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Out Break Investigation and identification of FMDV-Serotype Circulating in south west showa zone of Oromia region in Ethiopia

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ABSTRACT

Ethiopia is one of the countries in Africa with huge livestock resources. Despite this large resource base, the benefit derived from the livestock sector in Ethiopia is relatively low. Livestock diseases are among the many constraints which hinder the proper utilization of the resources for food security and national development. In Ethiopia Foot-and-mouth disease (FMD) is one of the contagious viral diseases that have great impact on economic development both in terms of direct and indirect losses. Despite the occurrence of several outbreaks in Ethiopia, only very few outbreaks are investigated for their economic impact and Confirmed by laboratory diagnosis. Proper outbreak investigation helps to identify the circulating serotype in the area to implement efficient vaccine-based FMD control strategy. Hence outbreak investigation was conducted from April 28 to May 10, 2020 in three districts of South west showa zone of Oromia region with the objectives of, detection and serotyping of FMDV circulating in the area. Purposive sampling was carried out and total of 150 accessible cattle were examined in the three districts and in the respective kebeles. 36% (n = 25/69) showed clinical signs of the disease. Appropriate Samples (7 tissue,8 probang and 10 swab) were collected from 25 clinically sick and healed cattle and processed in pool (pooled in to 15),first submitted for viral isolation in the cell culture laboratory and detection and serotyping of the virus has been done in the viral serology laboratory of NAHDIC. CPE is detected in 10 of the fifteen pooled samples.Atotal of 10 samples Four from Woliso, four from Ameya and two samples from Goro districts are positive for FMDV serotype SAT 1 tested with sandwich ELISA. Vaccination has been the main strategy of control of FMD in endemic area, therefore effective vaccination with formulated compatible vaccine containing SAT 1 recommended in each kebeles of the study area. Regular monitoring and early detection of FMD outbreaks is necessary to have further molecular based studies.

Keywords: Cattle, Outbreak, FMDV, Southwest showa, Oromia, cell culture, Sandwich ELSIA, Ethiopia

1. INTRODUCTION

Ethiopia is one of the countries in Africa with huge livestock resources, with an estimated population of about 60 million cattle, 30 million sheep, and 30 million goats (CSA, 2017)and whose agricultural sector is the biggest to its grand domestic product (GDP) 48% and the major contributor to its export earning is 90% (Tesfaye,2008).

According to Tesfaye(2008),livestock ownership currently contributes to the livelihood of an estimated 80% of the rural population in Ethiopia. Despite this large resource base, the benefit derived from the livestock sector in Ethiopia is relatively low. Livestock diseases are among the many constraints which hinder the proper utilization of the resources for food security and national development.Foot and mouth disease (FMD) is one of the most important and highest priority livestock diseases globally (FAO-OIE, 2012). In endemic regions of the world its annual economic impact in terms of visible production losses and vaccination costs is estimated between US$6.5 and 21 billion, whereas outbreaks in FMD free countries and zones cause losses of more than US$1.5 billion a year (Knight-Jones and Rushton, 2013).

In Ethiopia Foot-and-mouth disease (FMD) is one of the contagious viral diseases that have great impact on economic development both in terms of direct and indirect losses.According to the Office International des Epizooties (OIE), FMD ranks first among the notifiable infectious disease of animals (Alexanderson et al 2003).

FMD is caused by the genus Aphtho virus, from the family Picornaviridae which has seven distinct serotypes namely: A, O, C, SAT1, SAT2, SAT3 and Asia1 (Radositis et al 2000). Each serotypes of FMD virus is antigentically distnict (Kitching, 1987). All the seven serotype produce a disease that is clinically indistinguishable but immunologically distinct.Sofar it is believed infection with one serotypes does not confer immunity against the other (Richard, 1998).Sero surveys in different parts of Ethiopia reported FMD with different degrees of

prevalence reaching up to 26 %(Megersa et al 2009; Molla et al 2010; Bayissa et al 2011; Yahya et al 2013;Mishamo et al.,2018;Mekdes et al 2019). Outbreak incidence studies have also indicated that FMD occurs throughout the country with significant variation in geography and production systems (Jemberu et al 2015). Among the seven serotypes of FMDV, four of them (O, A, SAT 1, and SAT 2) have been reported in Ethiopia (Ayelet et al 2009;Jemberu et al 2015;,Beksisa &Daniel,2018;Sulayeman et al.2018).

The disease has an incubation period of 3-14 days.Excretion of the virus from the infected animals usually begins before the appearance of visible clinical signs (Kitching, 2002). Initial virus multiplication occurs mainly in the pre-pharyngeal area and the lung (Burrows, 1982). Irrespective of the portal of entry, once infection gains access to the blood stream, the virus shows a predilection for the epithelium of the mouth and feet and to less extent, the teat. Basically FMD is characterized by the appearance of vesicles in and around the mouth, feet and sometimes on the udder and teats.Loss of appetite,lamenes sand sudden drop in milk production are common symptoms. Mostly the lesions are susceptible to primary bacterial infection, at this stage, the animals are reluctant to eat and move. Death in calves may occur due to virus infection of the developing heart muscle. In susceptible animal population, morbidity reaches 100% but mortality is low especially in adults. (Woodbury, 1995).

Despite the occurrence of several outbreaks in Ethiopia, only very few outbreaks were investigated for their economic impact and Confirmed by laboratory diagnosis. Proper outbreak investigation helps to identify the circulating serotype in the area to implement efficient vaccine-based FMD control strategy. Therefore the current study was undertaken to investigate circulating serotypes of FMD that occurred in different district of South west showa zone of Oromia region, Woliso, Ameya and Goro districts.

2. MATERIALS AND METHODS

2.1 Description of the Study Area:

The study was conducted from 28 April- 10 May 2020 in the three district of South West showa zone in Oromia region of Ethiopia, which is located 114 km south west of Addis

Ababa.The study kebeles were Abado hole and Arbagaden kura from Ameya district; Gembela kebele from Woliso district and Gururа and Wayu kebeles from Goro district.The Zones have got a total land area of about 2.17 million hectare of land, and divided into three agro climatic zone. The low land located below 1500 m a.s.l which caver 17% of the total land area, the mid high land attitude from 1500 to 2500 m a.s.l covers 61% of the total land area and the high land cool temperature located above 2500 m a.s.l that covers 22% of the study area.South west showa zone has two rainy seasons, the long rainy season covering most of the place and occurs from June to September and the short rainy season occurs March to April with an average annual rain fall being 2900 mm.Almost 85% of the total land coverage used for crop production where as 15% is used for animal grazing. Generally the climate of the study area is suitable for both agriculture and livestock production.

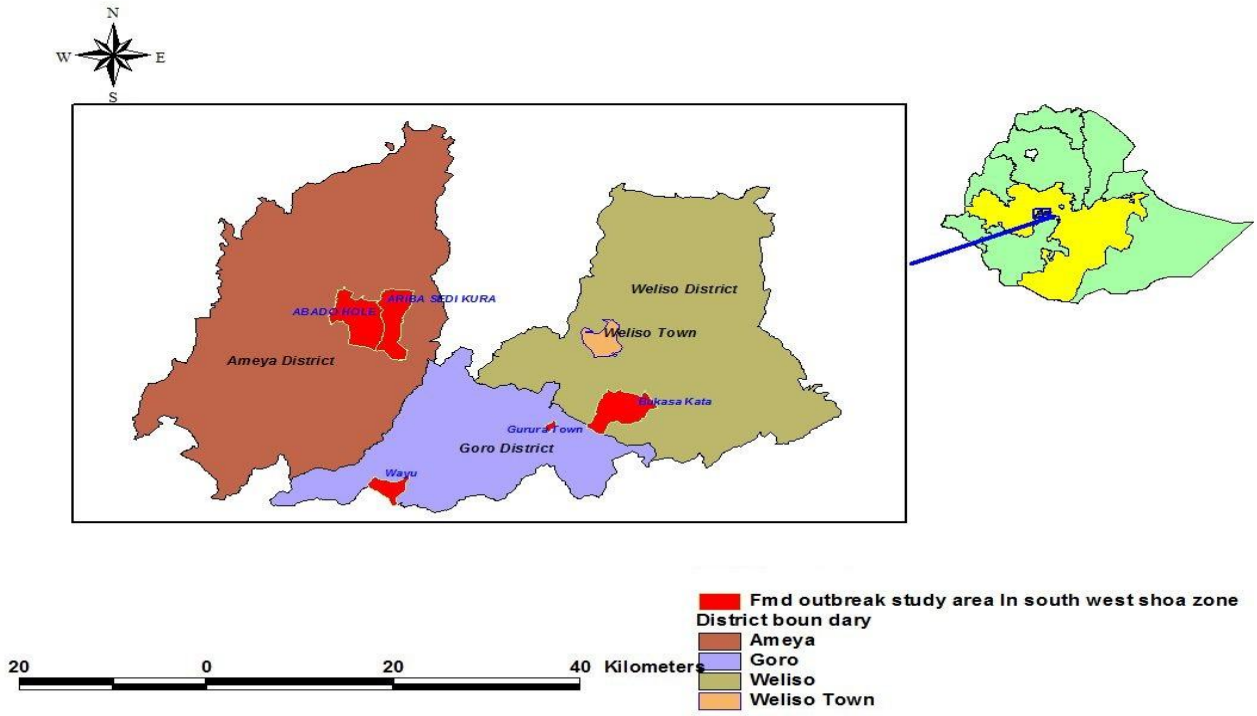

Figure 1: Map indicating study area of South west showa zone of Oromia region

2.2 Study animals:

The livestock population of the study area is estimated at 2.3 million of cattle, 619,000 Sheep, 172,000 Goat, 283,000 Equine and 1.4 million poultry. Agriculture, which is the main economy

sector of activities of the zones, provides livelihood for more than 90% of the population. The study was conducted in all age of cattle that were kept under extensive livestock production system.

2.3 Study Design:

Field level investigation and sampling was conducted purposively at a particular site of outbreaks within the study districts in different villages from April 28 to May 5, 2020 to determine the serotypes of circulating FMDV in clinically affected animals. Animals having clinical symptoms like lameness, and history of infection but having healed lesion were included in the purposive sampling.

2.4 Sample collection

As stated on OIE, 2012 animals that have manifested the signs of disease such as visible typical vesicular lesions or ruptured vesicle in the oral cavity, on the tongue, on the feet and teat as well as those having excessive salivation, lameness, anorexia and rise in temperature were considered as FMD clinically sick animals. In each outbreak area, as previously stated,Purposive sampling technique was applied. The mouth cavities of salivating animals were opened and examined for evidence of intact or ruptured vesicles and erosions on the tongue, dental pad, and mucosa of the oral cavity. The hooves of lame animals were washed with water and then examined for similar lesions on the coronary bands and inter digital spaces of the hooves. Other animals in the herd without these signs were similarly examined but sampling was done only when active lesions and suggestive of FMD cases were observed. About 1-2 gm Epithelial tissues collected from freshly ruptured vesicles and lesions from the interdigital space and swab samples were taken and placed in a universal bottle containing viral transport medium composed of equal amount of glycerol and 0.04M phosphate buffer saline (PBS) at pH 7.2–7.6 with some antibiotics and antifungal. Additionally Probang samples were collected from convalescent cases of FMD in the area by a probang cup and poured into 15 ml conical tube containing virus transport medium. A total of 10 swabs, 7 tissue and 8 probang samples were collected. All samples were properly labeled with district code, identification number, Species, Age and sex ,after packed transported to NAHDIC,

Sebeta by maintaining the cold chain in icebox containing ice packs during transportation and kept at -80^{o}C until laboratory tests have been conducted.

3. LABORATORY GIAGNOSIS

3.1 Virus Isolation:

The epithelial tissue samples collected and stored in freezer were thawed at room temperature and washed three times using sterile PBS at a PH of 7.2,while freezed swab and probang samples thawed under Bio-safety cabinet class II.the collected 25 samples pooled in to 10 based on their sample type and collection site. About 1 gm of epithelial tissue sample was grounded using sterile mortar and pestle by adding 10ml of sterile PBS containing antibiotic and antimycotic. The tissue suspensions were centrifuged at 5000 rpm for 15 min.The probang and swab samples individually centrifuged at 5000 rpm for 15 min then the supernatant was collected and filtered by Millipore filter of 0.22 m pore size. About 1ml of filtered suspension was inoculated on baby hamster kidney (BHK-21) monolayer cells grown on 25cm tissue culture flask and incubated at 37°C with5% CO2 in a humidified incubator for 48hrs. Cells were monitored for cytopathic effect (CPE) daily and frozen when CPE was exhibited. A second passage was performed on those samples not presenting CPEfollowing the same procedure as the first pass. According to OIE 2004, Samplesnot exhibiting CPE by 72 hours post-infection on thesecond passage was considered as negative,therefore those CPE positive samples transferred to Viral serology laboratory for FMDV detection and typing ELISA test.

3.2 Serotyping Sandwich ELISA

Serotyping was performed by antigen detection sandwich ELISA with selected combinations of anti FMDV monoclonal antibodies (MAbs), used as coated and conjugated antibodies. The test was applied for detecting and typing of FMD viruses. The kit was designed for detecting and typing of FMD viruses serotypes such as type O,A,C,Asia1,SAT 1 and SAT 2. The micro plates were supplied with catching MAbs.

10 samples were tested on a single micro plate containing 96 wells, one positive control for each FMD types O,A, C Asia1,SAT1 and SAT2 and negative controls were included in each plate. These controls were already incorporated into the ELISA micro plate trapped by the respective catching MAb.According to the manufacturer procedure: samples were diluted in 1:2 ratio in diluents buffer and 50µl of each diluted sample was distributed in 80 wells of column 1 to 10 Then, 50µl of sample diluents added in all wells of column 11 and 12 (positive and negative control respectively) then plates were incubated at 25˚C for 1hour. After incubation, all fluids on the plates were discarded and the remaining residual fluids were removed. Then 200µl of washing solution were added and incubated for 3min at room temperature, subsequently wells were emptied and the washing repeated twice (three washing cycles in total). Then all residual fluids were removed by tapping on clean absorbent paper and 50µl of conjugate A was added in all wells of rows A to F and the same volume of conjugate B was added in to all wells of rows G to H . Plates were covered and incubated at room temperature for 1hour. After incubation 50µl of substrate solution was added for color development to all wells and incubated at room temperature for 20minutes in the dark. The reaction was stopped by adding 50µl of stop solution (sulfuric acid (H2SO4)). Immediately after stopping, the color development the plate read at 450 nm wavelength using micro plate reader. The interpretation was done according to the manufacturer test protocol.

4. **RESULT**

4.1 Clinical examination

At the time of field clinical examination 36% (n = 25/69) cattle showed signs and lesions suggestive of FMDV infection. The major important clinical signs observed during the field trip were more of feet lesion on inter-digital space and the coronary bands of the infected animals(Figure 2). In Some cases the hooves of affected animals tended to separate from the coronary bands and reluctant to move and lagging behind herds and refusal of grazing were

observed, where as no apparent lesion were detected in and around the mouth and the udder or teat of clinically sick animals.

Fig. 2: Severe lesions at the skin-hoof junctions of 2 years and 8 month cattle

Table: 1 FMD outbreak districts with respective kebeles

Outbreak district	Kebeles	No of animal examined	No of affected animals	No of deaths
Ameya	Abadohole	15	4	4
	Arbaseden kura	10	4	
Woliso	Bukessa keta	15	8	1
Goro	Gurura	17	8	4
	Wayu	12	1	
Total		69		

4.2 Virus Isolation

From 15 pooled samples containing 7 epithelial tissues, 8 oropharengeal fluid and 10 swab samples collected from the three study sites Woliso,Ameya and Goro were subjected to virus isolation.CPE characterized by destruction of BHK-21 monolayer cell within 72 hrs was registered for 5 epithelial, 3 Oro-Pharyngeal fluid and 2 swab samples.

4.3 FMDV detection and serotyping ELISA

10 pooled cell culture suspension showing CPE were subjected for FMDV detection and serotyping Sandwich ELISA for the presence and serotyping of FMD viral antigen. From 8 samples taken from Woliso district tested in pool, four were positive for FMDV, whereas out

of eight samples taken from Ameya district and tested in pool, four were positive and out of 9 samples taken from Goro district, two were positive for FMDV (Table 2). All positive samples were identified to be FMDV serotype SAT 1.

Table 2. Showing Study area, sample type and its respective result

District	kebele	Species	Typeof sample	CPE status	Serotype identified
Woliso	Bukessa keta	Bovine	tissue	++	SAT 1
Woliso	Bukessa keta		tissue	++	SAT 1
Woliso	Bukessa keta		probang	++	SAT 1
Woliso	Bukessa keta		probang	_	ND
Woliso	Bukessa keta		sawb	++	SAT 1
Woliso	Bukessa keta		sawb	_	ND
Woliso	Bukessa keta		sawb	_	ND
Woliso	Bukessa keta		sawb	_	ND
Ameya	Hole		Tissue	_	ND
Ameya	Hole		Probang	_	ND
Ameya	Hole		Swab	++	SAT 1
Ameya	Hole		Swab	++	SAT 1
Ameya	Arbasaden		Tissue	++	SAT 1
Ameya	Arbasaden		Probang	++	SAT 1
Ameya	Arbasaden		Swab	_	ND
Ameya	Arbasaden		swab	_	ND
Goro	Gurura		tissue	_	ND
Goro	Gurura		tissue	+	ND
Goro	Gurura		tissue	+	ND
Goro	Gurura		probang	_	ND
Goro	Gurura		probang	++	SAT 1
Goro	Gurura		probang	_	ND
Goro	Gurura		probang	_	ND
Goro	Gurura		Swab	+	SAT 1
Goro	Wayyu		swab	+	ND

Oromia region

ND-Not detected CPE=Cytopatic effect,++=positive,_=negative

5. DISCUSSION

According to Quinn et.al, 2002 description, Foot and mouth disease (FMD) is a contagious acute viral disease of animals characterized by formation of vesicles in the mouth, on the feet, teats and sudden death of young stock and known to causes large scale economic losses and halt exports of animals and animal products. During this study the veterinary professionals

in the area informed us, FMD has been occurred every year in the area however vaccination against this disease largely not being practiced except only in few dairy herds containing exotic animals.

The results of the present study indicated that out of the total number of 69 cattle examined during the outbreak 36% (25/69) animals manifested clinical signs and healed lesions suggestive of Foot and mouth disease. This finding related with the previous clinical findings of Belachew (2014) who reported during outbreak of FMD in Ethiopia, 36.9% of animals manifests clinical sign of Foot and mouth disease. Another authors (Legesse, 2008) and(Nigussie *et al.*, 2011) reported that 53% and 28.2% sick animals showed clinical signs after conducting outbreak investigations in different parts of the country. In the current study the clinical lesion of the disease is limited to leg of the infected animals. This was justified by earlier reports by Kitching *et al*. (2005) and McLaws *et al*. (2006) who described that variations in clinical manifestations and severity were associated with the virus strains, infection dose of the virus and susceptibility of the host. In the study, FMD virus was isolated from all the three districts. FMDV serotype SAT 1 was identified to be the cause of the outbreak. In previous study seroprevalence of FMD was conducted in these areas and reported as 22% (Shanko *et al.,* 2015) but it is the first attempt to investigate the circulating serotype of FMDV that was responsible for the cause of the outbreak in the south west showa zone specifically in the three districts: Ameya, Woliso and Goro. The FMDV causing 2020 outbreaks in the study area were not closely related to previous Ethiopian isolates reported by Mishamo et al(2018) in the central part of Ethiopia. These new introductions are likely to have happened through uncontrolled trans-boundary movements of animals, which constitute a significant risk for virus transmission by crossing border from any directions. This is explained by due to lack of strong animal movement regulation across the border and with in the country and also the ability of the virus to convey long distance with the wind.

6. CONCLUSION

FMD is endemic in Ethiopia with variation in prevalence across several regions and zones of the country. High prevalence of FMD antibody was reported by Shanko and his colleague in

2015,at the time they recommend characterization of the circulating FMDV serotypes in the districts. Accordingly the current finding confirmed the circulating FMDV in Woliso, Ameya and Goro districts was identified to be single serotype SAT 1. FMDV serotype SAT 1 was reported in these areas for the first time.

7. RECOMENDATION

Regular monitoring of FMD outbreaks to have more detailed information of FMDV serotype and conducting Phylogenetic analysis is required. Vaccination has been the main strategy of control of FMD in endemic area, therefore effective regular vaccination with formulated compatible vaccine containing SAT 1 is recommended. Vaccination alone is unlikely to control the disease; therefore animal movement controls strategy should be initiate in the country.

LIST OF ABBREVATION

BHK-21	Baby Hamster kidney 21 Cell
CSA	Center of Statistics Agency
CPE	Cyto Pathic Effect
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
MAb	Monoclonal Antibody
OIE	World Animal Health Organization
SAT1	Southern African Territories Type 1
SAT 2	SouthAfrican Territories Type 2
US	United State

DECLARATION

Ethical approval and consent to participate

No need of ethical clearance

Consent for publication

No need of permission

Availability of data materials

The data and materials are available

Competing interest

None of the authors of this paper have financial or personal relationship with other people or organization that could inappropriately influence or bias the content of this paper by any means.

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Author Contribution

Ayelech , Kebede and Dr Dereje contributed equally in the entire work of the study.

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