Foot and Mouth Disease in Adama and Boset districts, East Shewa zone, Ethiopia: Seroprevalence and Virus serotyping

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Abstract

Foot and mouth disease (FMD) is consistently ranked as the most economically important viral disease, and ranks among the top five important livestock diseases in Ethiopia. The status of FMD is barrier for the international trade of livestock and livestock products; thus, continuous epidemiological studies are necessary to develop efficient control strategies. This study determined the seroprevalence of FMD, associated risk factors for its occurrence, and FMD serotypes in the Adama and Boset districts of the East Shewa zone of Oromia Regional State, Ethiopia. A total of 305 blood samples were randomly collected from cattle and subjected to a 3ABC enzyme-linked immune-sorbent assay (ELISA) to detect antibodies against non-structural proteins of FMD virus (FMDV) and Solid-Phase Competitive ELISA for FMDV serotype identification. Evaluation of the associated risk factors was conducted using a multivariable logistic regression model. In this study, an overall seroprevalence of 28.2% (95% CI: 25.7% - 36.2%) was recorded. The risk of being FMD positive was significantly higher in exotic crossbreds [38.60%; OR= 2.23 (95% CI: 1.34 – 3.71); $p<0.001$] as compared to local breeds. The seroprevalence of FMD was significantly higher in large herd sizes [54.41%; OR=4.2 (95%CI: 3.05 - 15.87), $P < 0.001$] compared to medium and small herd sizes. Three FMD serotypes namely serotype O, A, and SAT-2 were identified. Besides, mixed serotypes O and A, serotypes O and SAT-2, serotypes A and SAT-2, and serotypes A, O, and SAT-2 were recorded. By considering the detection of multiple serotypes of the FMDV and the lack of cross-protection among the serotypes, the use of polyvalent FMD vaccine against the serotypes of the virus circulating under field conditions is highly recommended.

Keywords: 3ABC-ELISA; Ethiopia; Foot and Mouth Disease; Risk factors; Seroprevalence; Serotypes.

Introduction

Ethiopia owns the largest livestock population in Africa with an estimated cattle population of 70 million (CSA, 2021). Livestock production plays an important role in the economies of farmers and pastoralists, and the country at large. The Ethiopian livestock contribution to the national economy is estimated at 19% of the total gross domestic product (GDP), 40% of the agricultural GDP, and about 20% of the country's export earnings (Shapiro *et al.,* 2017). Moreover, the livestock sector plays a vital role in the livelihood of millions of households in Ethiopia as a source of meat, milk, drought power, and income. The contribution of livestock to the country's economy primarily in terms of foreign currency gains via the export of live animals, meat, skin, and hides. Although there is substantial international demand for live animals and animal products, transboundary livestock diseases are a significant factor that hampers the trade of animals and animal products to the international market (Shapiro *et al.,* 2015; Jemberu, 2016). Among the transboundary animal diseases, foot and mouth disease (FMD) is the most important that has a significant socio-economic impact on Ethiopia.

Foot and mouth disease is a highly contagious viral disease of cloven-hoofed animals. The disease is characterized by fever and vesicular eruptions on the tongue, feet, snout, and teats, and sudden death in young animals. A non-enveloped RNA virus within the family *Picornaviridae* and genus *Aphthovirus* causes foot and mouth disease. 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. There is no cross-protection and immunity between the different serotypes (Davies, 2002). Therefore, disease control via vaccination must consider the evolving variants and the serotypes that are circulating in the field.

In Ethiopia, FMD is consistently ranked as the most economically important viral disease, and among the top five important livestock diseases (Shiferaw et *al.,* 2010; Jibat et *al.*, 2013). Among the seven serotypes of FMD viruses, currently, four of them (serotype O, A, SAT-1, and SAT-2) have been reported in different geographical settings of Ethiopia (Ayelet *et al.,* 2009; Negusssie *et al.,* 2011; Mohammed *et al.,* 2022). The national FMD outbreak report revealed that the disease is endemic in all regional states affecting more than a quarter of the country every year (Ayelet *et al.,* 2009; Jemberu, 2016). The status of FMD is an important determinant for international trade of livestock and livestock products (ILRI and MoA, 2015), and the existence of FMD is an effective barrier from the international markets (Jibat *et al.*, 2013; Shapiro *et al.*, 2015).

Outbreaks of FMD frequently occur in different parts of Ethiopia. This is mainly due to the lack of effective vaccines and vaccination strategies, the absence of livestock movement control, and the absence of systematic disease surveillance and reliable epidemiological data. Considering the various serotypes of FMDV and the absence of cross-protection among the different serotypes, there are strong recommendations to use polyvalent vaccines incorporating the dominant circulating serotypes in the country. To achieve this, continuous epidemiological studies including the identification of FMDV serotypes circulating in major livestock populations of the country are important. Therefore, this study was intended with the objectives of estimating the seroprevalence, assessing risk factors, and identifying circulating serotypes of FMDV in Adama and Boset districts of East Shewa, Oromia Regional State, Ethiopia.

Materials and methods

Study area

The study was conducted in two districts; namely, Adama and Boset districts of East Shewa Zone, Oromia Regional state, Ethiopia, which is located from 7^0 33′0″ to 9^0 08′56″N and 38^0 24′10″E to 40^0 05′ 34″E (Figure 1). About 93% of the total area of the zone is located along the Rift Valley. East Shewa Zone has a total of 1,147,173 cattle (CSA, 2021).

Adama is one of the districts in the East Shewa Zone and it is located at 8.54^0N 39.27^OE at an altitude of 1,712 masl. It receives an average rainfall of approximately 600 – 1,150 mm with annual average minimum and maximum temperatures of 18^0C and 32^0C (ESZLFD, 2017). Boset district is characterized by a semi-arid climate. Its' mean annual rainfall is ranged from 600 to 900 mm, while the mean annual temperature is 26 – 34°C. Almost 90% of the district is found in less than 1,500 masl (BDANRO, 2017). These districts were selected based on their large livestock population, accessibility for transportation, presence of huge fattening farms, and main routes for live animal exports.

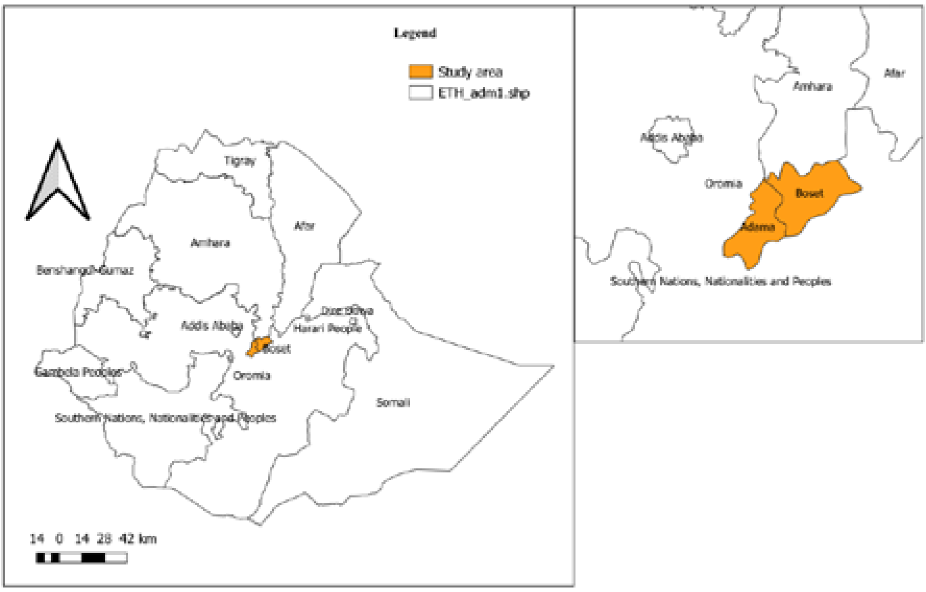

Figure 1: Map of Ethiopia showing the study areas. This map was developed from the Ethiopian

Administrative boundaries shapefile 2021 using QGIS version 3.1.1.2.

Study population, study design, and sample size

Study animals were local zebu and exotic crossbred cattle managed under extensive and semi-intensive systems. Cattle were kept together with either small ruminants (mixed herd composition) or only cattle without mixing with other livestock species. To avoid maternal immunity interference, cattle that were in age of < 6 months were not sampled. Animals were grouped into two age categories; 6 months to 2 years (young) and above 2 years (adult). During sampling, information such as age, sex, breed, herd size, composition, and previous vaccination status for the last six months was collected to determine whether FMD seropositivity was associated with potential risk factors. Herd sizes were categorized arbitrarily into small herds (< 15 cattle), medium herds (15 – 30 cattle), and large herds (> 30 cattle), in which the largest herd size contained 43 heads of cattle.

From January 2021 through June 2021, a cross-sectional study design was used. A multistage sampling method was applied to select 5 *Kebeles* from Adama district and 3 *Kebeles* from Boset district. A simple random sampling technique was used to select 305 cattle from the population.

The sample size calculation was determined according to Thrusfield (2007). The minimum sample size required for the study was calculated based on 26.8% individual animal-level prevalence of FMD (Sulayeman *et al.,* 2018) foot-and-mouth disease (FMD, with a 95% confidence interval, and 5% absolute precision. Accordingly, 305 blood samples were collected.

Blood sample collection

Samples were carefully collected following all ethical procedures for sampling, enrollment and sample collection. Blood samples were collected from the jugular vein of cattle using plain vacutainer tubes. About 10 ml of blood was drawn from each randomly selected animal and kept overnight in an upright position at room temperature. The serum samples were harvested, labeled, and transported using an icebox containing an icepack to Animal Health Institute (AHI), serology laboratory in Sebeta, Ethiopia. The samples were kept at -20°C until the serological test conducted.

Laboratory Diagnosis

The sera samples were tested for the presence of antibody against FMDV using competitive 3ABC ELISA (ID Screen®, ID Vet, Grabels, France) at the Animal Health Institute, Sebeta, Ethiopia. This test detects antibodies produced against non-structural proteins of the virus and can thus distinguish between FMDV infected animals and vaccinated with purified FMD vaccine. The tests were performed according to the manufacturer's instructions.

FMDV serotyping

Serum samples exhibiting strong antibodies against FMDV non-structural protein using 3ABC ELISA was selected for FMD serotyping. A total of 59 strong positive serum samples were selected and subjected to antigen-capturing sandwich ELISA (IZSLER, Brescia Italy). The assay was a monoclonal antibody based on an indirect sandwich ELISA that can detect and typing of

FMDV of serotypes O, A, C, Asia-1, SAT-1, and SAT-2. The test was performed according to the manufacturer's manual.

Statistical analysis

Data generated from the serological test was recorded in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 16 (STATA, 2019). A logistic regression analysis was used to measure the association of the risk factors with the seropositivity of FMD. The association of risk factors was screened out by using univariate logistic regression analysis and variables with p-value < 0.25 (maximum likelihood ratio test) were offered to the final multivariable model. The risk factors having a p-value < 0.05 were considered statistically significant. The multicollinearity and goodness fit of the models was checked using the variance inflation factor (VIF) and Hosmer and Lemeshow tests.

Results

Among the 305 sera tested using 3ABC-ELISA, a seroprevalence of 28.2% (95%CI: 25.7% - 36.2%) was detected.

Potential risk factors screened with univariable logistic regression analysis

With the univariate model, risk factors of age, sex, herd composition, and study districts, although observed with higher seroprevalence records in adult cattle, males, unmixed cattle herd, and Boset districts, respectively; and were found to be statistically not significant (p > 0.05). Significantly higher seroprevalence was recorded in exotic crossbreds [38.60%; OR = 2.23 (95% CI: 1.34 - 3.71), p = 0.002] and large herd size [54.41%; OR= 4.35 (95%CI: 2.34 - 8.10), p<0.001] with the model as compared to local breed and small-medium sized herds, respectively (Table 1).

Table 1. Univariable logistic regression analysis results of potential risk factors for FMD seropositivity

Variable	No. tested	Positive (%)	OR(95%Conf. Interval)	p-value
Study Districts				
Boset	155	67 (43.27)	Ref	
Adama	150	19 (12.67)	0.19 (0.11 - 0.34)	0.071
Breed				
Local	191	42 (21.99)	Ref	
Cross	114	44 (38.60)	2.23 (1.34 - 3.71)	0.002
Sex				
Male	60	23 (38.33)	Ref	
Female	245	63 (25.71)	0.56 (0.31 - 1.01)	0.053
Age				
Young	100	25 (25.00)	Ref	
Adult	205	61 (29.76)	1.27 (0.73 - 2.19)	0.387
Herd Size				
Small	144	31 (21.53)	Ref	
Medium	93	18 (19.35)	0.87 (0.46 - 1.68)	0.687
Large	68	37 (54.41)	4.35 (2.34 - 8.10)	<0.001
Herd composition				
Cattle, sheep, and goats	175	47 (26.86)	Ref	
Cattle only	130	39 (30.00)	1.17 (0.71 - 1.93)	0.547

Multivariable logistic regression rnalysis of risk factors with FMD seropositivity

From six hypothesized risk factors, only two variables (i.e. breed and herd size) showed a significant association and were included in the multivariate logistic regression model. The multivariate logistic regression analysis revealed the risk of being FMD seropositive was significantly higher in exotic crossbreds with a prevalence of [38.60%; OR= 2.23 (95% CI: 1.34 – 3.71; $p<0.001$] as compared to local breeds. The seroprevalence of FMD was significantly higher in large herd sizes [54.41%; OR=4.2 (95%CI: 3.05 - 15.9, $p < 0.001$] compared to medium and small herd sizes (Table 2).

Table 2. Multivariate logistic regression analysis of risk factors with FMD seropositivity

Variable	No. tested	Positive (%)	OR (95% Conf. Interval)	p-value
Breed				
Local	191	42 (21.99)	Ref	
Cross	114	44 (38.60)	2.23 (1.34 - 3.71)	<0.001
Herd Size				
Small	144	31 (21.53)	Ref	
Medium	93	18 (19.35)	0.91 (0.42 - 1.94)	0.802
Large	68	37 (54.41)	4.2 (3.05 - 15.9)	<0.001

Identification of FMD Serotypes

Sera samples exhibiting strong antibodies against FMDV non-structural protein using 3ABC ELISA was selected for FMD serotyping. From the total of 59 strong positive sera samples selected and tested using antigen-capturing sandwich ELISA, three FMD serotypes (Serotype O, A, and SAT-2 were identified. Serotype O was recorded with the highest proportion (69.36%) followed by serotype A (45.63%), and SAT -2 (28.73%). In this study mixed serotypes such as serotypes O and A (3.38%), serotypes O and SAT-2 (1.69%), serotypes A and SAT-2 (27.12%), and A, O, and SAT -2 (18.64%) were also recorded (Table 3).

Table 3. FMD serotypes identified at Adama and Boset districts

Types of FMD serotypes identified (%)				**Mixed serotype identified (%)**			
No. sera tested	**O**	**A**	**SAT-2**	**O & A**	**O & SAT-2**	**A & SAT-2**	**A, O & SAT -2**
59	41 (69.36)	27 (45.63)	17 (28.73)	2 (3.38)	1 (1.69%)	16 (27.12%)	11 (18.64)

Discussion

In Ethiopia, FMD is consistently ranked as the most economically important viral disease, and among the top five important livestock diseases (Shiferaw *et al.,* 2010; Jibat *et al.,* 2013). The status of FMD is an important determinant of international trade of livestock and livestock products (ILRI and MoA, 2015), and the existence of FMD is an effective barrier from the international markets (Jibat *et al.,* 2013; Shapiro *et al.,* 2015). In this study, an overall FMD seroprevalence of 28.2% was recorded in cattle in the study districts. This is in agreement with those of other previous reports in a different part of Ethiopia by Sulayeman *et al.* (2018) foot-and-mouth disease (FMD, Shurbe *et al.* (2022), and Dubie and Negash (2021) who reported a seroprevalence of 26.8% in Addis Ababa, Arsi and East Shewa, 26.8% in Gamo zone, and 19.8% in Afar region, respectively. However, the seroprevalence of this study was higher than Negusssie *et al.* (2011) (5.6%) and slightly higher than the study of Bayissa *et al.* (2011) who reported seroprevalence of 23%. The observed difference in prevalence in the current study could arise from the difference in sampling method, study design, agroecology, unrestricted herd mobility, and animal management systems.

In the current study, significantly higher seroprevalence was recorded in exotic crossbreds (38.6%) compared to local breeds. The risk of being FMD seropositive was significantly higher in exotic crossbreds [OR=5.51 (95% CI: 2.37 - 12.82), $p<0.001$] compared to the local breed. This might suggested that the local breed cattle are relatively resistant to the FMDV compared to exotic breeds. This finding is in agreement with Sulayeman *et al.*(2018), Negussie *et al.* (2011), and Ahmed *et al.* (2020) who reported a significant variation in seroprevalence among the different breeds of cattle. Similarly, Hunter (1998) reported that the direct impact of FMD on livestock in sub-Saharan Africa depends on the breeds of animals used. FMD is more severe in European breeds of cattle than the other breeds (Quinn *et al.*, 2005).

In this study, herd size was categorized into three groups namely < 15 cattle (small), 15 - 30 cattle (medium), and > 30 cattle (large). The seroprevalence of FMD was significantly higher in large herd sizes [54.41%; OR=4.2 (95%CI: 3.05 - 15.87), $p<0.001$] compared to medium and small herd sizes. Similar trends were reported by Sulayeman et al.(2018) and Dubie and Negash, (2021) where higher seroprevalence of FMD was observed in larger herd sizes. This suggests that higher herd size has a greater chance of animal contact and hence virus transmission between cattle herds.

Previous reports on FMDV serotypes in Ethiopia showed that FMD outbreaks were associated with serotypes O, A, C, SAT-2, and SAT-1 as diagnosed clinically, serologically, virologically, and molecular techniques during the period 1981-2018 (Jemberu *et al.*, 2016). In this study, three FMD serotypes namely serotype O, A, and SAT-2 were identified. The current result was in agreement with the previous studies conducted by Ayelet *et al.* (2009), Jemberu *et al.* (2016), Gizaw *et al.* (2020), and Mohammed *et al.* (2022) stated that FMD serotype O, A, and SAT-2 were recorded from outbreaks in the central parts of the country. Serotype O was recorded with the highest proportion followed by serotype A, and SAT-2. This was in agreement with the studies conducted by Ayelet *et al.* (2009), Negussie *et al.* (2011), and Gizaw *et al.* (2020). However, Jemberu *et al.* (2016) recorded serotype SAT-2 in a higher proportion compared to serotype A. Mixed serotypes with serotypes O and A (3.38%) serotype O and SAT-2 (1.69%); serotypes A, O, and SAT-2 were identified. This suggests the possibility of simultaneous infection with different serotypes of the FMD virus which is in agreement with Arzt *et al*. (2021) reported the occurrence of simultaneous infection of individual hosts by multiple FMD virus strains. In FMD-endemic countries, vaccination is a highly effective method for preventing FMD. In Ethiopia, the National Veterinary Institute (NVI) is producing inactivated FMD vaccine containing a cocktail of serotypes O, A, and SAT-2. Due to the growing trend of private investments in the livestock sector, vaccination of cattle against FMD has been practiced (Ayelet *et al.,* 2013; Jemberu *et al.,* 2020) in some market-oriented dairy farms and feedlot operations in urban and peri-urban areas. However, the FMD vaccines used in many FMD-endemic developing countries are of questionable quality (Sutmoller *et al.,* 2003). Quality of vaccine is a critical requirement for the successful control of animal diseases through immunization. Various factors may limit their effectiveness such as low potency, poor antigenic match between the field and vaccine strains, as well as short duration of protection (Ferrari *et al.,* 2016).

Conclusions

Foot and Mouth Disease (FMD) is a highly prevalent disease in the study areas. FMD serotypes O, A, and SAT-2 were identified in the study areas. Mixed serotypes O and A, serotypes O and SAT-2, serotypes A and SAT-2, and Serotype A, O, and SAT -2 were recorded. It was concluded that by considering the detection of multiple serotypes of the FMDV and the lack of cross-protection among the FMD virus serotypes, the use of polyvalent FMD vaccine against the serotypes of the virus circulating under field conditions is highly recommended.

Acknowledgments

The authors would like to thank the technical staff at Animal Health Institute (AHI) (the former, National Animal Health Diagnostic and Investigation Center, NAHDIC) for sample collection, laboratory work, and data analysis. Many acknowledgments would also go to the College of Veterinary Medicine and Agriculture of Addis Ababa University for all-around support.

Ethical considerations

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.

Authors' contributions

AA and AM were involved in data collection and laboratory work. HN and YJ were involved in the design, statistical analysis, and interpretation of data; drafted the paper and involved in the critical revision of the paper. All the authors have read and approved the paper for submission.

Competing of interest

The authors declare that they have no competing/conflict of interests.

Funding

This study was funded by Addis Ababa University Thematic Research Project (Ref: VPRTT/PY-137/2019). The funder had no role in the conception, design of the study, data collection, analysis, and interpretation of the data reported in this manuscript

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