antibodies to FMDV in the serum sample will result in the formation of immune complex and consequently reduce the amount of free antigen trapped by the immobilized rabbit antiserum. In turn, fewer guinea pigs anti FMDV detecting antibodies will react in the next incubation step after the addition of enzyme labeled (HRP) anti-guinea pig Ig conjugate. Following incubation, the substrate/chromogen solution, containing H_2O_2 is added to each well, before being stopped after 15 minutes by addition of sulfuric acid. A change in colour development is read with spectrophotometer at 492 nm filters, in comparison to antigen Control (Ca), containing free antigen only. The diagnostic threshold for this assay is set at 50% inhibition (50PI). If either or both replicate PI values of test serum fall above 50 PI, then that test serum fall above 50 PI, and then that test serum is tentatively considered to be positive. If both replicate PI value of a test serum fall below 50 PI then the test serum is considered as negative (Ferris, 2004).

Antibody detection by 3 ABC ELISA

The detection of antibody to the polyprotein 3ABC proteins is useful indicator of FMD virus infection with any of the seven serotypes of the virus (Mackay *et al*., 1998). Antibody to the 3ABC is only found in virus-infected animals but not in vaccinated animals (Diego, 1997). Briefly, the test is carried out as follows: Microtiter plates are supplied pre-coated with recombinant FMDV 3ABC viral antigen; dilutions of the samples to be tested are incubated in the well of these plates. Any antibody specific for 3ABC binds to the antigen in the wells and forms antigen-antibody complex on the plate well surface. Unbound material is removed from the wells by washing. Peroxidase labeled anti-IgG conjugate is added, which binds to the antibodies of the sample complex with the 3ABC antigen. Unbound conjugate is removed by washing, and the Tetra-methylbenzidinel (TMB) containing substrate is added to the wells. The degree of colour, which develops (optical density measured at 450nm), is directly proportional to the amount of antibody specific to 3ABC present in the sample. The diagnostic relevance of the result is obtained by comparing the optical density (OD), which develops in wells containing the samples with the OD from the wells containing the positive control (Diego, 1997).

Compared to the liquid phase blocking ELISA, 3 ABC ELISA allows differentiation between samples from infected (3ABC positive) and vaccinated (3ABC negative) animals (Hamblin *et al*., 1986a,). The 3ABC ELISA is also rapid test for screening of large number of sera. In areas where more than one serotypes exist, the test is also cheaper compared to the conventional liquid phase blocking ELISA, which has the disadvantage that each serum sample must be tested against all existing serotypes (Sangare, 2002).

Nucleic acid recognition methods

The polymerase chain reaction (PCR) can be used to amplify the genome fragments of FMD virus in diagnostic material. Specific primers have been designed to distinguish between each of the seven serotypes and in-situ hybridization techniques have been developed for investigating the presence of FMD virus RNA in tissue samples (Woodbury, 1995).

Unlike many living organisms where the hereditary information is enclosed within a DNA genome, FMD virus has an RNA genome that can be sequenced directly, but RNA is unstable and is usually first transcribed into cDNA prior to performing the nucleotide sequence. Reverse transcriptase (RT) when combined with PCR provides a rapid and powerful technique for studying diverse RNA genomes (Vosloo *et al*., 2002).

The molecular epidemiology of FMD is based on the comparison of genetic differences between virus isolates, and showing the genomic relationship between vaccine and field strains for all seven serotypes based on sequences derived from the 1D gene. Sequence differences of 30-55% of the VP1 gene are obtained among seven seroptypes while different subgroups (genotypes, topotypes) are defined by differences of 15-20% (Knowles and Samuel, 2003). Reveres-transcription PCR (RT-PCR) amplification of FMD virus RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform these comparisons (OIE, 2004).

2.7. Economic Importance

FMD is probably one of the most important livestock diseases in the world in terms of economic impact. The economic importance of the disease is not only due to the ability of the disease to cause losses of production, but also related to the reaction of veterinary services to the presence of the disease and to the restrictions on the trade of animals both locally and internationally (James and Rushton, 2002). FMD, therefore, threatens the livelihoods of simple farmers, large sophisticated farming practices and the national and the international economies of the countries (Asseged, 2005).

The direct production effects in extensive production system include loss of milk due to udder involvement and reduced draught animal power from lesions on the feet. FMD also causes lower rates of live-weight gain in growing animals due to reduced feed intake, and reduction in reproductive capacity by increased abortion rates of up to 10% in animals infected during pregnancy; the disease also causes up to 6% mortality in calves. Restrictions on animal movement and international trade can cause much more serious losses (James and Rushton, 2002). The loss in animal production and international trade restriction imposed following an outbreak makes FMD of a major concern for livestock owners. The control of outbreak (slaughter of infected and in contact, disposal of carcass in disease free zones) and the loss due to the ban on livestock exports costs several million USD (United State Dollar) for a single outbreak (Sellers and Daggupaty, 1990).

2.8. Control

Routine vaccination is used where the disease is endemic; in contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use strict movement controls and slaughter of infected and contract animals when outbreaks occur (OIE, 2004).

2.8.1. Endemic Areas

The disease is generally controlled by vaccination and movement restriction of animals. Although the upper respiratory tract is a favored site for infection and replication, FMD vaccines are able to protect animals against the development of clinical disease when given by the parenteral route. One mechanism for this is the transudation of serum antibodies into the mucosae thereby preventing virus attachment to susceptible cells, aggregating and distorting virus particles and facilitating opsonisation, in which virus-antibody complexes are removed from the blood by scavenging phagocytes (Asseged, 2005). Vaccination against FMD virus is achieved with inactivated vaccines that should induce protective immunity against each type of antigens incorporated in the vaccine

(Asseged, 2005). Therefore, when vaccinating animals, it is important that the vaccine contain the same subtype of virus as is in the area. This necessitates frequent checking of the serotype and subtype during an outbreak because FMD virus frequently changes during natural passage through various species. Protection induced by aqueous aluminum hydroxide vaccine can protect for 4-6 months while a double emulsion oil vaccine can protect for up to 1 year (Gonzalez *et al*., 1992).

2.8.2. Disease Free Areas

Stamping out

In the first instance, 'stamping out' policy, consisting of the slaughter of all affected and in-contact susceptible animals would be instigated, together with associated zoosanitory measures including, the imposition of movements restriction, to control the outbreaks. Such measures might also extend to preemptively slaughtering other herds in which there is no clinical evidence of the disease, but which have been epidemiologically linked with an outbreak, and may therefore contain infected animals. The stamping out is done with full compensation paid for animals slaughtered. The success of stamping out is recognized by the OIE in its guidelines on re- establishing trade following an outbreak (Asseged, 2005).

Emergency vaccination

Emergency vaccination, within an infected area, has gained more preference in recent years, in an attempt to reduce the amount of virus circulating and spreading beyond the restricted area. This so-called 'suppression' or 'dampening down' vaccination regime is now on the agenda of a European Commission working group setup to assist the Animal Health and Animal Welfare (AHAW) committee in establishing criteria for the eradication of certain infectious diseases including FMD. The use of emergency FMD vaccines has two clear objectives: Firstly, to provide protective immunity, as rapidly as, possible to susceptible stock, and secondly, to reduce the amount of virus released and thereby limit the spread of disease (OIE, 2004; Asseged, 2005).

Protective vaccination

The protective vaccination is used effectively in animals not already exposed to FMD virus. It would therefore be employed out side the 3 km protection zone and outside any predicted aerosol spread of virus from the infected premise. All vaccinates would be naive to FMD antigen, and would require a minimum of 3-4 days to develop protective immunity. This protective vaccination would thus form a ring around the infected area, preventing diseases spread, and allows the outbreak to expire within the protection zone, where infected herds would quickly be identified and slaughtered (Asseged, 2005).

2.9. Status of the Disease in Ethiopia

2.9.1. Disease Status

In Ethiopia, foot and mouth disease is endemic and a notifiable disease; the national animal and plant health regulatory directorate sends monthly and annually official reports to OIE (Leforban, 2005; MoARD, 2009). The disease is widely prevalent and previously used to occur frequently in the pastoral herds of the marginal lowland areas of the country. However, this trend has been changed and currently the disease is also frequently noted in the highlands of the country (Sahle, 2004).

2.9.2. Temporal Distribution

According to MoARD, division of animal and plant health regulatory directorate disease outbreaks report summary, FMD occurs at any time of the year however, the highest outbreaks of the disease are observed during the heavy rainy and extreme dry seasons of the years. Various researchers reported that this might be associated with factors such as drought. During dry seasons especially pastoralists are obliged to move their herds long distances in search of pasture and water and thereby transmission of highly contagious diseases like FMD exacerbated at herd gathering sites or communal points (Rufael, 2006; Legesse, 2008; Molla, 2009; Bayissa, 2009). The lower prevalence occurs on April and the higher prevalence was recorded in July (Alemayehu *et al.,* 2014).

2.9.3. Spatial Distribution

FMD is widely distributed in all areas of Ethiopia, although the level of the disease prevalence may show significant variations across the different farming systems and agro-ecological zones of the country. The disease is more prevalent in lowlands and those of pastoralist and agro-pastoralist (Megersa *et al.,* 2009). Animals from low altitude have higher seroprevalence than those from mid altitude and high altitude areas. This is due to scarcity of feed and water resources in the lowland leads increase animal movement and aggregations at water points (Yahya *et al.*, 2013).

2.9.4. Disease magnitude/prevalence

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

The overall sero-positivity of 66.7% in cattle, 9.3% in sheep and 7.2% in goats (Legesse, 2008) and 44.20% in cattle (Nigussie, 2009); 8.01% in cattle (Abunna *et al*., 2013); 21.4% in cattle (Desissa *et al.*, 2014); 11.6% in cattle (Yahya *et al.*, 2013); 21.59% in cattle (Duguma *et al*., 2013); 14.5% in cattle (Alemayehu *et al.,* 2014) were reported after laboratory diagnosis from blood samples collected in different study areas of Ethiopia.

2.9.5. FMD virus serotypes identified

Research findings and records from National Animal Health Diagnostic and Investigation Centre (NAHDIC) and National Veterinary Institute (NVI) of Ethiopia indicate that five of the seven FMDV serotypes (O, A, C, Southern African Territories SAT-1, and SAT-2) were identified in Ethiopia and the isolated serotypes were responsible for FMD outbreaks during 1974-2007. In terms of species, these serotypes were identified from bovine, swine, ovine, and caprine samples collected from the outbreak areas. Cattle were found to be infected with all circulating serotypes of FMDV, where as swine had only serotype O (Sahle, 2004; Gelaye *et al*., 2005; Legesse, 2008; Nigussie, 2009; Ayelet *et al*., 2009).

FMDV serotypes O and C were first recorded in Ethiopia in 1957 while serotype A was identified in 1969 (Martel, 1974; 1975). Serotype C was not identified after 1983. It seems to have disappeared from Ethiopia. However, a recent report of serotype C specific antibodies in cattle in Ethiopia indicates that circulation of serotype C viruses in the country may have gone unnoticed (Rufael *et al*., 2008).

The first isolation of SAT-2 was in 1989 in a sample collected from cattle raised on Leben Ranch, Borena Zone, in southern Ethiopia (Roeder *et al*., 1994). After an apparent gap of 16 years, serotype SAT-2 was recorded in 2007 from a bovine sample collected from Bambas, Benshangul-Gumuz, western Ethiopia bordering Sudan (Ayelet *et al*., 2009). SAT-2 may have been introduced by animal movement across the border with Sudan because SAT-2 is endemic in Sudan (OIE, 2001; Vosloo *et al*., 2002). The distribution of serotypes in different study area shown that, the most prevalent serotypes were serotype O followed by serotype A (Ayelet *et al*., 2013).

The impact of the disease in affecting the national export trade has been witnessed by import bans imposed by different countries at different times. Following the 2001 outbreak of FMD in the United Kingdom, Saudi Arabia and Indonesia had imposed trade bans of our export meat and pickled sheep and goat skin, respectively. Therefore, FMD causes substantial economic loss to farmers and to the nation from embargoes of livestock and livestock product trade (Megersa *et al*., 2009).The 2006 FMD outbreak detected in Egypt also became the major cause of live cattle import ban to the Egyptian markets (MoARD, 2006).

2.9.6. FMD control program in Ethiopia

At present, FMD is considered as one of the most important livestock diseases demanding urgent control intervention that should result in minimizing the impact of FMD to the level that won't be a major cause of international trade barrier. Conversely, the complex nature of the disease, its wider distribution across the country and absence of proper kind and amount of FMD vaccine within Ethiopia demanded that control strategies be implemented progressively on a short and medium to long-term basis. Measures such as disease free zone establishment and mass vaccination of the national cattle herds may have important contributions to minimize the impact of the disease. However, these measures will require huge financial and logistic resources that their consideration should be viewed from long term perspective (MoARD, 2006).

Therefore, the short term FMD vaccination program give emphasis to the control of all outbreaks occurring in the country through ring vaccination and vaccination of all export cattle before entering the quarantine stations. To protect export animals from contracting the disease while being kept in quarantine sites cattle found within 10 km radius of these sites could be vaccinated. All dairy animals should also be vaccinated (MoARD, 2006). The recommended dosage and route of administration of the vaccine is 4 ml per head of cattle subcutaneously, preferably in the dewlap region. The first vaccination requires two injections at 6 months of interval. Immunity develops 2-3 weeks after vaccination and may last for one year (DACA, 2006).

3. MATERIALS AND METHODS

3.1. Study Area

The research was conducted in two districts of Northwestern and Eastern Zone of Tigray Regional State, namely Sheraro and Seasie Tseada Emba districts, from November 2014 up to April 2015. Tigray region is the most northerly region of Ethiopia bordering Eritrea to the north, Sudan to the west, Afar region to the east and Amhara region to the south. Tahetay Adiabo (Sheraro) is bordering to the north by Eritrea, west by Kafta humera and Welkaite, east by Lealay Adiabo. Seasie Tseada Emba is bordering to the east by Afar region, North by Erob, West by Ganta Afeshum and South by wekro district. http://www.esgpip.com/Tigrai.html

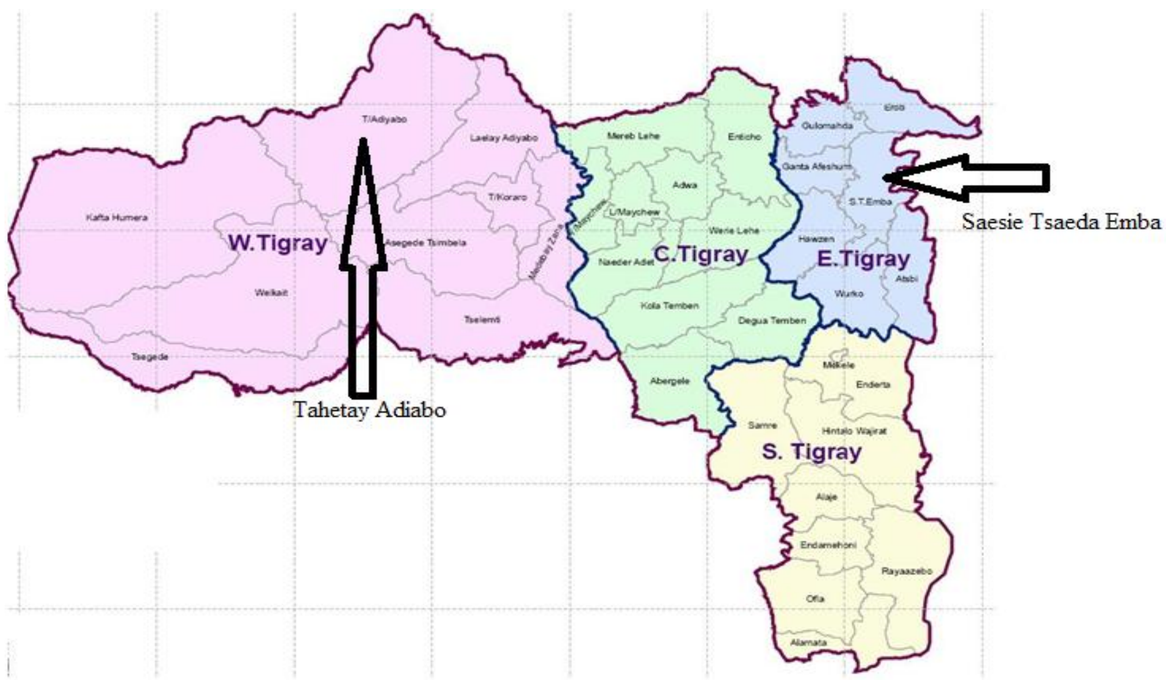

Figure 2: Map of Tigray region and study areas

Source: http://www.esgpip.com/Tigrai.html

Tigray region has five administrative zones such as Western, Northwestern, Central, Eastern and Southern zones (CSA, 2007). The region total population of cattle is 4,065,082, 1,381,622 sheep and 3,191,184 goats, 2,412 horse, 4,690 mule, 692,179 donkey, 52,541 camel, 5,242,699 poultry and 229,626 beehives (CSA, 2013). North West Tigray zone had 557 mule, 163,638 donkey, 14,929 camel, 1,557,283 poultry and in Eastern Tigray Zone 443 horse, 717 mule, 113,680 donkey, 763,542 poultry and 49,374 beehives (Table 1) (CSA, 2013).

Table 1: Livestock population in North western and Eastern zone, Tigray region

Zone	**Distance from A.A (km)**	**Livestock population**			**Human population**	**Ref.**
		Cattle	Sheep	Goat		
North Western	1500	1,436,922	11,247	1,194,801	102,492	(CSA, 2013)
Eastern	824	418,666	601,412	217,262	158,257	(TBoFED,2011)

The agro-climatic zones of the districts are lowland (kolla) and highland respectively. The Districts selected in this study represent the typical agro-ecology of the region, comprising lowland and highland areas of Sheraro, Northwestern and Seasie Tseada Emba, Eastern Zones with extensive animal production system and traditional housing and grazing of natural pasture are the predominant husbandry practices. There are two distinct seasons: wet and dry seasons. The wet season extends from June to September, while the dry season is from November to May (TBoARD, 2007). However there are four different seasones such as, Kiremt or Meher (Summer): June, July and August are the summer season. Heavy rain falls in these three months. Belg (Autumn): September, October and November are the spring season sometimes known as the harvest season. Bega (Winter): December, January and February are the dry season with frost in morning specially in January. Tseday (Spring): March, April and May are the autumn season with occasional showers. May is the hottest month in Ethiopia. http://www.ethiopiantreasures.co.uk/pages/climate.htm

The annual rain fall, temperature and cultivated land for Sheraro and Seasie Tseada Emba were 18.9-35.3°C, 54.8 mm, 64,486 hectare and 8.1-19.4°C, 30.9 mm and 18,836.25 hectare respectively (TBoFED, 2011) and the study areas have altitude range from 914 up to 2695 m.a.sl, GPS recorded for all the PA's in each district were describe on (Table 2).

Table 2: Geographical location and altitude of the selected PA’s in the two Districts

Districts and PA's		GPS Altitude (m.a.sl)	Latitude	Longitude
Sheraro	Aditsetser	1074	14.35691N	037.61098E
	Adiaser	914	14.22688N	037.59192E
	Gemahalo	1024	14.56830N	037.73639E
	Sheraroketema	1015	14.40551N	037.77613E
Seasie Tseada Emba	Tsenkanet	2328	13.98841N	039.56165E
	Hadishadi	2530	14.09918N	039.60942E
	Hadishhiwet	2695	14.18723N	039.56343E
	Senkata	2350	14.05764N	039.34727E
	Saze	2312	13.97740N	039.54768E
	Komasebha	2692	14.28387N	039.54658E

3.2. Study population, study design and sampling technique

Begait (breed of cattle that are found in the northern Ethiopia of Tigray region), local and cross breed (a mixture of Holestain Fressian with local breed cattle found in Tigray region) cattle that are kept under traditional management system were studied. The study animals were selected from two zones of the existing five zones namely, Northwestern and Eastern zones, Tigray Region.

A cross-sectional study design was implemented to determine the seroprevalence of FMD in the study area. Two Districts and ten kebelle/PA were selected purposively based on difference in agro-ecologies, cattle population, accessibility, previous outbreak report, route of trade, transboundary animal trade and contact with wildlife. However, systematic random sampling was used for household and individual animal selection. In Sheraro out of 18 PA’s four PA’s were selected and from Seasie Tseada Emba out of 27 PA’s six PA’s were selected. The numbers of animals included in the study were distributed proportionally over the selected Districts.

Breed, age, sex physiological status, altitude (location), District, PA, herd size, vaccination status, outbreak report, contact with small ruminants and wildlife contact history (Annex 2) were included in the data collection during the study to evaluate them as risk factors for the epidemiology of FMD. Serum was collected and tested using IDEXX 3ABC-ELISA test to determine the prevalence of the antibody. Sampled animals were grouped into three age categories based on their dental eruption status and stage of puberty (Annex 3).

3.3. Sample Size Determination

Total sample size was estimated according to Thrusfield, (1995) by using 95% confidence interval and 5% precision. The previous prevalence reported by (Afera *et al*., 2012) in Tigray region (Gantaafeshum, Raya Azebo and Maykadra Districts) was 39.2%. Therefore, by using the formula 366 samples from both districts was needed but 400 samples were collected.

i.e. $$n = \frac{1.96^2 \, P\exp\,(1 - P\exp)}{d^2}$$

Where, n= the total sample size; Pexp= expected prevalence; d= absolute precision

3.4. Questionnaire survey

A semi-structured questionnaire was developed and tested. The questionnaire was administered to those cattle owners who were willing to participate. These willing owners were a subset of farmers (as some farmers were not willing) from whom bleeding of cattle for sera was conducted. The questionnaire was designed with the following main objectives of assessing: the perception and understanding of the communities about FMD, access to veterinary service, economic impact by FMD, livestock production constraints in the study area with particular reference to FMD and the most important epidemiological risk factors that could be associated with FMD so as to avoid bias which would most likely misguide the study. For the present study a total of 60 households were interviewed individually using structured questionnaire. Questionnaire survey was the first step of the investigation process where tentative diagnosis of the disease can be made (Putt *et al*., 1988). The questionnaire indicated in (Annex 1).

3.5. Study Methodology

3.5.1. Sample collection

A total of 400 samples were collected from cattle in the study Districts. The aim was to determine the level of antibody in the serum for vaccinated and non vaccinated animals. Blood sample were collected by jugular-veins puncture with plain vacutainer tube. The samples were labeled accordingly to allow identification of each animal number and studied area and kept in slanted position overnight to allow serum separation via clotting blood samples. Serum was decanted and aliquoted into 2 ml sterile cryovials before transported and temporary stored in cold boxes. Then transported in an icebox to Mekelle regional laboratory for refrigeration and stored at -20°C. Finally, at the end of each sampling sera will be transported in cold chain to National Veterinary Institute (NVI) laboratory for serological examination (OIE, 2004).

3.5.2. Laboratory analysis

FMD-3ABC ELISA

The FMD-3ABC ELISA was used to determine the seroprevalence of FMD. IDEXX-FMD-3ABC ELISA allows the detection of serologically positive animals and able to differentiate vaccinated animals from infected animals. The IDEXX FMD 3ABC Ab Test has high specificity (100%) in IDEXX validation tests with samples from multi-vaccinated animals. The 3ABC ELISA was performed as follows. https://www.idexx.com/pdf/en_us/livestock-poultry/fmd-3abc-ab-info-sheet.pdf

3ABC ELISA Procedure

The ELISA protocol was conducted as per the Manufacturer procedure (IDEXX Switzerland AG). First all reagents must be allowed to come to 18-26°C before use. Reagents should be mixed by gentle swirling or vortexing. About 100µl of prediluted samples and controls (1:100 in IDEXX FMD 3ABC sample diluents) was dispensed into the appropriate wells of the microtiter plate pre-coated with recombinant FMDV 3ABC viral antigen (Annex 4). The plates were covered with a lid and incubated for 60 minutes (± 10 min) at 37^0C (± 3^0C) in a humid chamber. After incubation microtiter plates were filled with about 300µl washing solution and washed three times. Aspirate liquid contents of all wells after each wash and finally firmly tap residual wash fluid from each plate on to absorbent material. Then 100µl of the ready to use Anti-Ruminant-IgG-Po-Conjugate was dispensed into each well and incubated for 60 minutes at 37^{0}C in a humid chamber. After washing the plate, 100µl of TMB-substrate was dispensed into each of the 96 wells and incubated at room temperature at 18-26^{0}C for 15 minutes. Finally, the reaction was stopped by adding 100µl of stopping TMB-solution. The result was read using a spectrophotometer at 450nm wavelength within 2 hours of stopping the reaction. The stop solution should be dispensed in the same order and at the same speed as the substrate. The reader, connected to the computer loaded with ProComm and word packages, was used to automate the reading of OD value. To validate assay the optical density (OD) of the positive control (PC A450) should not exceed 2.00 and the OD of the negative control (NC A450) should not exceed 0.5. The percentage positivity (PP) for test samples in relation to the negative and the positive controls was calculated as follows: The OD of the positive control (OD pos) and the OD of the samples (OD sample) are corrected by subtracting the OD of the negative control (OD neg):

$$\text{Value (\%)} = \frac{\text{OD sample} - \text{OD neg control}}{\text{OD pos control} - \text{OD neg control}} \times 100$$

Interpretation of results

S/P %	<20 %	≥20 % and <30%	≥30 %
Interpretation	Negative	Suspect	Positive

The cut off value provided by the Manufacturer was used to determine the percentage positivity.

3.6. Data Management and Analysis

Microsoft Excel-2007 and SPSS-20 statistical software programmes were used for data entry and analyses, respectively. The data were coded before the entry. Descriptive statistics was used to determine the frequency of proportion (prevalence) and to determine the questionnaire data gathered. The total prevalence was calculated by dividing the number of 3ABC ELISA positive animals by the total number of animals tested. Univariable logistic regression was used to assess association FMD positivity with sex, age, breed, physiological status, location, altitude, contact with wild life and herd size by using SPSS -20 software. All variables $P < 0.1$ in the univariable analysis were further tested by multivariable logistic regression model to assess their effect on FMD sero-positivity. In all the analyses, 95% confidence level and $p \leq 0.05$ were set for significance.

4. RESULT

4.1.Risk factor Positivity by Box plot

The distribution of ODs of the tested animals was shown in Figure 3. The horizontal dotted line indicated cut off-value for FMD positivity. Number of FMD positive cattle appeared higher in <1500m.a.sl than > 1500m.a.sl, Begait breed than local breeds and large herd size (Figure 3).

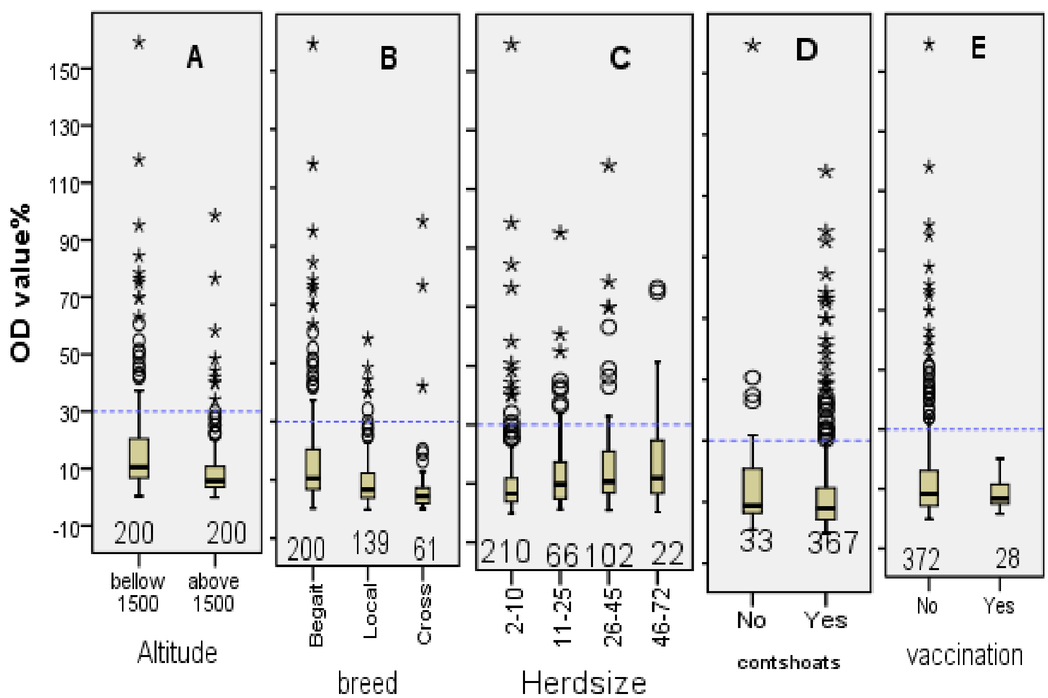

Figure 3: Box plot description of the risk factors

4.2.Seroprevalence of FMD and Associated Risk Factors

The overall seroprevalence of FMD was 9.8% (39/400). The sero-prevalence of FMD in selected Districts was 15% in Sheraro and 4.5% in Seasie Tseada Emba. Significantly higher sero-prevalence was observed in lower altitude, Sheraro District, Adiaser PA, Begait breed, 4-10 age, lactating cows and cattle that have contact with wildlife (Table 3). Whereas, sex (7.1% in male and 11.9% in female, X^2 =2.58, p=0.108, contact with shoats 18.2% in No and 9% in Yes, X^2 = 2.906, p=0.088, vaccination status 10.2% in No and 3.6% in Yes, $X^2 = 1.306$, p=0.253 and outbreak 6.1% in No and 10.1% in Yes, $X^2 = 0.556$, p=0.456) were not significant.

4.3. Univariable logistic regression

The logistic regression was used to test the strength of associations between the risk factors and the prevalence of the disease. Sheraro had 3.74 times more odds of FMD sero-positive than Seasie Tseada Emba. Large herd size of 46-72 cattle had 4.45 times higher odds of FMD infection. Lactating cows were found 6.69 times higher FMD sero-positivity than bulls. In this analysis age ≤4 years, local breed, male, 2-10 herd size, >1500m.a.sl, Seasie Tseada Emba, bull and no contact with wildlife were used as a reference category (Table 4).

Table 3: Seroprevalence of FMD by different risk factors

Variables		N	Positive	Prevalence (%)	95% CI	P-value
Altitude						
	< 1500m.a.sl	200	30	15	14.46,15.53	0.000
	> 1500m.a.sl	200	9	4.5	4.20,4.79	
District						
	Sheraro	200	30	15	14.46,15.53	0.000
	Seasie Tseada	200				
	Emba		9	4.5	4.20,4.79	
PA						
	Aditsetser	52	6	11.5	10.57,12.41	0.036
	Adiaser	47	9	19.1	17.85,20.34	
	Gemahalo	56	8	14.3	13.30,15.29	
	Sheraroketema	45	7	15.6	14.44,16.75	
	Tsenkanet	29	0	0		
	Hadishadi	28	1	3.6	2.89,4.03	
	Hadishhiwet	29	4	13.8	12.44,15.15	
	Senkata	28	1	3.6	2.89,4.30	
	Saze	34	1	2.9	2.32,3.47	
	Komasebha	52	2	3.8	3.27,4.32	
Breed						
	Begait	200	30	15	14.46,15.53	0.002
	Local	139	6	4.3	3.95,4.64	
	Cross	61	3	4.9	4.34,5.45	
Age						
	≤ 4	177	8	4.5	4.18,4.81	0.007
	4-10	198	28	14.1	13.57,14.62	
	> 10	25	3	12	10.64,13.35	
Herd size						
	2-10	210	13	6.2	5.86,6.53	0.032
	11-25	66	9	13.6	12.71,14.48	
	26-45	102	12	11.8	11.13,12.46	
	46-72	22	5	22.7	20.70,24.69	
Physiology						
	Heifer	77	5	6.5	5.93,7.06	0.013
	Bull	96	3	3.1	2.74,3.45	
	Ox	86	10	11.6	10.88,12.31	
	Pregnant	20	1	5	4.02,5.98	
	Lactating	107	19	17.8	17.00,18.59	
	Dry	14	1	7.1	5.70,8.49	
Contact wildlife						
	No	280	20	7.1	6.78,7.41	0.007
	Yes	120	19	15.8	15.08,16.51	
Total		400	39	9.8	9.49,10.10	

Table 4: Univarient logistic regression by different risk factors

Variables		Odds Ratio	95% CI (OR) Lower	Upper	P-value
Age	≤ 4 year	Reference	-	-	-
	4-10/≤ 4	3.4794	1.5417	7.8528	0.0027
	> 10/≤ 4	2.8807	0.7109	11.673	0.1383
Breed	Local				
	Begait/Local	3.9118	1.582	9.6725	0.0031
	Cross/Local	1.1466	0.2772	4.742	0.8502
Sex	Male	Reference	-	-	-
	Female/Male	1.7604	0.8769	3.5343	0.1117
Herd size	2-10 HS	Reference	-	-	-
	11-25 HS/2-10 HS	2.3927	0.9732	5.8826	0.0573
	26-45HS/2_10HS	2.0205	0.887	4.6027	0.094
	46-72HS/2-10HS	4.457	1.4196	13.9933	0.0105
Altitude	> 1500m.a.sl	Reference	-	-	-
	< 1500/> 1500	3.7451	1.7289	8.1126	0.0008
District	Seasie Tseada Emba	Reference	-	-	-
	Sheraro/Seasie Tseada Emba	3.7451	1.7289	8.1126	0.0008
PA	Komasebha	Reference	-	-	-
	Adiaser	5.921	1.208	29.011	0.028
	Sheraroketema	4.605	0.905	23.436	0.066
	Gemahalo	4.167	0.842	20.623	0.08
	Aditsetser	3.261	0.626	16.973	0.16
	Hadishhiwet	4	0.685	23.342	0.123
	Hadishadi	0.926	0.08	10.684	0.951
	Senkata	0.926	0.08	10.684	0.951
	Saze	0.758	0.066	8.695	0.824
	Tsenkanet	0			0.998
Contact wildlife	No	Reference	-	-	-
	Yes/No	2.4455	1.2531	4.7726	0.0088
Physiology	Bull	Reference	-	-	-
	Pregnant/Bull	1.6316	0.1609	16.5417	0.6787
	Heifer/Bull	2.1528	0.4979	9.3074	0.3047
	Dry/Bull	2.3846	0.2305	24.6662	0.466
	Ox/Bull	4.0789	1.0838	15.3507	0.0376
	Lactating/Bull	6.6932	1.9137	23.4094	0.0029
Outbreak	Yes/No	1.7379	0.3997	7.5564	0.4611
Vaccination	Yes/No	0.3255	0.0431	2.4605	0.2768
Contact shoats	Yes/No	0.4446	0.1712	1.1544	0.0959

4.4.Multivariable logistic regression

Multivariable logistic regression analysis for significantly associated risk factors was conducted simultaneously. Sex, breed, herd size, physiological status and contact wildlife were exclude from final model as they didn't have significant association after the effect of age and altitude was removed (Table 5). District was removed from the model due to its multi-colinearity with altitude.

Table 5: Multivariable logistic regression for age and altitude

Risk factors		Coefficient	Odds Ratio (OR)	S. E.	95% CI (OR)	P-Value
Age	4-10/≤4	1.0782	2.9394	0.4214	1.287-6.713	0.0105
	>10/≤4	0.7734	2.1672	0.7247	0.5236-8.9699	0.2859
Altitude (m.a.sl)	<1500/>1500	1.1762	3.242	0.4002	1.4798-7.1026	0.0033
Constant		-3.6698		0.4489		0

4.5.Knowledge of the farmers on their animal performance and economic impact of FMD

All the respondents (n = 60) from the two districts indicated heifers reach puberty at average age of 3.5 year. This study indicated that the cows daily produce on average of 2.67L milk for about 9 months. All the respondents said their cattle were used common watering point and grazing land (Table 6).

Table 6: Reproductive and productive performance of Begait and local breed cows as per the knowledge of respondents

Parameter	Mean ± SD	Minimum	Maximum
Puberty age (year)	3.51± 0.74	2	5
No. matting to conceive (times)	1.63± 0.61	1	3
Dam first delivery (year)	4.53± 0.74	3	6
Conceive after birth (year)	1.29± 0.47	0.3	2

No. of calves per lifetime	6.22± 1.34	4	11
Daily milk yield (liter)	2.67± 1.58	0.5	6
Lactation length (month)	9.25± 4.02	6	24

Farmers clearly have the knowledge of clinical manifestation and economic impact of FMD outbreak (Table 7).

Table 7: The clinical manifestation and economic impact of FMD outbreak when it occurs as per the farmer's knowledge

Risk factors	No. positive respondents	% of respondent
Death by FMD	13/60	21.7
Lameness	59/60	98.3
Abortion	22/60	36.7
Reduce milk yield	59/60	98.3
Emaciation	59/60	98.3
Reduce sale price	59/60	98.3
Withdrawal of cow from milking	37/60	61.6

The present study indicated that 17(28.3%) responded sheep were more infected than goats 11(18.3%) by FMD. From all respondent about 50 (83.3%) responded that there was shortage of feed and water for their animals especially in dry season (Table 8).

Table 8: Knowledge of farmers on FMD transmission, host range and intervention

Risk factors	Frequency	% of respondents
Sheep infected by FMD	17/60	28.3
Goat infected by FMD	11/60	18.3
Animal introduced from other area	31/60	51.7
Feed and water shortage	50/60	83.3
Inadequate vet. Service	36/60	60
Cattle FMD non-vaccinated	54/60	90

Above half of the respondents respond that they were used the traditional treatment method during the outbreak of FMD. The traditional methods include use of honey on the affected area, keep the animal in shaded area and giving palatable feed, wash the affected area by their urine and warm water with salt. This shows the respondents know FMD before longtime and use their own method but not report to veterinarian.

Most of the respondents reported that FMD was mostly common After Rain (Autumn) and also at dry seasone (Winter) (Figure 4).

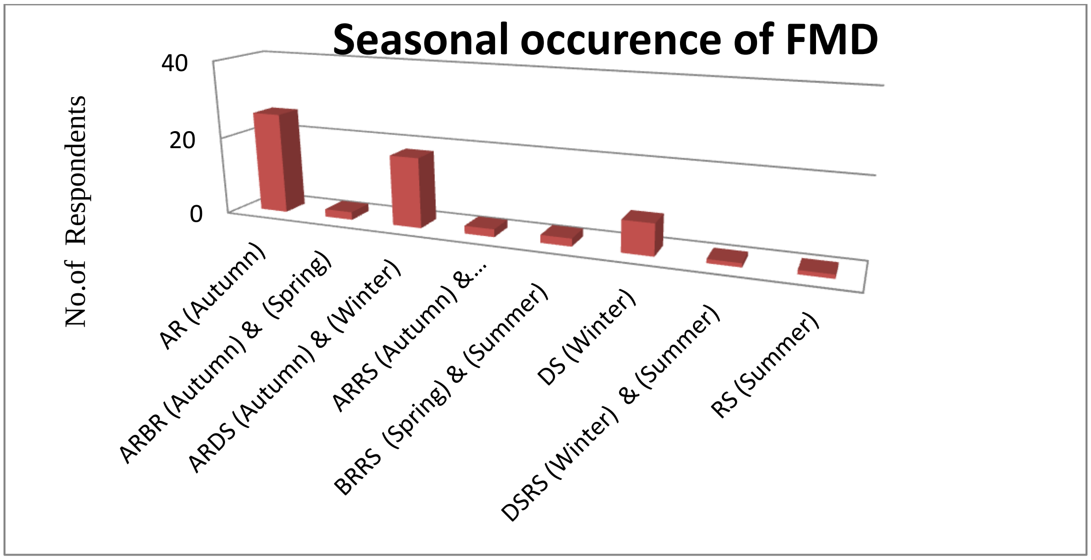

Figure 4: Seasonal difference for FMD outbreak

About 55% of the respondents reported that during FMD outbreak they use the traditional treatment methods like using honey, wash the infected part by warm water with salt and keep the animal in shaded area (Figure 5).

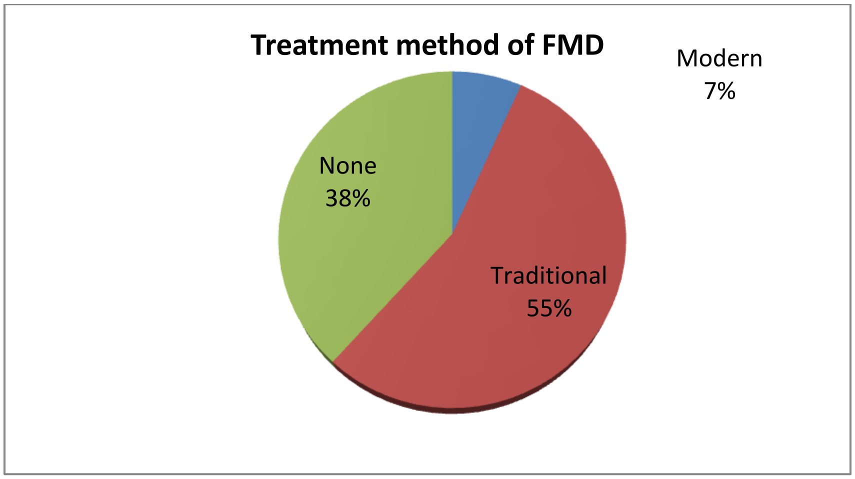

Figure 5: Treatment method of FMD

5. DISCUSSION

5.1.Questionnaire survey

The current questionnaire survey results have allowed us to generate relevant epidemiological information not shown by serological survey result. Hence, the long standing nature of the disease in the area and the communities' understanding about FMD was determined.

The present finding of 2.67L of milk per day found was higher than low-producing cows of 1.87L milk per day in Borena cattle (Bayissa *et al*., 2011). The results of other parameters were also comparable with findings of 4.8 years at first calving and 8.1 months of lactation length. 61.6% of respondents reported that they don't milk FMD infected cow until recovery. Although further research is needed to look more specifically at milk losses due to FMD, it is likely that a reduction in milk supply would force households to either sell cattle or other assets to purchase grain for food.

Most of the herd keepers verified that these risk factors act as source of infection and FMD transmission to their animals. This also implies that farmers of the study area also know some important epidemiological risk factors that could be associated with the disease. Similar opinion was reported by (Belachew *et al*., 2012) in selected districts of Gambella and (Nigusse *et al*., 2011), study in Ethiopia, stated that the high incidence of the disease may be associated with extensive movement of livestock and high rate of contact of animals at herd gathering sites.

In the study Districts, livestock production system was extensive, animals used communal grazing land and watering points. Free animal movement was a common practice in the area. Introduction of new animals into individually owned herds following purchase for replacement, herd expansion and receiving cultural gifts was usually exercised without any screening for the health status of the new animal.

The respondents (60%) claimed that there was shortage of animal health centers in the district because too far distance of the available clinics and the available clinics are not well equipped with facilities. Therefore, about 55% of the respondents were used the traditional treatment method instead of reporting during the outbreak of FMD, this might be related with no animal health center in their surrounding and also shows long time history with FMD occurrence in the area.

Temporally, FMD was most commonly occurs after rainy season (Autumn) and rainy season (Summer) of the year in the study area. Studies conducted in Mali (Sangare *et al*., 2004) and in Ethiopia by (Nigussie, 2009) also indicated that FMD is more prevalent in the cold and rainy seasons of the year. FMDV transmission requires proper humidity and temperature are maintained FMD virus can be carried up to 250 km across the sea and up to 60 km across the land as reported by (Kitching, 1992)

The roles and involvement of wildlife in the occurrence and transmission of FMD were not known by all the interviewed respondents. In contrary to (Belachew *et al*., 2012) report, in the current study almost below quarter of respondents recognize effect of FMD on sheep and goats and they can be taken as a risk for the occurrence of FMD.

Even though, there was no outbreak report in this study period, the result indicated that there was a higher risk/warning for FMD outbreak occurrence. This shows there was a high level of carrier animal within each herd with higher positive value. This might be due to the virus was circulating in the population with few cases and the owners were alert for the signs/lesions then they give treatment by traditional method.

5.2.Seroprevalence of Foot and Mouth Disease

3ABC ELISA was used in the current sero-prevalence study. Cattle which react positively to the 3ABC ELISA could be persistently infected or become carriers of FMD as described by Alexandersen *et al*. (2002) and Vosloo *et al*. (2002). These carrier animals could present a risk to susceptible animals and play a role in the maintenance of the agent as well as the epidemiology of the disease in the area.

In the present study the overall prevalence of FMD was 9.8% and 15% in Sheraro and 4.5% in Seasie Tseada Emba Districts. The prevalence in this study was lower than the previous reports by (Afera *et al*., 2012) who reported 39.2% in selected Districts of Tigray region. This might be due to in 2011/12 there was an outbreak in Tigray region that might increase the number of affected animals. It was also lower 23% at Borena pastoral and agro-pastoral system by (Bayissa *et al.,* 2011), 21.59% at Bale Zone (Duguma *et al.*, 2013), 24.6% at Borana and Guji Zones (Mekonen *et al.*, 2011) and 14.5% Central Ethiopian cattle feedlots (Alemayehu *et al.*, 2014) reported respectively. However the present study result was higher than the work in Afar Pastoral area 5.6% by (Jenbere *et al*., 2011), Southern Ethiopia 9.5% by (Megersa *et al.,* 2009) and Dire Dawa and its Surroundings 8.01% by (Abunna *et al.,* 2013). The differences might be due to uncontrolled transboundary movement of animals between border of Eritrea and Sudan, difference in husbandry system, ecology and breed.

Breed variation between Begait, local and cross breed was statistically significant indicating Begait breed was more affected. This might be attributed to large herd size of Begait breed with uncontrolled movement and extensive management system. This study was in agreement with Afera *et al.* (2012) in Tigray Region and Duguma *et al*. (2013) in Bale Zone that reports higher prevalence on local breed than cross and exotic breeds, even if it was not significant. This study was in agreement with (Kitching and Alexanderson, 2002) that, the disease is considerably less obvious or sub-clinical in breeds of cattle indigenous to Africa and Asia, where FMD is endemic.

No significant difference ($P>0.05$) was observed in the prevalence of FMD between female and male in this study agrees with the previous findings (Abunna *et al.,* 2013; Yahya *et al.,* 2013 and Megersa *et al.,* 2009) indicating both sexes are equally affected by FMD.

There was a higher seroprevalence on large herd size than small and medium herd size. Other studies conducted in selected districts of Gambella and Borana pastoral and agro-pastoral system area reported a similar effect (Belachew *et al.,* 2012 and Bayissa *et al.,* 2011). Moreover, for infectious disease in densely populated animals FMD may spread rapidly because of the high level of challenges from infected animals (Radostits *et al.,* 2000). This could be due to the contagious nature of the disease and mode of transmission which is enhanced by crowding and frequency of contact.

Significant sero-prevalence association was observed between cattle having contact with wildlife versus those not communicated with wildlife. This is a good indicator of the likelihood of FMD transmission from wildlife to cattle and vise-versa. According to Hargreaves *et al*. (2004) and FAO (2006) report, wildlife plays a unique and important role in the epidemiology of the disease in Africa although this aspect has been adequately investigated only in Southern Africa. FMD potential reservoirs such as African buffalo, wild pig, giraffes, deer, antelopes may become infected with FMD viruses and may be acting as source of infection for cattle (OIE, 2004; Rweyemamu *et al*., 2008). This study was consistent with (Belachew *et al.,* 2012) report that those frequently having contact with wildlife was more likely to be at risk than those with rare contact.

Higher age specific prevalence was observed on adult (14.1%) than young (4.5%). The current study agrees with results of (Musema, 2008) higher prevalence in adults (26%) than young (20%) and higher prevalence in adult (6.45%) than young animals (5.49%) by (Abrha, 2008). In line with this, (Rufael *et al*., 2008) has also reported lower prevalence of FMD in young animals from Borana area. According to Murphy *et al*. (1999) as age increases, the chance of exposure to the disease increases. This might be because adults have acquired the infection through repeated exposure to the different serotypes of the virus and could get access to mix with other herds at market places and communal pasture land.

Animals from low altitude (<1500m.a.sl) had significantly higher seroprevalence than those from high altitude (>1500m.a.sl) areas. This study was in agreement with (Yahya *et al.,* 2013) and (Megersa *et al*., 2009) who reported that higher prevalence in lowland than highland. This is due to scarcity of feed and water resources in the lowland increase animal movement and aggregations of animals at water points. The climatic stress factors in the lowland suppress the immunity of individual animals with subsequent susceptibility that may result in dissemination, sporadic occurrence of FMD in tropics (Seifert, 1996).

Higher FMD prevalence was recorded in Sheraro than Seasie Tseada Emba District. The seroprevalence between districts were significantly different ($p<0.05$). This might be attributed to the presence of extensive livestock movements in search of pasture and water in Sheraro than Seasie Tseada Emba. Cattle in Sheraro had access to neighboring country like Eritrea which is FMD endemic country.

As comparing, PA’s there was a significantly higher FMD prevalence recorded on Adiaser 19.1% and lower prevalence was seen in Saze 2.9%. This difference might be due to agro-ecological variation, management system, high contact of animals in lowland as compare to high land and effect of herd size.

This shows FMD control by vaccination doesn’t seem to be effective as vaccination coverage itself is limited and in some cases, animals vaccinated against FMD using bivalent vaccines containing the two serotypes A and O only were found to be affected by severe outbreak of another serotype or subtype (Sahle, 2004). The previous outbreak were due to serotype O Sudan type affected cattle and small ruminant in Tigray region in 2011/12. Movement of animals wasn’t limited among the administrative Regions of the country as well as the neighboring countries. This poses serious problem due to the transmission of various disease causing agents like FMD virus (Sahle, 2004).

6. CONCLUSION AND RECOMMENDATION

Despite the absence of an outbreak in the current study, 9.8% FMD prevalence is detected. It indicates that the cattle population of the area harbors carrier individuals that could lead the disease to strike again in the population. The positivity of the animals to FMD infection was associated with altitude, age, herd size, breed, functional state of the animal and contact with wildlife. In this line, higher level of FMD prevalence was observed in Sheraro district, Adiaser Peasant Association, adults, Begait breed, contact wildlife, large herd size and lower altitude. Farmers appears to have good knowledge of the manifestation, seasonality and economic impact of FMD but not aware of the interspecies transmission of the disease between shoats, wildlife, and due to contacts at watering and grazing points.

Therefore, based on the above concluding remarks the following recommendations are forwarded:

- ✓ An extensive regular surveillance in the epidemiology of the disease and further detailed investigation in wildlife and small ruminant is needed to determine their roles in FMD virus maintenance and transmission.
- ✓ The participation of the government and other stakeholders in regulation of animal movement among the different Regions and across international borders should be encouraged to control the disease spread and establishment of quarantine station around the border area is mandatory.
- ✓ The government and other organizations may give attention to the current status of the disease and give financial support for strict vaccination program to control the disease.
- ✓ Separation of animals grazing and watering points should be promoted to reduce the spread of disease from one animal to another animal and from one Peasant Association to another as animals from different Peasant Association have common grazing and watering points.

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https://www.idexx.com/pdf/en_us/livestock-poultry/fmd-3abc-ab-info-sheet.pdf Accessed on February 2015

8. ANNEXES

Annex 1: Questionnaire format survey

Date:_____________

Household level questionnaire format to determine the risk factors associated with FMD seroprevalence and to assess the economic importance of FMD infection in Tigray region.

Districts ______________________ PA/ Kebelle________________

Village/household ______________ Altitude___________________

1. Type of animal production system in the area (Tick)
   - Intensive
   - Mixed Crop livestock production
   - Agro pastoral
2. Agro climate of the area
   - Humid
   - Semi arid
   - Arid
3. Major problems of livestock production in the area (rank):
   - Disease _____if yes, list types of diseases ____________________________________
   - Feed and water shortage ______
   - Inadequate veterinary services _______
   - Others/specify _________________________
2. Impact of FMD:

Say yes or no for the following FMD impacts	If yes Rank	If yes, how many animals from the following age groups for each disease outcome listed from 1 to 7					
		Calf	Heifer	bull	cow	Pregnant	Oxen
1. Death (yes / no)							
2. Abortion (yes / no)							
3. Emaciation (yes / no)							
4.Lameness (yes / no)							
5.Reduced milk yield (yes / no)							
6. Reduction of traction power (yes / no)							
7. Reduction of sale price (yes / no)							

3. Grazing habit of the livestock
   - Grazing all neighbors' livestock together as one herd. Yes ☐ No ☐
   - Grazing household herd separately Yes ☐ No ☐

- Mixing at watering points Yes ☐ No ☐

4. Animal origin:
   - Born in the herd Yes ☐ No ☐
   - Introduced from other area Yes ☐ No ☐
5. Performance of the cattle population in the area

Female factors	Numerical score
Estimated Puberty age for females	
Estimated No. of mating to conceive	
Estimated Age of dam at first delivery	
Estimated Days spent to conceive after birth	
Calving interval in months (age between the younger vs. elder)	
Total No. of offspring per female in her life span	
Estimated daily milk yield	
Lactation length (total months spent producing milk post delivery)	

6. Do you milk FMD infected cows? Yes ☐ No ☐
   - How many times do you milk these cows? ___________________
7. Do you use FMD infected ox (en) for plowing? Yes ☐ No ☐
   - If no, how many days on average you don't use them for plowing? ________days
8. What do you do when your oxen are infected with FMD?

   ____
9. Are the animals in the area vaccinated for FMD
   - Before 2008/2000e.c Yes ☐ No ☐
   - 2008 – 2013/2000-2005 Yes ☐ No ☐
   - In 2014/2006 Yes ☐ No ☐
   - None Yes ☐ No ☐
10. Rank the month for FMD occurrence? ______________________

	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	Jun	Jul	Aug
Total sick												

11. Is there animal movement, if yes to where? _______________________________________
12. Other animals infected with FMD
    - Sheep
    - Goat
    - Camel

Annex 2: Check list for sample collection

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Altitude					
District					
PA					
Animal ID					
Owners name					
Breed					
Sex					
Age					
Herd size					
Physiological status					
Outbreak report					
Vaccination status					
Contact shoats					
Contact wildlife					

Annex 3: Study animals age estimation on the basis of dental eruption

	Eruption of teeth	Age estimation in year
Cattle	One incisor	Less than 2
	Two incisors Three incisors	From 2 - 3
	Canine Wear of teeth	Greater than 3

Source: (Merck veterinary manual, 1998)

Annex 4: Plate layout used for FMD-3ABC ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	NC	1	2	3	4	5	6	7	8	9	10
B	PC	PC	11	12	13	14	15	16	17	18	19	20
C	87	81	21	22	23	24	25	26	27	28	29	30
D	88	82	31	32	33	34	35	36	37	38	39	40
E	89	83	41	42	43	44	45	46	47	48	49	50
F	90	84	51	52	53	54	55	56	57	58	59	60
G	91	85	61	62	63	64	65	66	67	68	69	70
H	92	86	71	72	73	74	75	76	77	78	79	80

Annex 5: Advantage of IDEEX 3 ABC ELISA

IDEXX FMD-3ABC Bo- Ov ELISA

The IDEXX FMD 3ABC Ab Test Kit is an enzyme immunoassay for the detection of antibodies against Foot-and-Mouth Disease (FMDV) in serum or plasma samples of bovine and ovine origin. This test allows differentiates between samples from infected (3ABC positive) and vaccinated (3ABC negative) animals.

- Detects anti-3ABC antibodies

- High specificity with samples from multi-vaccinated animals
- Ready-for-use reagents
- Easy automation
- Easy data management when used with xChek® software

https://ca.idexx.com/livestock-poultry/ruminant/foot-and-mouth.html

The IDEXX FMD 3ABC Ab Test demonstrated 100% specificity in IDEXX validation tests.

Advantage of the taste

- Now you can give clients same-day FMD results with the IDEXX FMD 3ABC Ab Test:
- 3-hour protocol
- No overnight incubation
- Ready-to-use reagents
- Fast, familiar ELISA format for manual or automated work flows

https://www.idexx.com/pdf/en_us/livestock-poultry/fmd-3abc-ab-info-sheet.pdf