Outbreak Investigation and Sero-Type Identification of FMD in Selected Zones of Amhara Region

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Abstract: Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-footed animals and it is one of the most economically important diseases of livestock. A cross sectional study was conducted on investigation of FMD outbreak from November 2011 to April 2012 in selected districts of Amhara region, Ethiopia. A total of 430 animals were clinically and serologically examined for presence of specific lesions and nonstructural protein for foot and mouth diseases respectively. Of which, 122 (28.75%) manifested clinical signs and lesions suggestive of FMD, and 47 (10.93%) were sero-positive. From a total of 3419 animals observed and recorded on a designed format in five districts, 963 (28.16%) were infected, and 39 (1.6%) died during outbreaks of FMD. Epidemiological investigations revealed that the morbidity rate of the disease was 1.6% in Legambo districts, whereas the mortality rate was <2% in all districts. Furthermore, the mortality and case fatality rates were relatively higher, 1.6% and 6.06% in calves than the other age groups, respectively. From a total of 16 bovine epithelial tissue-cultured samples, all showed cytopatic effect for foot and mouth diseases virus, in which 16 samples had serotype O and high incidence of FMD outbreak in different districts of Amhara region. Generally the result of the present study showed that FMD is an important cattle disease in the study area. Thus, an appropriate control strategy has to be designed and applied which could involve regulation of transboundary animal movement and vaccination using the circulating virus strain.
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1. Introduction

The dominant economic feature of Ethiopia is the agriculture based; of which livestock is an important and essential component. Ethiopia has the largest livestock populations in Africa, possessing more than 43.1 million cattle, 48 million small ruminants, 15 million camels, 7million equines and 52 million chickens (CSA, 2008).

FMD also known as aphthous fever from the Greek word alpha means vesicle, in the mouth, was the first animal viral diseases established (Alexandersen *et al*., 2003). FMD virus belongs to the genus *Aphthovirus* in the family *Picornaviridae* and possesses a single strand of positive-sense RNA genome. It has a high mutation rate because the viral RNA–dependent RNA polymerase lacks proof-reading ability (Sahle, 2004), resulting in 7 immunogenically distinct serotypes (O, A, C, Southern African Territories [SAT] 1, SAT 2, SAT 3, and Asia 1) and numerous and constantly evolving variants showing a spectrum of antigenic diversity (Radiostits *et al*., 2000; OIE, 2004).

The disease spreads rapidly by movement of infected animals or mechanically on fomites such as clothing, shoes, vehicles, and veterinary instruments. The reasons for the rapidity of spread to fully susceptible populations is due to the highly infectious nature of the virus, the production of high titer in respiratory secretions and the large volumes of droplets and aerosols of virus shed by infected animals, the stability of virus in such droplets, the rapid replication cycle with very high virus yields and the short incubation period (Sellers and Daggupaty, 1977).

A foot and mouth disease is a highly contagious viral diseases, multi-species animal disease with a devastating impact on national economies and trade (James and Ruston, 2002. It is one of the most economically important diseases of livestock still a major constraint on economic growth, reduction of poverty, and food security Bronsvoort *et al.*, 2004). It can cause a number of deaths in young animals and production loss in adults (James and Ruston, 2002).

The economic importance of the disease is not only due to the ability of the disease to cause loss of production, but also related to reaction of veterinary services to the presence of the disease and the restriction on the trade of animals both locally and internationally, with subsequent reduction in value of animals and animal products (Tesfaye, 2006).

Foot-and-mouth disease is probably the most important livestock disease in Ethiopia in terms of economic impact. 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, in

which Ethiopia lost more than US$14 million, being a recent memory (Leforban, 2005).

In Ethiopia, factors which facilitate the occurrence of FMD are the presence of high number of susceptible animals, wild and domestic animals sharing common grazing pasture and watering points in areas where wild life occur, lack of control of animal movement, lack of effective vaccine, absence of systematic diseases surveillance and absence of reliable epidemiological data (Sahle *et al*., 2004).

Livestock are at risk from endemic strains as well as from antigenic variants prevailing in neighboring countries. The official data may not exhibit the reality of the disease due to the insidious nature of the disease, the unreported cases by farmers, as well as the few samples submitted to WRL for FMD, Pirbright, UK, for identification. However, records from the National Animal Health Diagnostic and Investigation Center and National Veterinary Institute of Ethiopia indicated that serotypes O, A, C, SAT1 and SAT2 were responsible for FMD outbreaks during 1974–2007 (Sahle 2004; Gelaye *et al*. 2005; Legess 2008).

FMD was first recorded in Ethiopia in 1957 when serotypes O and C were found while Type A and SAT2 were not identified until 1969 and 1989, respectively (Ayelet *et al.*, 2009). Moreover SAT1 and SAT2 were isolated recently from Meazan Teferi and Benishangul-Gumuz areas bordering Kenya and Sudan respectively from 2007 collected samples (Gelagay, 2008).

Records from 1997 to 2006 indicated that FMD outbreak occurred everywhere throughout the country with the highest in central part, particularly in North Shoa in which 128 out breaks were reported (Ayelet *et al.*, 2009). The occurrence of FMD in Ethiopia is increasing and in 1999 almost 15% of cattle were under risk of infections and in 2000 and 2001, a total of 27 and 88 diseases out breaks were reported, respectively (Esayas *et al*., 2005). Because three virus can produce clinically indistinguishable lesions in domestic animals, laboratory, diagnostic tests are required to identify the FMD virus and distinguish among seven serotypes (Kahrs, 2001).

Vaccination is an effective way to control FMD; however, the protection conferred by vaccination or infection is usually serotype specific and sometimes incomplete within a serotype (Sahle, 2004). The difficulties to vaccination are first vaccination programs may not be properly designed and secondly not only great change in antigencity but the virulence may also change dramatically (Quinn *et al*., 2009). In country such as Ethiopia where FMD is endemic, and where large number of susceptible domestic and wild ruminants exist with limited vaccinations on some dairy farms, serological survey are a pre-requiste to understand the epidemiology of FMD (Mestin *et al*., 2006).

This research will be conducted in North Eastern part of Ethiopia (Eastern Amhara region) to investigate the outbreak due to lack of awareness livestock owers to inform the outbreak the concerned bodies and there is no coordinated and designed bodies to investigate each FMD outbreak those leads to multiple serotypes circulate in the area even new serotype may involve that may result series loose in animal population.

So that I tried to minimize such problems by investigating each outbreak to identifying circulating sero type and it is a pre-condition for effective vaccination. Therefore, the objectives of the present study were investigation of FMD outbreak and isolate serotype circulating in the study area. So this study will contribute in filling the information gap of identifying the serotype in specific out break and will give a clue for knowing the serotype in specific area to taking appropriate corrective measures to limit FMD distribution. Finally this paper will give serve as a source of information for further investigations and to take corrective measures.

2. Materials and Methods

A number of materials were required for both study of the disease and writing of this thesis. The most important materials used were universal bottles, ice box, Pistle and mortar, scissors, bottle, test tubes, centrifuge, laminal airflow, syringe, Milliporefilter incubator, micropipette tips and single channel pipett, multichannel pipet, graduated cylinder, FMD kit. Equipment used for blood collection, (needle, needle holder, vaccutainer tubes). For tissue isolation the Minimum essential media, BHK Cells, Pbs (phosphate buffer solutions) and antibiotics required. For ELISA reagents and materials (Annex 2 with procedure).

2.1. General description of study areas

The study was conducted in Eastern part of amhara regional state bordering in the East with Afar regional state and, in the North by Tigray region and on North west by Debub gonder and in the West: Eastern Gojjam and in the South, Oromia region. This region covers about an area of 80,000 $km^{2,}$ and the area is inhabited by 3 ethnic groups namely Amahara, Agew and Oromia.

A total of five FMD outbreaks; three in Waguehumera zone of districts Dahana, Zkoala and Abergela and, one semen shewa zone of Moretnajiru districts and one outbreak in Southwello of Legambo districts. The Amhara regional State is located in North-western part of Ethiopia. It covers an estimated area of 170,752 km2, and FMD outbreaks occurred in mixed type of farming system (Central Statistical Authority, CSA 2008).

Table 1: Description of the study area

No.	Zone	District	Latitude	Longitude	Altitude (masl)	Temperature	Rain fall (mm/yr)	Farming system
1	Waguehamra	Abergele	$12^0$47`35.2``N	$38^0$59`77.4``E	1750	23-43c^0	250-750	pastoral
2	Waguehamra	Dahana	$12^0$27`582``N	$38^0$55`35.5``E	2757	12-30c^0	550-1000	mixing
3	Waguehamra	Zkola	$12^0$35`13.7``N	$38^0$45`47.2``E	1965	21-40c^0	300-800	Semi pastoral
4	Semen Shewa	Moretnajiru	$9^0$89`339``N	$39^0$14`572``E	2641	16-18^0	850-1300	Mixing
5	South wello	Legambo	$39^0$36`345``N	$10^0$80`208``E	1500-3700	12-20^0	800-1350	Mixing
6	South wello	Dessie	$11^0$8`N	$39^0$38`E	2470-2550	15-22^0	800-1250	Mixing
7	South wello	Kombolcha	$11^0$5`N	$39^0$44`E	1842-1915	24-28.2^0	750-900	Mixing

2.2. Study population and sampling methods

The study population consisted of cattle that were manifesting clinical signs of FMD and those in close contact with FMD outbreaks areas. The investigation of outbreaks was in three administrative Zones (South wello, wagu humra and Semen shewa), and five Districts were included for the occurrence of FMD outbreaks (Abergele, Dahana, Zkola, Moretenajuru and Legambo). The sampling was based on temporal feasibility to investigate. Cattle and small ruminants of all age groups, sex, and different management practices were recorded. Accordingly, 430 serum and 16 epithelial tissue samples were collected.

2.3. Study design

A cross sectional study was conducted to from November, 2011 to April, 2012 to investigate FMD outbreak in three zones of Amhara region that it can helps to get an understanding of the current status of the problem by describing it in relations to the occurance of FMD in cattle and small ruminants or livestock present in the study areas and clinical and epidemiological information was recorded on forms prepared for the purpose and samples of animals was clinically examined and specimens collected for diagnostic testing.

2.4. Sampling method

The sampling method was purposive sampling when an active outbreak of FMD was reported, a field investigation was conducted at the specific site of the outbreak, and in each village, and epidemiological information was gathered by interviewing village leaders, livestock owners, District animal health workers, and development agents. Clinical and epidemiological information were recorded, and animals were clinically examined for presence of FMD lesions on the mouth and feet, and specimens were collected for diagnostic testing.

2.5. Study methodology

2.5.1. Questionnaire survey on impact of FMD outbreaks

The investigation process was initiated by gathering data on epidemiological information on a specific outbreak. Data like geographical location, altitude, farming system, and management practices were gathered. Information on length of time since outbreak and population at risk, number of affected or number of dead animals by the disease were also collected. These data were collected in order to determine the characteristics of specific outbreaks (Annex 4).

2.5.2. Clinical examination

In each outbreak, animals were clinically examined from a distance for evidence of salivation and lameness. Salivating and/or limping animals were restrained in a crush pen for thorough examination and sampling. The mouth cavities of salivating animals were widely 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 of the hooves. Other animals in the herd without these signs were similarly examined, but sampling of epithelial tissue in such instance was done only when lesions were suggestive of FMD (Annex 6).

2.5.3. *Sample collection*

Tissue samples: Epithelial tissues were collected from un ruptured or freshly ruptured vesicles and placed in a bottle with transport medium composed of equal amounts of glycerol and 0.04-M phosphate-buffered saline solution pH 7.2–7.6 with antibiotics (OIE 2004). Species, identification number, sex, age, village, and type of tissue were labeled, and samples were immediately placed in a cooler containing ice for transport to National Veterinary Institute (NVI), Debre Zeit. Once the samples arrived at NVI, they were stored at +4°C until processed and placed at −20°C until analysis.

Serum sample: Serum samples were collected from cattle showing clinical signs (fever, depression, hyper salivation, lameness, vesicles, loss of appetite, and weight), those who had been potentially exposed, and those with no known exposure to foot-and-mouth disease virus (FMDv) including small ruminants. About 10 ml of blood sample was collected from the

jugular veins of each animal using plain vaccutainer tube, and the tube containing the blood sample was kept and protected from direct sunlight in a slant position for about 4 hr or until the blood clotted or alternatvely put the blood sample at room temperature within 24hrs until serum separated. Each serum was transferred into a sterile cryovial, bearing the names of the owner and herd size, age and sex of sampled animal and was transported in an icebox to kombolcha regional laboratory, kombolcha, and stored in deep freeze at -20C^{0}until transported and Laboratory investigation was conducted at the National Veterinary Institute (NVI), Serology laboratory, Debre-zeit.

2.5.4. ELISA test for seropositivity determination.

3ABC ElISA provides an effective method for detecting antibodies responsible for FMD virus in serum sample of bovines and shoats origin. FMD - 3ABC allows different ions between samples from infected (3ABC-posetive) and non-infected (vaccinated) (3ABC-Negative) animals. The 3ABC Elisa is also rapid test for screening of a large number of sera. In areas where more than one serotype exist, the test is also cheaper compared to the conventional blocking EIISA, which has the disadvantage that each serum sample must be tested against all existing serotypes. Briefly the test is carried out as shown in annex 2.

2.5.5. Cell culture for virus isolation

The epithelial tissue samples were thawed at room temperature and washed three times using sterile phosphate-buffered saline (PBS) at a pH of 7.2–7.6 under laminar air flow hood class II. About 1 g of epithelial tissue sample was grounded using sterile mortar and pestle by adding 9 ml of sterile PBS containing antibiotic. The tissue suspension was centrifuged at 3,500 rpm for 10 min. The supernatant was collected and filtered by Millipore filter of 0.22-µm pore size. About 0.5-1 ml of filtered tissue suspension was inoculated on baby hamster kidney (BHK-21) monolayer cells grown on 25 cm2 tissue culture flask and then flashed with growth media and incubated at 37°C and 5% CO_2 **in** a humidified incubation for 48 h. Cells were monitored for cytopathic effects (CPE) daily and frozen when CPE was developed or after 72 hr post-infection. A second pass was performed on those samples not presenting CPE following the same procedure as the first pass. Samples not exhibiting CPE by 72 hr post-infection on the second pass were considered Virus-negative (Annex 3).

2.5.6. Serotype identification

Tissue-cultured FMD virus samples that showed CPE were labeled using the following format: three-letter country code/isolate number/year (e.g., ETH/7/2008). The three letter country codes were designated as outlined by the World Reference Laboratory for FMD. The samples were submitted to National veterinary institution (NVI) and World FMD Reference Laboratory, Pirbright, UK, for serotype characterization. According to Kitching and Donaldson (1987), specimens were submitted to the World Reference Laboratory following the recommended international standards.

2.6. Data management and analysis

The epidemiological data and samples were collected by taking into the the impact of the occurrence Of FMD. Selected areas were coded first and different animal code. The data and laboratory results were first coded and managed into Microsoft Excel and analyzed using Statistical Package for Social Sciences software version 15.5. In all the analyses, confidence level was at 95%, and $p<0.05$ was set for significance. Descriptive epidemiological measures were also used to determine the morbidity, mortality and case fatality of FMD. Serotype identification for FMD virus was done at WRL for FMD, Pirbright, UK.

3. Results

3.1. Clinical examination

Cattle and small ruminants were carefully examined for presence of characteristic clinical signs of FMD. But small ruminants are subclinical. In each outbreak, animals manifesting the characteristic signs of FMD like vesicular lesions (ruptured vesicles) in oral cavity and on the feet and teats, lameness, and rise in temperature were considered as clinically affected by FMD. Out of 185 cattle examined physically, 47 (25.6%) animals showed clinical signs and lesions suggestive of FMD. The total numbers of animals have been shown were 122(28.37%) as indicated Annex 1. Even though small ruminants are subclinical, some rarely clinical sign have been seen. The principal clinical signs were salivation and lameness. Mouth lesions consisted of erosions and ulcers mainly on the tongue and dental pad. Foot lesions comprised of erosions on the interdigital spaces and the coronary bands. On the latter, the lesions were so severe that the hoof tended to separate from the coronary band. Most affected cattle showed both mouth and foot lesions.

3.2. Results of questionnaire survey on impacts of FMD outbreaks

From the total of 3419 animals observed during outbreak investigation of FMD in five Districts, 963 (28.16%) and 39(1.14%) cattle were found affected and dead during the outbreak of FMD, respectively. The morbidity rate, mortality rate, and case fatality rate were analyzed by considering different risk factors like Districts, age, sex, and husbandry system. The highest morbidity rate was recorded at Dahana district (32.4%), and the lowest was recorded at Legambo District (25.5%). Although, high morbidity rate (32.4%) was observed in animals kept under extensive production system (8.5%). The overall mortality and case fatality

of the disease were 1.6% and 4%, respectively. The highest mortality rate was recorded in Legambo District (1.6%), and there was a mortality in all outbreak districts in varies degree, and the lowest mortality was recorded in Zkola District (0.87%), but the case fatality rate was highest (6.08%) at Legambo districts.

Table 2: Result of questionnaire survey on impacts of FMD outbreaks

District of out break	Population at risk	Affected population	Dead population	morbidity rate %	Motality rate %	Case fatality rate%
Abergele	588	206	11	25.5	1.37	5.34
Zkolla	566	229	7	28.6	0.87	3.05
Dahana	442	216	6	32.4	0.89	2.65
Moretnajiru	400	164	6	28.8	1.05	3.65
Legambo	421	148	9	6.06	1.6	6.08
Total	2417	963	39	28.16	1.6	4

The age was categorized into two exclusive age groups as Young (≤3 years, and adult (>3years) (Annex 5). There was a statistically significant difference observed among different age groups of cattle with mortality and morbidity rates ($p<0.005$). Morbidity rates of cattle in different age groups were different in young, and in adult. The age specific mortality and case fatality rates were higher in young. The morbidity rates in all districts were 100% in adult animals but differ in young animals in different districts. The mortality and case fatality were higher in young animals in different districts of outbreak occurrence. As compared with the mortality in different districts of outbreak, the highest mortality (37.5%) was occurred in Legambo district but the lowest mortality (10.14%) was found in Zkola district. The case fatality (60%) also highest in Legambo district and the lowest case fatality (11.29%) was occurred Zkola district.

Table 3: Morbidity, mortality and case fatality of FMD in cattle and small ruminants with respect to age

District	Animal species	Age group	Affected population	dead	Morbidity %	Motality %	Case fatality rate%
Abergel	Cattle and shoats	Young	49	11	81.7	18.3	22.45
		Adult	160	0	100	0	0
Zkola	Cattle and shoats	Young	62	7	89.5	10.14	11.29
		Adult	168	0	100	0	0
Dahana	Cattle andshoats	Young	28	6	82.4	17.64	21.42
		Adult	187	0	100	0	0
Moretnajiru	Cattle and shoats	Young	16	6	72.7	27.27	37.5
		Adult	149	0	100	0	0
Legambo	Cattle and shoats	Young	15	9	62.5	37.5	60
		Adult	132	0	100	0	0
Total			966	39	96.11	3.9	4.05

3.3. Virus isolation and serotype Identification

Table 4: FMD virus isolated in different districts

Outbreak	Species	Type of sample	FMDv serotype isolated
Abergele	Bovine	Tissue	O
Zkola	Bovine	Tissue	O
Dahana	Bovine	Tissue	O
Moretnajiru	Bovine	Tissue	O
Legambo	Bovine	Tissue	O

Of the total 16 bovine epithelial tissue-cultured samples, all samples showed FMDV cytopathic effect (CPE) on BHK-21 monolayer cell cultures for FMD virus (Table 3). The CPE was characterized by a fast destruction of the BHK-21 monolayer cells, and infected cells were found singly, and the cell was found round in shape. Complete destruction of the cell sheet was mostly seen within 48 h of inoculation. Those samples that showed CPE were sent to WRL for FMD, Pirbright, UK, for further sero-typing. Of these, 16 tissue samples had serotype O.

3.4. Sero-positivity using -3ABC ELISA test (Cedi test)

From the total of 430 cattle and small ruminants examined for the presence of antibodies to the 3ABC non-structural protein of FMD virus, 47% cattle were found positive with respect total cattle population and 10.93% with the total animal population of 430. The sero-positivity of FMD varied from District to District, and the variations were not statistically significant ($\chi 2=4.529$, df=6, p=0.606). The highest seropositivity was recorded in Moretnajiru (18.75%), and the lowest was recorded in Kombolcha (6.52%). Similarly, there was no statistically significant (p=0.527) and (p=0.676) difference between sex group of animals and agro ecology of animals respectively, but there was a statistically significant (p=0.006) association between intrinsic host risk factors with age and species of the animals highly associated the with sero posetivity (p=0.000, $x^2=69.881$) and seropositivity (Table 1).

Table 5: Serological results by FMD-3ABC-ELISA

	Risk factor	No. of tested	No of positive	Sero-positive (%)	X^2-value	P - value
District	Dessie	81	9	11.11		
	Legambo	82	13	15.85		
	Moretnajiru	32	6	18.75		
	Kombolcha	92	6	6.52		
	Abergele	31	5	15.6		
	Dahana	34	5	14.7		
	Zkola	37	3	8.1		
	Total	**430**	**47**	**10.93**	**4.529**	**0.606**
Age	Young	196	13	6.63		
	Adult	234	34	14.52		
	Total	**430**	**47**	**10.93**	**6.833**	**0.006**
Sex	Female	213	23	10.8		
	Male	217	24	11.05		
	Total	**430**	**47**	**10.93**	**0.008**	**0.527**
Agro-ecology	Woynadega	123	11	8.94		
	Dega	233	28	12.02		
	Kola	74	8	10.81		
	Total	**430**	**47**	**10.93**	**0.783**	**0.676**
Species	Bovine	185	47	25.4		
	Ovine	130	0	0		
	Caprine	115	0	0		
	Total	**430**	**47**	**10.93**	**69.881**	**0.000**

Discussion

A total of 430 animals (small ruminants and cattle) were clinically and serologically examined for the presence of typical clinical signs and nonstructural protein for FMD. Out of these 122 (28.37%) animals were found manifesting clinical signs and lesions suggestive of FMD. The signs and lesions observed in sick animals were comparable with those reported by different researcher and literatures (Radostits *et al*., 2007; Quinn *et al*., 2005; McLaws *et al*., 2006).

From185 only 47 cattle (25.4%) animals were found seropositive. The highest seropositivity (18.75%) was found in Moretnajiru which indicates that the previous exposure of animals to FMD were present in the that District and these high percentages might be associated with the low immune states due to working power of cattle and the type of production system. These indicated that indigenous breeds appeared more positivity to the FMD virus's endemic region that might not showed the clinical episodes of FMD. But the lowest seroposetivity was found in Kombolcha that indicate the FMD might be present before certain periods.

On the other hand seroposetivity of small ruminants were almost almost all negative that indicates small ruminants are the carrier (reserviour) of FMDv that might not produce antibody The disease is considerably less obvious or sub-clinical in breeds of sheep, and goats indigenous to Africa and Asia, where FMD is endemic (Kitching 2002; Kitching and Hughes, 2002).

As the comparative of the seroposetivity with the age animals; as age increase seroposetivity also increases showed that age-specific seropositivitity have been revealed that adult and old animals were higher in seropositivity. This might be due to the cumulative experience of the population with the agent (FMDv) (Murphy *et al.*, 1999). Therefore, those animals aged greater than 3 years, might have acquired the infection from multiple serotypes, and could produce antibodies against those serotypes of FMD. The relatively low seropositivity in age group less than 3 years might be indicative of the existence of passive maternal immunity and low frequency of exposure (as they infected, the chance of mortality high).

The questionnaire survey on FMD indicated that the disease was endemic in most parts of the study areas and mostly occurred in the rainy seasons of the year. This observation agreed with Sangare *et al.,* (2004) also reported that FMD is more prevalent in the cold and rainy seasons in Mali from November to February and from June until September, respectively.

In the study areas, most animals used common pasture and watering points. There was no restriction on the movement of animals from region to region because of the absence of such regulations. This observation

was in agreement with Mersie *et al.* (1992) who reported that the high incidence of the disease in Ethiopia may be associated with extensive movement of livestock and the high rate of contact between animals at marketing and common grazing places as well as at watering points.

Vaccination against FMD was practiced in areas where cattle were kept in Sekota zones, Southwello zones (Legambo) and Semen shawa zone (Moretnajiru), respectively during the last 5 years in the districts of the study areas except some dairy farms. This finding was in agreement with Sahle *et al*. (2004) who stated that the only attempt to date by the government in Ethiopia to control the disease was by limited vaccination campaigns in dairy herds.

The morbidity rate of the disease was very high, reaching up to 32.4% in Dahana districts, in Sekota zone, and the mortality rate was very low (<2%) in all Districts. Furthermore, the mortality and case fatality rates were found relatively higher in, 1.6% and 6.08% in young (calves) than any the other age groups, respectively that are found in legambo districts. this result agree with Mersie *et al*. (1992) who indicated that outbreak of FMD in Ethiopia caused 6% mortality in calves. The mortality in calves might be due to cardiac involvement, and frequent deaths occurred due to acute heart failure. The morbidity rate of the disease was relatively higher in indigenous breed of cattle (25.5%) without consideration of the other breeds because this paper only involved indigenous breeds the fact that no exotic breeds in outbreak areas.

Out of seven serotype of FMD virus, the existence of serotype O confirmed during the study period from bovine samples collected from different Districts in Eastern Amhara region in Ethiopia. This is in agreement with Gelaye *et al*. (2005) who reported that serotypes O, A, and SAT2 were isolated from cattle during outbreaks from 1982 to 2000. Similarly, Gelaye *et al.* (2005) reported that serotypes O, A, C, SAT1, and SAT2 were also isolated during 1981–2007 from most regions of the country. Dejene, (2004) reported that serotypes O, A, C, and SAT2 were also isolated from samples collected during outbreaks in dairy farms in and around Addis Ababa. In 2005, serotypes O, A, and C were isolated in Ethiopia (FAO, 2007).

In this study, serotype O was responsible for most of the outbreaks. This showed that sero type O was highly prevalent and a dominant serotype causing outbreaks in Eastrn Amhara region, Ethiopia, and this finding agreed with the survey of Buxton and Faser, (1977) that there is a tendency for type "O" strain to occur most frequently in the outbreak area. Gelaye *et al*. (2005) and Ayelet *et al*. (2009) also reported that serotype "O" was a dominant FMD virus serotype circulating in Ethiopia. Klein, (2009) indicated that it is the most prevalent serotype worldwide of serotype O was investigated within 16 FMD tissue samples submitted to WRL for FMD. This also agrees with a previous study on molecular epidemiology of serotype O by Samuel and Knowles, (2001).

This suggested that the outbreaks due to these isolates were most probably spread by uncontrolled movement of animals, and these have a big risk on the transmission of the virus across the district in any directions because there is no strong animal movement regulation across the border and the ability of the virus to transmit with the wind. This statement is supported by Samuel *et al.* (1999) who demonstrated that closely related viruses could either be from the same outbreak or from viruses temporally closely related Sangare, (2005) also reported on the presence of transboundary (transdistrict) and transregional transmission of viruses in any direction which the FMD were present.

Serotype O isolated in three outbreaks in Eastern Amhara regional state especially in sekota zones, Moretnajiru and Legambo districts. This indicated that outbreaks due to these isolates were from the same origin. These might be due to free movement of livestock, and livestock products among various markets in different regions and states play an important role in the dissemination of the virus proved that these viruses were closely related with one other which indicated that these viruses isolated from the same outbreaks.

Conclusion and Recommendation

Foot-and-mouth disease is endemic in Ethiopia as in most parts of Africa. The presence of foot and-mouth disease in the country is a major obstacle to the development of agriculture because of its adverse effects on livestock production and agricultural exports. A total of 430 animals (small ruminants and cattle) were clinically and serologically examined for the presence of typical clinical signs and nonstructural protein for FMD. Out of these 122 (28.37%) animals were found manifesting clinical signs and lesions suggestive of FMD. There was a statistically significant difference observed among different age groups of cattle with mortality and morbidity rates ($p<0.005$). Morbidity rates of cattle in different age groups were different in young, and in adult. The age specific mortality and case fatality rates were higher in young (calves). On the other hand seroposetivity of small ruminants were almost all negative that indicates small ruminants are the carrier (reserviour) of FMDv In Ethiopia, factors such as the presence of high numbers of susceptible domestic animals, absence of regulation for prohibition of animal movement, presence of limited vaccination, high contact of animals at marketing and common grazing place as well as at watering points contributed to the occurrence of FMD and to the difficulty in

controlling the outbreaks. Based on the findings of this study the following recommendations are forwarded:

- ✓ To restriction of animal movement must be enforced to minimize further transboundary (Trans district) transmission of the disease During the study periods,
- ✓ Further detailed studies on the evaluation of available vaccines and the development of a vaccine which contains cocktails of antigens of FMD virus strains in the country should be encourage.
- ✓ Ministry of Agriculture and Rural development (MoARD) should design and implement an effective FMD control policy.
- ✓ There should be a legal guideline enforcing the control of livestock involvement with in the country and across the borders with neighboring countries.
- ✓ To reduce the mortality and morbidity of the disease on high risk groups of animals should be vaccinated using the circulating virus strain.
- ✓ Further investigation should be done to determinations roles of different hosts including small ruminants and wild animals.
- ✓ Development or adoption of viral diagnostic techniques should be encouraged in Ethiopia to diagnose accurately of this economically important viral diseases like FMD.

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Annexes

Annex 1: Summarized History of five outbreaks investigated from November2011 to March 2012**.**

1. Abergele district

Location, Abergele district found in Waguhamra zone of Amhara regional state, is located 84 km North, Sekota town.$12^0$47`35.2``N and $038^0$59`77.4``E Farming, semi-pastoral system Census 76,632 cattle 28,597sheep and 45,867 goats Breeds All cattle and small ruminants were indigenous breeds.

Outbreak history, the outbreak was reported on 5 January 2012. During investigation of the outbreak 25 clinical cases of FMD (all indigenous breeds of cattle) were observed and two tissue sampling were taken from cattle. Most of the affected cattle had healing lesion at that time. However small ruminants were subclinical with only 2 goats had showed slightly lame. The most likely source of infection was contact and free grazing with animals to Tigray region where FMD outbreak were present, geographical location might be contributed transmission through wind.

Sampling and examination: 18 cattle, 10 sheep and 10 goats. All most the cattle showed clinical signs but no suggestive clinical lesion were observed in either sheep or goats. The specified village in which FMD outbreak occurred so called Adezlay.

Pre-history: There was no outbreak of FMD in the area.

2. Zkola

Location –zkola district is 65km West of sekota town. It is one the seven district in waguhamrazone. It located $12^0$35`13.7``N and $038^0$45`47.2``E.

Farming system, semi pastural, mixed farming.

Census 70,642 cattle, 32,854 sheep and 53,486 goats.

Breed, all cattle sheep and goat are indigenous breeds.

Outbreak history: The outbreak was reported on January 2012. During investigation of the outbreak there were few cases with freshly ruptured vesicles in the mouth and on the feet, from which two epithelial tissue samples were taken from cattle. Around 20 cattle

of indigenous bread showed clinical signs but in shoats the subclinical disease were observed during the examination and sampling. This outbreak might be suggested that there have been contact at free grazing and might be through wind in nearby geographical district of the Abergele.

Sampling and examination: 18 cattle, 10 sheep and 10 goats were serum was collected and the outbreak occurrence village was Tsetska. The control of FMD by vaccination had never been attempted in this district.

Previous history: there was not an outbreak of FMD in the study area of Zkola, Village of Tsetseka.

3. Dahana

Location, Dahana district is found in Wague humera zone, 70 km South West of sekota town. It is located $12^0$27`58.2``N and $038^0$55`35.6``E.

Census 73,692cattle, 21,381 sheep and 48,693 goats Breed indigenous breeds of cattle, sheep and goats.

Outbreak history: There was a report of FMD outbreak on January 2012 which was the same as other outbreaks in Waguhamra zone. During investigation of the outbreak most the affected animals had freshly ruptured vesicles in the mouth and at the feets of cattle and most affected animals were in acute phase; with excessive salivation and rectal temperature above 40 ^{0}c. There were more than 27 animals shown clinical lesion. Only one goat was observed with vesicular lesion in the inter digital space of feet which result lame ness and only four tissue sample were taken. The outbreak was suggested to be precipitated by movement of animal for communal grazing and watering areas near by Zkola district where FMD outbreak occurrence and geo graphical location bordered by Zkola; by which wind might be involved in FMD transmission.

Sampling and examination: A total of 18, 10 and, 10 serum samples were taken from cattle, sheep and goats respectively. Three bovine tissue samples were collected. The outbreak occurrence villages were Menshewa and Amdewerk. Vaccinations of FMD have not been practiced in the area.

4. Mortanajiru

Location, moretenajiru district is found in Semen shoa zone of Amhara regional state at a distance of 70km West of Debreberhan town $09^0$89`339``N and $39^0$41`572``E.

Farming mixed farming

Census, 73,428cattle, 27,717Sheep and 41,299 Goats.

Bread, all indigenous bread

History of outbreak: The outbreak was reported on January 2012. The investigation revealed that there were about 24 cattle had shown freshly vesicular lesion in the mouth and feet which maked them inappetance and lame, lied down on the ground respectively. Four tissue sample were taken from cattle. Sheep and Goats yet have not showed clinical lesion. This FMD outbreak in the region (study district) was common after the introduction of exotic dairy breeds nearby areas (Deneba woreda). This outbreak was suggested that when immune status of animals lower due to the use of threshing power and low food access might be caused FMD outbreak not only this area but also neighboring woredas FMD outbreak occurred.

Sampling and examination: A totalof 18, 10 and 10 cattle, sheep and goats respectively. The village of outbreak occurrence was Wabin (Gulale). Vaccinations yet have not been practiced in the area.

5. Legambo

Location, -It's found 40km from Dessie town, in Amhara regional state.

$39^0$36`345``N and $10^0$80`208``E.

Farming mixed farming.

Census, 72,183 cattles, 118,191 sheep and12, 164 goats.

Breed, all indigenous breed.

History of outbreak: This outbreak was reported on 12 February 2012. It was known that around last days of January and continuous to the next month the year. The outbreak investigation had been revealed that there were about above 31 cattle clinically affected and most affected animals were acute phase with excessive salivation, vesicular lesion in the mouth, feet and teat. Even though small ruminants are subclinical one sheep and one goat showed that clinically lame. This outbreak was suggested be precipitated by uncontrolled movement of communal grazing watering due to FMD outbreak has been occurred far distance area last year was one suspected. In fact it was suspected that the second largest market in South wello was found near outbreak occurrence in which animals contact and dropping saliva in the area.

Sampling and examination: A total of 38 cattle, 30 sheep and 30 goats. Serum sample were taken from cattle, sheep and goat respectively. Four tissues from bovine were taken from the cattle. The FMD (Afteiger) had been encountered in the village of Tincha. Animals have never been vaccinated against FMD.

Annex 2: principle, reagents, materials, preparation of washing solution, procedure, and evaluating 3ABC-ELISA test.

Descriptive Priciples

Micro titer plates are supplied precoated with recombinant FMDV 3ABC viral. Dilution of samples to tested are incubated in the wells of these plates. Any antibody specific for 3ABC binds to the antigen in wells and forms an antigen/antibody complex on the plate well surface. Unbound material is removed from the wells by washing. A peroxides –labeled anti-igG conjugate is added, that binds to the antibodies of the sample complexed with the3ABC antigen. Un bound conjugate is removed by washing, and the TMB-

containg substrate is added to the wells. The degree color that develops (optical density measured at 450 nm) is directly proportional to the amount anti-body specific for 3ABC present in the sample. The diagnostic relevance of the result is obtained by comparing the optical density (OD) that develops in wells containing the sample with the OD from the wells containing the positive control.

Reagents

CHEKIT-FMD-3ABC-Microtiterplate, precoated with recombinant FMDV 3ABCprotein.

CHEKIT-FMD-3ABC-bo-ov-Anti-Ruminant-igG-PO-conjugate, labeled with horseradish peroxides.

CHIKIT-FMD-3ABC-bo—ov positive positive control serum.

CHEKIT –FMD-3ABC-bo-ov Negative control Serum.

CHEKIT-FMD-3ABC sample Diluents.

CHEKIT-10X Wash concentrates.

CHEKIT-TMB Substrate Solution.

CHEKIT-Stop solution TMB.

Specimen information:.

5µl of blood serum was needed for each sample well. fresh, refrigerated, or previous frozen serum may be tested.

Preparation of reagents:

Preparation of washing solution is needed for to know how much solution was required.

100x5x3ooµlx3x2=900000µl (the total volume of washing solution) =900000 µl

1000ml

900ml is the total volume of wash solution. To know the amount of sample diluents CV=c_2v_2

10xv =c_2v_2

10xv=1x900ml

V=90ml of washing solution was added to 810 ml distilled water to make 900ml of mixed or diluted washing solution.

Procedure

1.100µlof serum added to the micro titer plate using single channel pipette from serum that is found in cryovials.

2. 45µl of diluents added to another micro titer plates by using multichannel pipette.

3. 5µlof serum was added to micro titer plates that containing 45µl diluents from micro titer plates containing 100 µl of serum.

4.90µl of diluents added to pre-coated micro titer plate with recombinant FMDV3ABC antigen.

5. 5 µl of negative and positive control and 10 µl of prediluted anti body to all micro titer of pre coated with recombinant FMDV3ABC antigen.

6. Covered by plaster paper in order to avoid evaporation and agitated at in incubator at $37c^0$ for one hour in humid chamber.

7. 300µl diluted CHEKIT FMD 3ABCwashing solution were added to each wells by multichannel pipette and removed washing solution (3 times).

8. Then drying and aspirate by clean and disinfected clothes at inverted wells.

9. 100µl CHEKIT-FMD-3ABC-Anti-ruminant –igG conjugate that was labeled peroxides and dispensed in to each well and covered by plaster paper, incubated for 60 minutes at $37c^0$ in humid chamber.

10. Washing anti-immuglobin G by repeated step seven (7).

11. Repeat step eight (8) for drying and aspirate.

12. Then add 100µl of TMB – containing substrate in to each 96 well and incubated at room temperate for 15 minutes.

13. Finally adding 100µl CHEKIT-stopping solution TMB per each well.

14. Then the result was read by using by spectro photometer at 450 nm wave length within two (2) minutes of adding stopping solution.

The result was indicated that 47 samples were positive but others were negative with respect to the positive and negative control values. The negative control should not exceed 0.5 of spectro phometer reading value and the positive control should not 2 of photo spectrometer reading value.

Analyze the samples in relation to the negative and positive controls with the formula

$$\text{Value (\%)} = \frac{\text{OD sample} - \text{ODnegative}}{\text{ODposetive} - \text{OD negative}} \text{x100}$$

Value	<20%	≥20% to<30%	≥30%
Interpretation	Negative	suspected	Negative

But all the samples were have been clearly positive and negative.

Annex 3: To isolate the virus FMD required principle, reagents, materials, procedures and evaluating the cultured tissue sample.

Principle: to determine whether the tissue sample contains the virus or not. If the tissue sample contains virus cause specific morphological changes of cellular monolayer after 24hrs-48hrs of inoculation of the virus (cytopatic effect) in to the BHK cellular cultured flask that had Minimum essential media.

Reagents

Minimum essential media, BHK Cells, Pbs (phosphate buffer solutions) and antibiotics.

Materials.

Pestle and mortar, scissors, bottle, test tubes, centrifuge, laminal air flow, syringe, filter, incubater, micropipette tips and single channel pipette.

Procedures:

1. Take the sample from the deep freeze and put into incubator at 37.7C^0 to warm the sample and take out the sample from the incubators.

2. Take 1 gm of tissue sample and washed thoroughly using Pbs with antibiotics in the mortar.

3. Washed at least 3 times using Pbs has antibiotic and discard the Pbs after washing tissue sample.

4. Then crush the tissue samples using scissors in the mortar.

5. The tissue sample is further crushed using pestle and mortar until the tissue sample is not visible.

6. Add 9 ml of Pbs with antibiotic into the mortar using the pippet and mix tissue sample thoroughly.

7. Transferred the suspension into the test tube and add anti biotic, then centrifuge in the test tube at 3500 rpm for 10 minutes.

8. Take the supernatant from the test using syringe and inject in the bottle trough the filter paper.

9. Wash the BHK cell culture medium using Pbs solutions.

10. Inject 0.5 ml of filter solution in to cell cultured flask media.

11. Place the cell culture flask media in the incubator at 37 ^{0}c for 1 hr (absorption).

12. Discard the supernatant solution from the cell culture flask and 10 ml mm (minimum essential medium) in to cell cultured flask for only maintenance level if above this level cells became aged.

13. Incubate the cell cultured flask at 37C^0 until CPE is produced with in 24hrs-48hrs.

Result: There were morphological changes monolayers BHK cells (break down monolayer cells). Interpretation: The result indicated the morphological change was due to FMD virus presence in tissue.

Annex 4: Questionnaire Format for investigate the impact of FMD outbreak.

I. General Information

1. Name of the respondent _____

Sex _____ Age _____

2. Address zone _____ Woreda _____ Kebele _____

Longitude _____ latitude _____

3. House hold Leader

Level of education Illiterature ☐ Elementary ☐ Above ☐

4. Marital status:-

Not Married ☐ Married ☐ divorced ☐ windowed ☐

II. The impacts of outbreak in study area

1 how many animal populations do have----------

2 what are the animal population do have

a. Cattle ☐ c. goats ☐

b. sheep ☐ d. all species ☐

3. What are the diseases common at this time

a. sheep pox ☐ c. mange ☐

B. Foot and mouth diseases ☐ d. PPR ☐

E. Afteiger ☐

4. Which diseases characterize in both mouth lesion and lameness

a. Foot and mouth diseases ☐ c Afteiger ☐

b. maze ☐ d. afumar ☐ E. All ☐

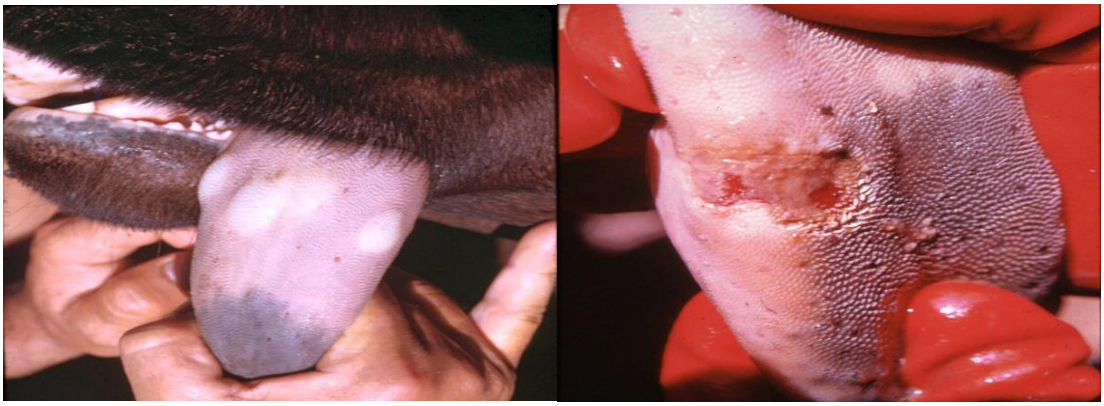
5. How many animals affected by this diseases----------

6. How many of which died due to Foot and mouth diseases----------

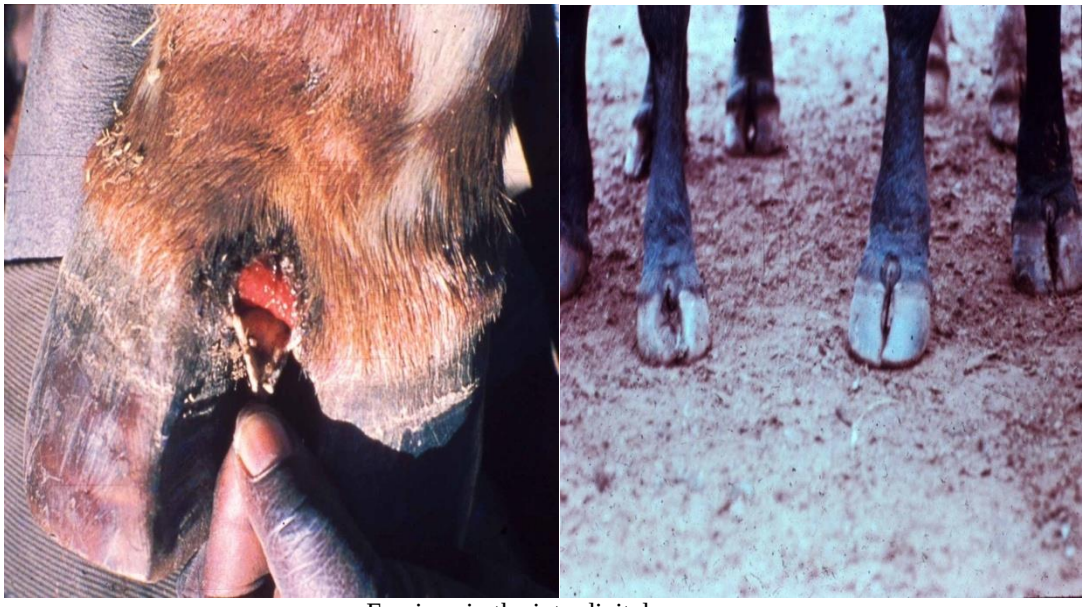
7. Which species mostly affected----------

8. Which age group mostly affected----------

Annex 5: FMD lesions found in different outbreaks



Vesicules and erosions oftongue



Erosions in the interdigital space

7/3/2017