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Research Article

Seroprevalence, Serotyping and Associated Risk Factors of Foot and Mouth Diseases in Bovine in Western Amhara Regional State, North Western Ethiopia

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ABSTRACT

Foot and Mouth Disease (FMD) is the most contagious disease of animals. The study aimed to determine seroprevalence of FMD, its serotypes and associated risk factors. A total of 389 sera samples were collected from cattle in four districts of the North Western Amhara region and subjected to a 3ABC Enzyme-Linked Immunosorbent Assay (ELISA). The overall seroprevalence of Foot and Mouth Disease Virus (FMDV) was 5.66% (22/389); (95%; Confidence Interval (CI): 3.34% to 7.98%). The 22 positive samples were subjected to solid phase competitive ELISA to identify specific serotypes. The occurrence of FMD was higher in Adet (Odds Ratio (OR)= 12.8), greater in the semi-intensive than extensive production systems (OR=10.4) and highly occurred in the cross breed than local breed cattle (OR=3.56). Serotypes identified in the four districts were type O, SAT2 and A. This study revealed that FMD is a prevalent and endemic disease. Thus, awareness creation to the stakeholders on the control and prevention of a disease is required. Further epidemiological investigation and vaccine trials should be conducted.

Keywords: Foot and Mouth Disease; Risk factors; Seroprevalence; Serotype

INTRODUCTION

The livestock sub-sector is an integral part of agriculture that provides food, milk, draft power, manure fertilizer for crop production and in turn, utilizes the crop residues as feed. Transboundary Animal Diseases (TAD) are one of the infectious diseases that have an impact on the livestock sector through decreased productivity, restricted international trade access, loss of entire herds, biodiversity loss and the loss of valuable genetic resources. The important bottleneck for the development of the livestock sector includes infectious and non-infectious diseases, lack of food for animals, lack of appropriate disease control policy, lack of appropriate veterinary services and lack of attention from the government [1]. The most important constraints are widespread endemic diseases including viral, bacterial and parasitic infestation. Among the health constraints, infectious disease like FMD is considered as one of the livestock diseases which cause for the major socio-economic problem in Ethiopia.

FMD is the most contagious disease of animals. It is a leading cause of high losses in susceptible cloven-hoofed animals and negatively affects the national economy of a country [2]. The virus has seven different serotypes (A, O, C, Asia1, South African Territories (SAT) 1, SAT 2 and SAT 3). All serotypes are clinically indistinguishable but immunologically distinct [3]. The disease has clinical signs of vesicular formation and erosions of the epithelium of the mouth, nose, muzzle, feet, teats and udder, tongue, lips and between the hooves [4]. The virus can be transmitted either directly *via* contact with an infected host or indirectly *via* contact with a contaminated environment. In addition to contact virus can transmit to a new susceptible animal either orally or *via* the respiratory tract [5].

FMD epidemiology is the study of the distribution of serotypes,

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prevalence rates and risk factors that contribute to the occurrence of FMD. FMD is found all over the world and classified as a notifiable disease by the World Organization for Animal health [6].

FMD is endemic in Ethiopia, with complex epidemiological nature, wider geographical distribution and broad host range. It has the ability to establish carrier status and its antigenic diversity leading to poor cross-immunity, variant type of viral genes and relatively short duration of immunity. The incidence of FMD is high in Ethiopia due to the presence of free animal movement, a high rate of contact among animals at commercial markets, the presence of communal grazing areas and watering, poor diagnostic facilities, poor surveillance and limited prevention and control strategies of the government [7].

The epidemiology of FMD should be studied on a regular basis in Ethiopia because of occurrence of FMD repeatedly recorded every year [8]. For prevention, control, mitigation and eradication of the disease, baseline information about seroprevalence, serotype and risk factors are essential. Therefore, the objectives of this study were to estimate the seroprevalence of FMD in the study area, to assess risk factors associated with the disease and to identify serotypes of foot and mouth disease virus circulating in the study area.

MATERIALS AND METHODS

Study area

The research was carried out in the North-Western part of Ethiopia's Amhara regional state located between the latitudes of 8° 45'-13° 45' N and the longitudes of 35° 15'-40° 20' E, covering an area of 157,127 km^2. The average annual rainfall in the region ranges between 598.3 mm and -1692 mm. Country side areas are home to 87.3% of the population, while urban areas are home to 12.7%. The region's main farming system is a mixed crop-livestock production system [2,3]. Samples were collected from four districts (Adet, Banja, Simada and Ebenat) in western Amhara region, Ethiopia (Figure 1).

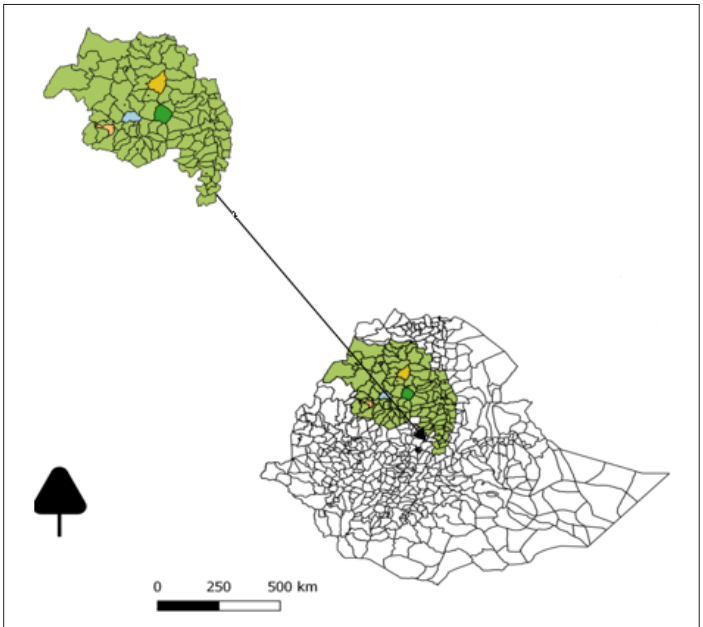

Figure 1: Map showing the study area. **Note:** Four districts in Amhara region, Ethiopia; ( ): Adet; ( ): Banja; ( ): Ebenat; ( ): Simada; ( ): Amhara region; ( ): Ethiopia.

Study population

The study population consists of cattle of various ages, sexes, herd sizes and reared under various production systems and farming types. This region has a nearly similar population density with a mixed crop-livestock production system and similar animal management, except beef and dairy farms in the urban areas with intensive and semi-intensive production systems.

Study design

A cross-sectional study was carried out with a multistage sampling method from region to individual animal level but within each stage, purposive, convenient and random sampling method was applied. Study districts were selected due to accessibility and relatively good security but the peasant associations in the district were selected by considering agro-ecology. Half of the animals in one herd were used as sampling units, but if the owner had a small number of animals, all of them were included.

Sample size determination

The sample size was determined using a sample size formula based on the desired precision and previous prevalence in the study area [4]. According to the previous report of (12), the seroprevalence of FMDV in cattle in the area was 14.3%.

$$n = 1.96^2 \times P_{exp}(1 - P_{exp}) / d^2 = ((1.96)2 \times (1 - 0.143)) / (0.05)2 = 188$$

Thus, a sample size of 188 was determined. However, it was maximized to 389 to increase precision and accuracy. Where Z a/2 value of the critical region=1.96, n=sample size, P_{exp}=expected prevalence, d=absolute precision used as a significance level and 95% confidence interval. The sample size in each district was calculated using the disproportionate stratified sampling method, which divides the total sample size by the number of strata or districts, as well as a similar flow at the peasant association level.

Study methodology

Sample collection: Blood was drawn from the jugular vein of each animal using a 10 ml plain vacationer tube. Each vacationer tube was carefully inspected. During collection, the serum was carefully harvested and labeled for each individual animal before being placed in cryovials. The sera samples were kept at -19°C until the laboratory investigation was completed. The research was carried out at the National Animal Health Diagnosis and Investigation Center (NADIC) in Sebeta, Ethiopia.

Serological examination with non-structural 3ABC competitive ELISA

Serum samples were subjected to Non-Structural Protein (NSP) Competition 3ABC ELISA (ID Screen ®, ID. Vet and Montpellier France). A total of 389 sera were tested. A test was performed as per the manufacturer's instructions with series of procedures with 96 well micro plates. Positive results from non-structural 3ABC competitive ELISA was screened again for specific serotype antibody detection using a solid-phase competitive ELISA.

Data management and analysis

The serum data was entered into Microsoft excel spreadsheet and coded for analysis. The overall seroprevalence and prevalence over each factor was analyzed by Stata software Version 13 with chi-square statistical tools. Risk factors were analyzed by univariable

and multivariable logistic regression analysis.

RESULTS

Seroprevalence of FMD

The overall seroprevalence of FMDV was 5.66 % (22/389) with (95%; CI: 3.34% to -7.98%). Statistically significant (P-value=0.001) higher seroprevalence was recorded in Adet 14.44% (n=13) as compared to other districts as shown (Table 1). The districts in the south Gondar zone had relatively similar seroprevalence, 3.39% in Ebenat and 3.85 % in Simada were recorded. In general, the prevalence of FMDV varied among districts.

Table 1: The prevalence of foot and mouth disease virus among districts.

Variable	No. of test	No. of positive test	χ^2	P-value	HI
District	Simada	104	4(3.85%)	17.54	0.001
	Ebenat	118	4(3.39%)		
	Adet	90	13(14.44%)		
	Banja	77	1(1.30%)		

Note: No. of test: Number of animals tested; No. of positive test: Number of positive sample for foot and mouth disease; χ^2: Chi-square.

FMD prevalence between risk factors

Seroprevalence was compared in various risk factors such as age group, gender, number of animals, production systems and animal rearing practices. Even though higher seroprevalence was recorded in female 5.86% (n=15) than male 5.26% (n=7), there was no statistically significant difference (p-value=0.8). And also, age group was not statistically significant (p-value=0.37) in this study. It was higher in semi-intensive production system (28.57%) than intensive (17.65%) and extensive (3.4%) production systems. Significantly higher seroprevalence (P=0.014), was also recorded in cross breed (15.15%) compared to local breed (4.78%) cattle and more descriptions including the χ^2 value for each risk factor (Table 2).

Table 2: Seroprevalence of FMD across corresponding risk factor.

Variable	Categories	No. of test	No. of positive test	χ^2	P-value
Sex	Female	256	15(5.86%)	0.058	0.8
	Male	133	7(5.26%)		
Age	Adult	281	14(5%)	0.8	0.37
	Young	108	8(7.34%)		
Breed	Local	356	17(4.78%)	6.09	0.014
	Cross	33	5(15.15%)		
Production system	Intensive	16	3(17.65%)	27.75	0
	Extensive	353	13(3.70%)		
	Semi-intensive	20	6(28.57%)		

Note: No. of test: Number of animals tested; No. of positive test: Number of positive sample for foot and mouth disease; χ^2: Chi-square; FMD: Foot and Mouth Disease.

Seroprevalence across herd size, farm type and new animal added per year

Lower herd size had lower seroprevalence (5.06%) than higher herd size (6.06%), but the difference was not statistically significant. Seroprevalence was found to be significantly (P=0.003) higher in dairy farms (20.8%) than in smallholder farms (4.75%) and in beef farms with no prevalence. There was no statistical difference in seroprevalence among new animals added within a year or not (P= 0.79). Seroprevalence corresponding to risk factors is described below (Table 3).

Table 3: Seroprevalence of FMD across corresponding risk factor among farm types, herd size and new animals added.

Factors	Categories	No. of test	No. of positive test	χ^2	P-value
Herd size	Above 5 per herd	231	14(6.06)	0.17	0.67
	Below 5 per herd	158	8(5.06%)		
New animal added per year	No	347	20(5.76%)	0.07	0.79
	Yes	42	2(4.76%)		
Farm type	Small holder	358	17(4.75%)	11.3	0.003
	Dairy farm	24	5(20.8%)		
	Beef farm	-	-		

Note: No. of test: Number of animals tested; No. of positive test: Number of positive sample for FMD; χ^2: Chi-square; FMD: Foot and Mouth Disease.

Logistic regression analysis

The occurrence of FMD seroprevalence was compared among districts using a univariable logistic regression analysis. The diseases occurred 12.8 times more frequently in Adet than in Banja and it was statistically significant (P=0.015). The effect of breed indicates that FMD occurs 3.56 times more frequently in cross breed cattle than in local breed cattle, with a significant difference (p=0.02). FMD occurred 10.4 times more frequently in semi-intensive than in extensive production systems, with a significant difference (P=0.000) as shown (Table 4). The occurrence of FMD seroprevalence among farm types showed that it was 5.3 times more frequently occurred in dairy farms than in smallholder farms and it was statistically significant (P=0.012). But there was no significant difference (p-value= 0.67) in herd size of number of animals per herd above 5 or below 5 as shown (Table 5). Also, new animals added within a herd in this study, did not show statistically significant difference (P=0.7) for the occurrence of FMD. From the risk factors included in this study, only districts and production systems showed a significant difference in the occurrence of FMD in multiple logistic regression analysis. Among districts, the FMD prevalence in Adet was 12.6 times higher than in Banja (P=0.016). It was 3.09 and 2.7 times more common in Simada and Ebinat, respectively, than in Banja, but there was no significant difference. Foot and mouth diseases virus was 14 times more common in semi-

intensive production systems than in extensive production systems, with a significant difference (p=0.003) (Table 6).

Table 4: Univariable logistic regression result of FMD seroprevalence among mentioned districts.

Factor	Categories	OR	P-value	CI
District	Adet	12.8	0.015	1.63- 100.5
	Ebenat	2.66	0.38	0.29- 24.3
	Simada	3	0.32	0.33- 27.75
Breed	Cross	3.56	0.02	1.22- 10.37
Production system	Semi-intensive	10.4	0	3.47- 31.14
	Intensive	5.57	0.014	1.42- 21.8

Note: OR: Odds Ratio; CI: Confidence Interval; FMD: Foot and Mouth Disease.

Table 5: Univariable logistic regression result of FMD seroprevalence among farm types, herd size and new animals added.

Factor	Categories	OR	*P-value*	CI
New animal added within year	No *vs.* Yes	1.2	0.7	0.27-5.42
Farm type	Cross	3.56	0.02	1.22- 10.37
Herd size	Number of animals per herd above 5	1.2	0.67	0.49-2.45

Note: OR: Odds Ratio; CI: Confidence Interval; FMD: Foot and Mouth Disease.

Table 6: Multi variable analysis on risk factors.

	Factors	OR	P-value	CI
District	Adet	12.6	0.016	1.6-98.8
	Ebenat	2.7	0.38	0. 28-24.5
	Simada	3.09	0.31	0. .33-28.8
Production system	Semi-intensive	14	0.0033	2.53-79.8
	Intensive	13.5	0.016	1.65-117.9

Note: OR=Odds Ratio; CI= Confidence Interval.

Serotype identification

FMD virus serotypes O, A and SAT 2 were identified as the causal serotypes from antibody detection in four districts. In Adet, 11 samples were serotype O and 3 samples were A. Three O and one SAT 2 serotype samples were identified in Simada. O serotype was identified in Banja and Ebenat.

DISCUSSION

Seroprevalence of FMD

Foot and mouth diseases virus was endemic in our country and found in all regions of Ethiopia, the prevalence and distribution varied across districts, which could be attributed to differences in land scape, agro ecology and animal movement within Ethiopia [9-11]. Prevalence varies from place to place with seropositivity that ranges from 5.6% to 42.7% in cattle. The overall seroprevalence of FMDV in the North Western Amhara region was 5.66% in this study, but in another study, the overall prevalence in the entire Amhara region was 11.04 %. Most of the districts chosen for this study had low prevalence compared with previous studies, Banja having a relatively low proportion of FMD prevalence of 4.2% and in similar this district having a prevalence of 1.3 % in this study [11,12]. This could be due to the area being in a relatively central location or it being a non-boundary area with less movement of animals. This may be due to exporting of animals than importing in the area. In South Gondar Zone districts, Tesfaye and his colleagues reported that the prevalence of the disease in the area was 9.6% [13]. However, it was 3.6% in the current study; this may be due to variation in selected peasant association. Another scholar, Abunna and his colleagues reported that the overall prevalence of FMD at Dire Dawa and its surroundings, Eastern Ethiopia was 8.01% [14]. This finding was in agreement with the present study. In this study, foot and mouth disease was present in all ecosystems. Cross-bred cattle had higher percentages of foot and mouth disease seropositivity than local breed cattle. It was 3.56 times higher in cross breed than in local breed cattle in the present study. Similarly, in previous studies, conducted in central Ethiopia, crossbred cattle were 2.79 times more likely than local cattle in seropositivity of the disease and in another study from West Shewa Zone, Ethiopia, crossbred cattle were 6 times more likely than local cattle in seropositivity [15,16] In this study, sex group was not statistically significant and it was in agreement with many previous studies [9,17,18].

There was no statistically significant difference among age groups in this study and it is congruent with the report of from Bale Zone, Oromiya regional state, Ethiopia [5]. However, studies conducted from different parts of the country showed that age groups were statistically associated with disease occurrence [9,10,18]. The difference could be attributed to age delegation among researchers. The prevalence of FMD in the semi-intensive production system was 28.57%, while the prevalence in the extensive production system was 3.57%. This difference may be due to animal density variation, as the semi-intensive production system had a higher density of animals in a fixed area than the extensive production system. FMD prevalence had increased in densely populated than scattered one, so ventilation and animal density may cause for variation in occurrence and distribution of the disease.

The occurrence of foot and mouth disease in dairy farm was 5.3 times more than occurrence of the diseases in small holder farms (p=0.012). There was no seroprevalence in beef farm, this may due to unavailability of animals constantly in the farm for a long period of time. In this study, serotype A, O and SAT 2 were identified and similarly Sulayeman and his colleagues from central Ethiopia reported that the same type of serotypes were detected [5]. Negussie et al., [19] found serotype O to be the most prevalent and dominant serotype in Adet, causing the majority of outbreaks in the Amhara region. In agreement to this serotype O was also identified in Adet, Simada, Ebenat and Banja in this study. Serotype O considered as the most widely studied and common FMD serotype in the world and in this study, serotype O was the most dominant serotype [20].

Another scholar indicated that it was the most prevalent in central Ethiopia [13,21,22]. The three serotypes, O, A and SAT 2 were found in the central part of the country, Debre Birhan, Debrezyiet and Addis Abeba and those serotypes were identified in the current study areas. SAT 2 was also the cause for FMD outbreak in Afar Region, Ethiopia [7,10].

CONCLUSION

The present study indicated that FMD is more prevalent and endemic disease in in North Western Amhara region on rural communities as well as dairy and beef farms because of free movement of livestock in different regions and ineffective control measures. The seroprevalence of foot and mouth diseases varies among districts with a minimum prevalence of 1.3% in Banja to a maximum prevalence of 14.4% in Adet. The average prevalence in this study is 5.66%. In addition, different serotypes were identified. As a result, livestock owners should be aware about the clinical and economic consequences of FMD and try to control and prevent their animals. Vaccine trials, more work on serotypes and detailed epidemiological investigations should be conducted.

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