Epidemiological and clinical characteristics of the foot and mouth disease outbreaks in cattle in central Ethiopia

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Abstract

Foot and mouth disease (FMD) is an acute highly contagious viral disease of all cloven-hoofed animals that causes significant economic problems in Ethiopia. The objectives of this study were to assess the morbidity and clinical features of FMD in sick cattle and identify causal serotypes of FMD outbreaks in central Ethiopia. Outbreaks of FMD were investigated in a total of 150 herds of cattle from January 2021 to April 2021. Seven epithelial tissue and 23 oral swab samples were collected and subjected to a real-time polymerase chain reaction (rRT-PCR) and Sandwich Enzyme-linked Immunosorbent Assay (ELISA) for detection and serotyping of FMD virus, respectively. A total of 150 herds of cattle were examined, of which 114 (76%) herds of cattle were clinically affected with FMD. In this study, 75.9% animal-level morbidity was recorded. Exotic breeds and adult cattle were more affected by Foot and Mouth Disease Virus (FMDV) with morbidity of 100% and 77.4%, respectively. The clinical features in sick cattle showed that profuse salivation was the most frequently observed clinical sign (40%) followed by oral cavity vesicle formation (30%), and interdigital space lesion (15%). Out of 30 samples subjected to rRT-PCR and ELISA test, 28 (93.33%) and 27 (90%) samples were found positive, respectively. In this study, three types of FMD serotypes were detected in which SAT-2 (n = 13) was the predominant serotype followed by serotype O (n = 9), and serotype A (n = 5). The current study revealed that FMD serotype SAT-2 was highly responsible for the occurrence of FMD outbreaks in central Ethiopia. Although the FMD vaccine produced in Ethiopia contains all the identified

serotypes, detailed studies on topotypes identification have to be performed to provide full protection.

Keywords: Cattle; Central Ethiopia; Epidemiology; FMD; Outbreak; Serotypes

Introduction

Ethiopia is one of the countries that possess a huge number of livestock populations in Africa, and where 17% of the National Gross Domestic Product and 39% of the Agricultural Gross Domestic Product (GDP) is directly contributed by the livestock sector (Shapiro *et al.,* 2017). However, compared to its potential the contribution of the livestock sector to the national economy is still minimal. Although there is substantial international demand for live animals and animal products, the exports of livestock have suffered from repeated trade bans due to importing countries' concerns over animal diseases. Among the transboundary animal diseases, foot and mouth disease (FMD) is the most important livestock disease that has a significant socio-economic impact on Ethiopia (Shapiro *et al*., 2015). FMD is consistently ranked as the most economically important viral disease, and among the top important livestock diseases in Ethiopia (Shiferaw *et al*., 2010; Jibat *et al*., 2013).

FMD is an acute highly contagious disease of cattle, sheep, goats, pigs, and cloven-hoofed wildlife species (Davies, 2002; Kitching *et al*., 2007). The disease is characterized by fever and vesicular eruptions on the tongue, feet, snout, and teats, and sudden death in young animals (OIE, 2021). FMD is caused by a non-enveloped RNA virus within the family *Picornaviridae* and genus *Aphthovirus*. Foot-and-mouth disease virus (FMDV) exists in seven distinct serotypes; A, O, 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 (Davies, 2002; OIE, 2021).

FMD is endemic in Ethiopia with almost five out of seven serotypes prevailing so far (O, A, C, SAT-1, and SAT-2) (Ayelet *et al.,* 2009; Negusssie *et al.,* 2011; Jemberu *et al.,* 2016). Several outbreaks are reported each year due to several factors, including the virus's complex epidemiological nature, its wider geographical distribution and host range (Davies, 2002), the ability to establish carrier status (Alexandersen *et al.,* 2002), poor cross-immunity caused by

antigenic diversity (Domingo *et al.,* 2005), and the relatively short duration of immunity (Belsham, 2020).

Although the mortality rate of adult animals is very low, FMD causes high morbidity. Despite the impacts of the disease, there is limited government strategy to control the disease. This is evidence of the increasing occurrence of FMD outbreaks since 1990 (Ayelet *et al*., 2009; Wubshet *et al*., 2019) throughout the country due to high numbers of susceptible animals, sharing grazing lands and watering points in places where wild animals reside as well as lack of control of animal movement (Negusssie *et al*., 2011), and no official control strategy except vaccination in some market-oriented farms (Jemberu *et al*., 2020). Currently, a trivalent vaccine with serotypes O, A, and SAT-2 is used as a prevention method in Ethiopia (Tesfaye *et al*., 2020). In endemic countries like Ethiopia, the first step for the control of FMD is understanding the epidemiology of the disease in the country. Thus, continuous epidemiological studies including the identification of FMDV serotypes circulating in the field are important to aid the selection of appropriate vaccine strains. This will help to improve the currently used polyvalent FMD vaccine in the country and design effective FMD control programs. Therefore, the objectives of this study were to assess the morbidity and clinical features of FMD in sick cattle and identify causal serotypes of FMD outbreaks in central Ethiopia.

Materials and methods

Description of study areas

FMD outbreaks were investigated from January 2021 to April 2021 in central Ethiopia such as Bishoftu, Modjo, Fitche, Debre birhan, and Addis Ababa (Figure 1.)

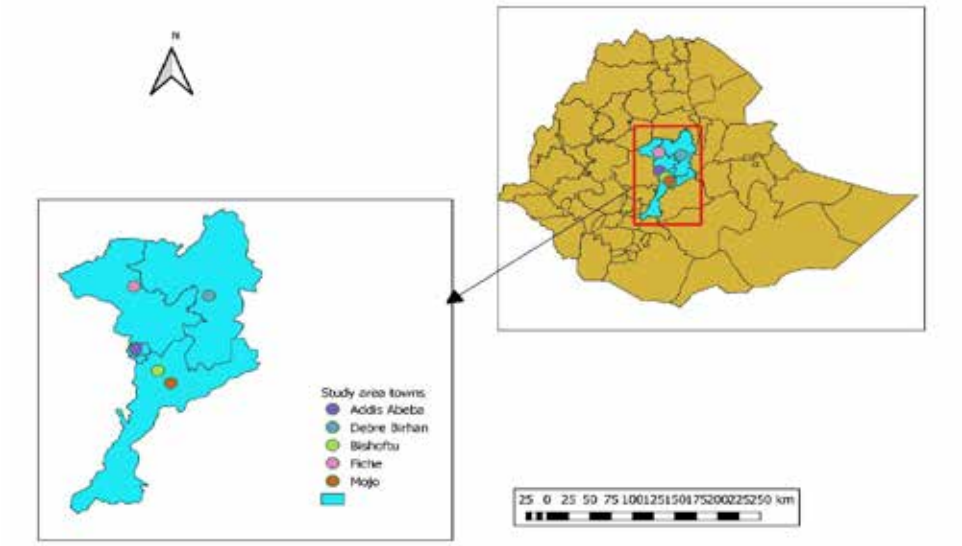

Figure 1: Map of Ethiopia showing outbreak investigated areas

FMD outbreaks were investigated in Addis Ababa city administration especially in the Akaki-Kality sub-city, which is located at 9° 2,'N latitude and 30°42'E longitude and lie at an altitude of 2,050 meters above sea level (masl). Outbreaks of FMD were also investigated in the North Shewa zones of Amhara (Debre Birhan) and Oromia (Fiche) regional states. Geographically, Debre Birhan is situated at an altitude of 2,780 masl and 9°36'N latitude and 39°38'E longitude whereas Fiche is found at an altitude of 2,738 masl and latitude and longitude of 9°48′N and 38°44′E. The total cattle population of the zones is estimated to be 1,704,407 and 1,323,045, respectively (CSA, 2021). Outbreaks were also investigated in the Bishoftu and Modjo districts of East Shewa zone, Oromia regional state. Bishoftu is located between 8°43`- 8°45`N latitude and 38°56`-39°01`E longitude and has an elevation of 1,920 masl in the central highlands of Ethiopia whereas Modjo is located at 8°39′N 39°5′E with an elevation between 1788 and 1825 masl. East Shewa zone has a total of 1,109,685 cattle population (CSA, 2021).

Study population

The target populations were cattle managed in various production systems (intensive, semi-intensive, and extensive) in central Ethiopia. Investigations of FMDV infected farms were conducted in 150 herds of cattle in central Ethiopia. During outbreaks of FMD, the data collected in each herd include the age, breeds (local, exotic, and crossbred), sex, and clinical features of the disease were recorded and appropriate representative samples were collected. In this study, the age of animals was categorized as an adult (> 3 years old) and young (< 3 years old) according to Mesfine *et al*. (2019).

Study design

For this study, FMD outbreak was declared when at least one animal showed typical clinical signs of FMD, including vesicles/lesions on the feet, mammary glands, and the oral cavity in a farm or herd. An outbreak investigation was conducted from January 2021 to April 2021 in central Ethiopia. When active cases of FMD outbreaks were reported, field investigations were performed at the particular site of the outbreak. In each outbreak, animals were clinically examined from a distance for evidence of salivation and lameness. Animals that showed suggestive clinical signs such as salivation and/or lameness were thoroughly examined. The mouth cavities of salivating animals were widely opened and examined for the presence 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 the presence of lesions on the coronary bands and interdigital spaces. Appropriate samples were randomly collected for identifying the causal FMD serotypes.

In each outbreak, the number of animals at risk, age, and sex of animals, the number of affected and died due to FMD were recorded. Animal-level morbidity was determined as the number of clinically affected animals divided by the total number of animals at risk. The herd-level morbidity was determined as the number of positive herds (herds with one or more clinically affected animals) divided by the total number of herds surveyed. Mortality was determined as the number of animals that died due to FMD divided by the total number of animals at risk (Thrusfield, 2005).

Sampling and sample preparation

Epithelial tissues samples were collected with viral transport medium (VTM) containing an equal amount of glycerol and 0.04M of phosphate-buffered saline (PBS) supplemented with 1µg/ml gentamycin (Invitrogen, Paisley, UK), 1 mg/ml streptomycin (Certa, Braine l'Alleud, Belgium), 1 mg/ml kanamycin (Sigma, St. Louis, MO, USA), 1000 U/ml penicillin (Continental Pharma, Puurs, Belgium) and 5 µg/ml amphotericin B (Bristol-Myers Squibb, New York, USA) (OIE, 2021). Oral swabs were also collected with cotton-tipped swabs by swabbing under the tongue and areas of contact between the lower gum and inner surface of the lower lip and then immersed in 2 ml of VTM into sterile plastic screw-cap tubes as recommended by OIE (2021). Samples were labeled and transported using an icebox containing ice pack to the National Animal Health and Diagnostic Investigation Center (NAHDIC) and placed at -20 °C until analysis.

About 1 gm of epithelial tissue sample was grounded using sterile mortar and pestle by adding 10ml of sterile PBS containing antibiotics. The tissue suspension was centrifuged at 5000 rpm for 15 min. About 1ml of filtered suspension was taken for analysis. Similarly, the swab samples were vortexed and then centrifuged at 5000 rpm for 15 min, and then about 1ml of suspension fluid was taken for FMDV detection.

Extraction of viral RNA

Total RNA was extracted from 140 µL suspensions using QIAamp Viral RNA Mini Kit (Qiagen, USA) according to the manufacturer's instructions with the final volume of 60 µL of RNA. Extracted RNA was kept at −80 °C until further analysis.

Detection of FMDV by real-time RT-PCR

The one-step real-time reverse transcription-polymerase chain reaction (rRT-PCR) assay was applied for primary detection of the FMDV. Real-time RT-PCR was performed using primers and probes targeting the conserved 3D(pol) gene encoding the viral RNA-dependent RNA polymerase according to Callahan *et al*. (2002). Universal primers were used to detect the presence of FMDV RNA; FMD 3D forward (5'ACT GGG TTT TAC AAA CCT GTGA-3'), FMD 3D-

reverse (5'GCG AGT CCT GCC ACG GA-3'), and TaqMan probe (5'- [6FAM] TCC TTT GCA CGC CGT GGG AC [TAM]-3').

Real-time RT-PCR was carried out using a Superscript III platinum Taq one-step rRT-PCR kit (Invitrogen™). The optimal volume of nucleic acid was 5 µl per reaction, which was added to 20 µl of rRT-PCR reaction mix containing 12.5 µl of 2× reaction mix, 0.5 µl of Superscript III/Platinum Taq enzyme mix, 2 µl of each forward and reverse primers, 1.5 µl of Tag man Probe and 1.5 µl of RNAse-free water. Negative control (nuclease-free water) and positive control (field isolate) were included in each run. The optimal thermal cycling conditions were done with reverse transcription (cDNA synthesis) at 50°C for 30 min, followed by 50 cycles of initial denaturation at 95°C for 10 min, denaturation at 95°C for 15 sec, annealing at 60°C for 1 min, and extension at 72°C for 30 seconds. One positive and 1 negative control were included in each reaction.

The PCR amplification was carried out in the thermal cycler Rotor-Gene Q (Qiagen®, Germany). FMDV was detected through threshold cycle (Ct) values based on baseline and graphs. The Ct value < 32 is classified as positive, samples with 32 < Ct < 40 were ambiguous and marked for retesting and no Ct (undetected) was considered as negative.

Serotype identification using Sandwich ELISA

FMD virus serotype identification was performed using antigen-capturing sandwich ELISA (IZSLER, Brescia, Italy). This test kit has diagnostic specificity of 99.7% and a sensitivity of 93.9% according to the information of the manufacturer's validation data report (Brocchi *et al*., 2006). The kit was precoated and conjugated with monoclonal antibodies (MAbs) that can detect and typing of FMDVs of type O, A, C, Asia-1, SAT-1, and SAT-2. The test was performed according to the manufacturer's guidelines. The optical density of each well was measured at a wavelength of 450 nm using Microplate Reader and the result was interpreted according to the kit protocol.

Data Management and Analysis

Data generated from field and laboratory investigations were recorded by using a Microsoft Excel spreadsheet. Risk factors and serotype identification results were recorded and tabulated. Descriptive statistical analysis was used

using SPSS version 20 software (SPSS Inc., Chicago, USA) for the different variables.

Results

Characteristics of outbreaks

In this study, of 150 herds of cattle surveyed, 114 (76%) herds of cattle showed suggestive clinical signs of FMD (Figure 2).

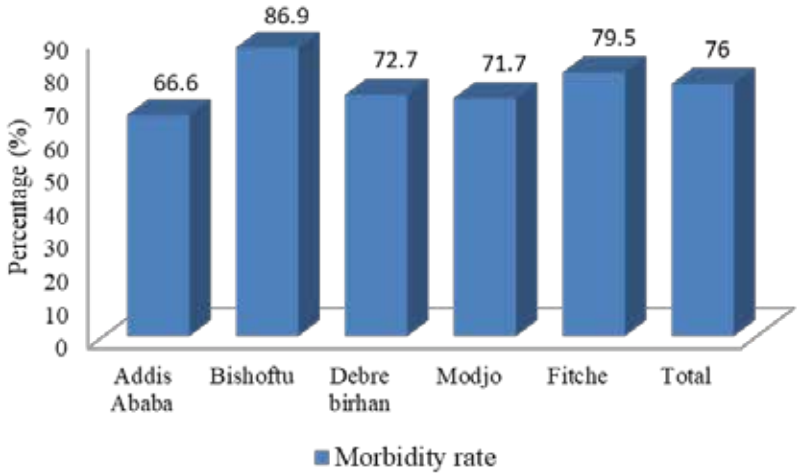

Figure 2: Foot and mouth disease morbidity rate at herd level

In this study, death associated with FMD was not recorded. However, 75.9% morbidity rate was recorded at the animal level in which exotic breeds were more affected with the morbidity rate of 100%, followed by crossbreed (79.8%) and local breed (64.7%). Similarly, high morbidity was recorded in adults (59.36%) compared to young (40.64%), and in females (54.75%) than males (45.25%) as shown in Table 1.

Table 1. Animal level morbidity rate with the different risk factors

Variables	Animals examined	FMD infected animals	Morbidity rate (%)
Sex			
Female	617	459	74.3
Male	510	397	77.8
Age			
Young	458	338	73.7
Adult	669	518	77.4
Breed			
Local	40	263	64.7
Crossbred	639	510	79.8
Exotic	83	83	100
Total	1127	856	75.9

In affected cattle, lesions were observed in the mouth especially on the tongue and gum, interdigital space, and teat (Figure 3). The other observed clinical signs include fever (mean of 39.8 ^{O}C), excessive salivation, and lameness.

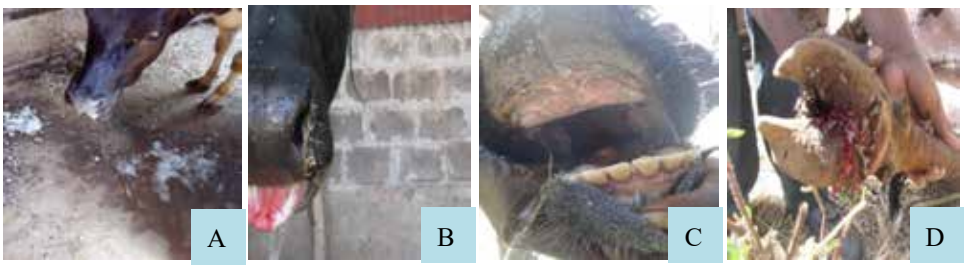

Figure 3: Typical clinical signs of FMD in cattle such as excessive salivation (A), erosive lesions on the tongue (B) and gum (C), and lesions in the interdigital space (D)

The clinical features in sick cattle showed that profuse salivation was the most frequently observed clinical sign (n = 342; 40%) followed by oral cavity vesicle

formation (n = 257; 30%), and inter-digital space lesion (n = 128; 15%) as shown in Figure 4. All sick animals had raised body temperature ≥ 38.5^{O}C.

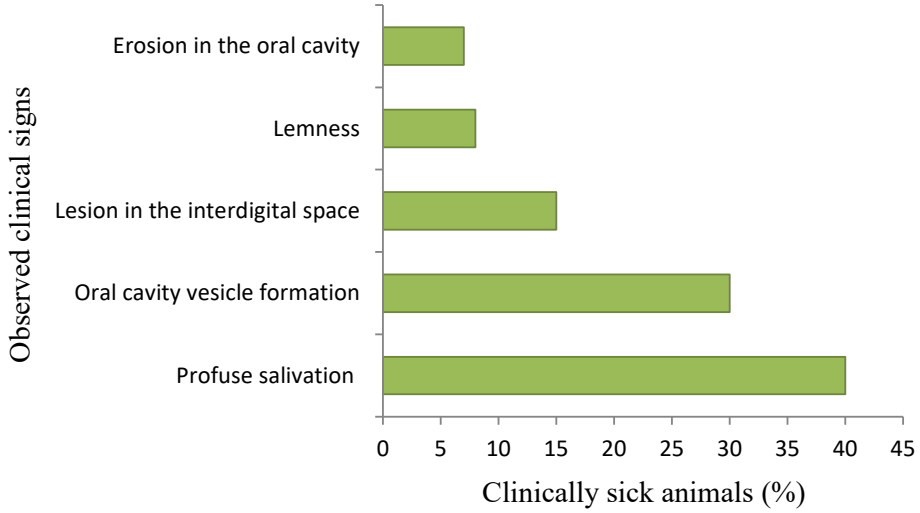

Figure 4: Percentage of clinical signs observed in sick animals during outbreaks

Detection of FMDV genome

The presence of FMD viral genetic material was detected using the rRT-PCR method. Out of 30 samples tested by rRT-PCR, 28 (93.33%) samples were found positive having a Ct value ranging from 17.77 to 30.48 and the fluorescence of samples rose above the background fluorescence as shown in Figure 5.

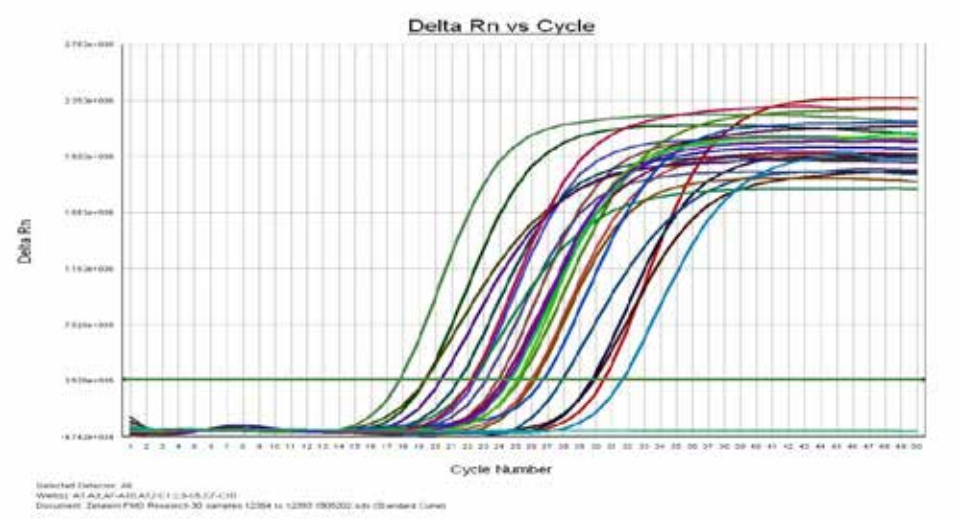

Figure 5: Real-time RT-PCR result showing amplification curve

FMDV serotyping using sandwich ELISA

Antigen detection sandwich ELISA was performed to identify the serotypes circulating in the study areas. Out of 30 samples subjected for ELISA, 27 (90.0%) samples were found positive. In this study, the three types of FMD serotypes were detected in which SAT-2 (n = 13; 43%) was the predominant serotype followed by serotype O (n = 9; 30%), and serotype A (n = 5; 17%) and FMDV was not detected in three samples (10%). FMD serotype SAT-2 was detected from samples collected from Addis Ababa, Bishoftu, Debre Birhan, and Fitche districts. Only SAT-2 was detected from samples collected from Addis Ababa, Bishoftu, and Debre Birhan. From samples collected from Modjo, serotypes O and A were detected whereas serotypes O, A, and SAT-2 were detected from samples collected from Fitche as shown in Table 2.

Table 2. Summary of FMDV serotypes virus identified by Antigen Detection sandwich ELISA

Study sites	No. of samples tested	No. of positive samples	No. of non-detected samples	FMD Serotypes		
				O	A	SAT-2
Addis Ababa	1	1	-	-	-	1
Debre Birhan	4	4	-	-	-	4
Bishoftu	3	3	-	-	-	3
Modjo	9	8	1	6	2	-
Fitch	13	11	2	3	3	5
Total	30	27	3	9	5	13

Discussion

In this study, typical FMD clinical signs were observed in cattle found in five districts of the central parts of Ethiopia namely Addis Ababa (Akaki Kality sub-city), Bishoftu, Debre Birhan, Modjo, and Fitche, from which three different FMD serotypes O, A and SAT 2 were identified. Out of 150 herds of cattle examined during FMD outbreaks in the study areas, 114 (76%) herds of cattle manifested suggestive clinical signs and lesions of FMD such as excessive salivation, formation of vesicles, erosion on the tongue, gum, and dental pad, as well as lesions in the inter-digital spaces and coronary bands on the feet. The current finding was much higher than the previous findings of Sulayeman *et al*. (2018) who reported 28.8% of animals manifested a clinical sign of the disease during FMD outbreaks. This result was in line with the study of McLaws *et al*. (2009) who stated that variations in clinical severity and manifestations were associated with the virus strains, viral infection dose, species, breed susceptibility, management system, and exposure to the previous infection. In this study finding, adults (> 3 years old) were more affected by the disease. This might be associated with a high probability of exposure to FMDV related. This might be due to (1) high mobility during marketing, watering, and grazing, (2) contact with humans during milking, and (3) high production stress for lactating cows. These all might facilitate the transmission of FMDV among cattle.

The current findings revealed that exotic breeds are highly susceptible to FMD with a morbidity rate reaching 100%. FMD is more severe in European breeds of cattle than the other breeds (Quinn *et al.*, 2005). Negusssie *et al.* (2011) recorded a high morbidity rate in the exotic breed of cattle, which might be asso-

ciated with the variation of the genetic factors among breeds of cattle and the type of production system. Mortality was not recorded in the infected animals during the study period. The type of breed is one of the major factors associated with the morbidity and mortality rate of the disease (Jemberu *et al*., 2016).

In the current study, out of 30 samples tested by real-time PCR for the presence of genetic material targeting the 3D regions of FMDV in the sample, 93.33% (n = 28) were found positive. Previously, Paixão *et al*. (2008) suggested that real-time RT-PCR that targets the 3D region of the viral genome is a powerful technique for reliable detection of FMDV which now becoming a key diagnostic test used to confirm FMDV presence in field samples. The negative results for those 2 (6.67%) samples may be attributable to the small quantity of viral RNA in the original samples, which might have resulted from late sample collection after the first appearance of clinical signs.

The lowest Ct value (15.06) was recorded in the bovine epithelial tissue sample collected from the Fitche district, whilst the highest Ct value (31.19) was found in the oral swab samples collected from Addis Ababa (Akaki-Kality). In this study, epithelial tissue samples had lower Ct values than oral swab samples this might indicate a higher level of viral RNA concentration. In support of this observation, OIE (2021) and Reid *et al*. (2000) reported that the preferred sample for FMDV detection is the epithelial tissue.

Previous reports on FMDV serotypes in Ethiopia revealed that FMD outbreaks occurred due to any of serotypes O, A, C, SAT-2, and SAT-1 as diagnosed clinically, serologically, virologically, and molecularly during the period 1981-2018 (Jemberu *et al*., 2016). In our study, serotyping of FMDV showed FMD serotypes SAT-2, O, and A were recorded in central parts of Ethiopia. The current result was in agreement with the studies conducted by Ayelet *et al*. (2009), Jemberu *et al*. (2016) and Gizaw *et al*. (2020) which reported FMD serotype O, A, and SAT-2 from outbreaks in the central parts of the country. Serotype SAT-2 showed an increase in the relative frequency of occurrence in Ethiopia (Jemberu *et al*., 2016). The high incidence in central Ethiopia could be associated with trade-related animal movements. In Ethiopia, animal prices are higher in urban centers, the largest of which is Addis Ababa which is found in

the center of the country and livestock usually move toward the center from other parts of the country.

Conclusions

FMD outbreaks are a big problem for cattle in central Ethiopia. The disease causes a 75.9 % and 76% morbidity rate at the individual animal and herd level. Death associated with FMD was not recorded. FMDV serotypes A, O, and SAT-2 respecitvley were identified from outbreaks in central Ethiopia and serotype SAT-2 was the predominant serotype circulating in the area. Although the vaccine produced in Ethiopia contains all the identified serotypes, detailed studies on topotype identification have to be performed to provide full protection.

Acknowledgments

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Ethical Consideration

Ethical approval for this study was granted from the animal research ethical review committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University (Reference number: VM/ERC/07/04/13/2021). All methods were performed in accordance with relevant guidelines and regulations. Before conducting the research, cattle owners were informed of the objectives and the benefits of the study and they gave consent for their animal's inclusion in the study. Cattle owners gave verbal consent for their animal's

inclusion in the study because they were unable to write and read. These consents were taken in the presence of a third independent party.

Competing interests

The authors declare that they have no competing interests.

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