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Seroprevalence and serotype distribution of foot and mouth disease virus and associated risk factors in cattle across various export livestock sourcing districts of Bale Zone, Ethiopia

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Abstract

Background Foot and mouth disease (FMD) is a contagious and economically important viral disease affecting cloven-hoofed animals. A cross-sectional study was conducted from January to March 2022 in selected districts of Bale zone, Ethiopia to determine the seroprevalence and serotypes of FMD virus circulating in cattle. Blood samples were collected from cattle and tested for antibodies against non-structural proteins (NSP) of FMD virus using a 3ABC enzyme-linked immunosorbent assay (ELISA). Positive samples were further tested for serotype-specific antibodies using solid phase competitive ELISA (SPCE). Descriptive statistics, both univariable and multivariable logistic regression were used to analyse FMD serostatus and associated risk factors.

Results A total of 962 cattle sera were tested and 200 samples, 20.8% (95% CI: 18.3–23.5) were positive for antibodies against NSP of the FMD virus. The highest seroprevalence was observed in the Seweyna district (35.2%, 95%CI: 26.8–44.7) and the lowest in the Dinsho district (13.0%, 95% CI: 9.5–17.5). The seroprevalence of FMD virus antibody was significantly associated with district ($P < 0.05$). The seroprevalence among different age groups was statistically significant ($P < 0.05$). The odds of FMD infection for males was 0.76 (95% CI: 0.534–1.082) compared to females, indicating that male cattle have a lower risk for FMD infection. Out of 200 samples tested for serotype O, A, SAT 1, and SAT 2, 85(43%) were found to have serotype O, 59(30%) serotype A, 142(71%) serotype SAT 1, and 75 (38%) serotype SAT2. Furthermore, multiple FMD serotypes were observed in 15–40% (30–80) of animals tested.

Conclusions Serotype-specific antibodies against the FMD virus indicate the occurrence and distribution of serotypes O, A, SAT1, and SAT2 in cattle across various districts of the Bale zone in Ethiopia. These findings also highlight the importance of continuously monitoring the seroprevalence of FMD virus serotypes circulation in export

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livestock sourcing areas. The results indicated that four FMD virus serotypes were distributed across the studied districts. This study supports the inclusion of all four FMD serotypes in vaccine production.

Keywords Seroprevalence, Serotype, FMD, Cattle, Bale, Ethiopia

Background

Foot and mouth disease (FMD) is a contagious transboundary viral disease affecting cloven-hoofed animals, caused by a non-enveloped RNA virus in the *Aphthovirus* genus. There are seven serotypes of the FMD virus: O, A, C, South African territories 1 (SAT1), SAT2, SAT3, and Asia1 [1]. FMD has high morbidity but low mortality in adult animals, although young animals can develop myocarditis, which leads to death. The disease causes vesicles in the mouth and on the coronary band of feet in all cloven-hoofed animals [2, 3]. FMD significantly affects the international livestock trade [4, 5].

Infection with one serotype of FMD virus does not provide immunity against other serotypes [6]. Distinct subtypes are identified through biochemical and immunological tests [7, 8] which necessitates separate immune protection [8].

The identification of the first FMD serotypes in Ethiopia dates back to 1957, when Serotypes O, A, and C were first recognized [9]. Since then, FMD outbreaks have occurred consistently throughout the years with reports originating from all regions of the country. Ethiopia is endemic for FMD virus serotypes O, A, SAT2, and SAT1 [10, 11].

Sequence analysis of FMD virus from 2008 to 2019 confirmed the presence of serotypes O, A, and SAT2 [11]. No SAT1 sequences were obtained between 2008 and 2019 [11]. However, the SAT1/IX lineage was the only serotype sequenced in 2007 [12]. There have been no sequences of SAT 1 serotype in Ethiopia since that time. FMD serotype C has not been isolated since 1984 in Ethiopia [10].

The FMD virus Serotype O, specifically EA-4 and EA-3 lineages, has been identified and characterized in Ethiopia by Ayelet et al. [10] and Gizaw et al. [11]. This serotype has been associated with significant economic losses in the country, as reported by Jemberu et al. [13]. The co-occurrence of different FMD virus lineages has been observed in Ethiopia, as documented by Gizaw et al. [11]. Among the SAT serotypes, SAT1 and SAT2 are more predominant and typically restricted to sub-Saharan Africa [14]. FMD virus Serotype O, A, and SAT2 are endemic in Ethiopia mostly in southern, central, and north-western parts of the country [11]. Currently, there is no evidence of the existence of the SAT3 serotype in Ethiopia [11].

FMD cases are often underreported in Ethiopia, making it challenging to determine the true incidence of the disease. Various studies have assessed the seroprevalence of FMD virus antibodies in cattle in Ethiopia, revealing a range from 4.8 to 72.1% [15–19]. A literature review spanning from 2007 to 2021 found an average seroprevalence of 21.39% [20]. While most studies in Ethiopia have focused on FMD seroprevalence, very few studies investigated the serotype distribution of FMD virus, which has implications for FMD vaccination strategies [11]. Many studies on FMD centred around central and northern areas of the country, while information on export livestock sourcing areas, such as the Bale, is scarce. Trade restrictions resulting from FMD outbreaks significantly impacted the international trade of animals and animal products [4]. This study addresses the information gap regarding the seroprevalence and serotypes distribution of FMD virus in the export animals sourcing areas of the Bale zone. It also aimed to investigate the geographical distribution of FMD and identify associated risk factors for transmission among cattle.

Results

A total of 962 cattle sera were subjected to FMD NSP ELISA for antibodies against the non-structural protein (NSP) of the FMD virus. Out of these, 200 samples (20.8%, 95% CI: 18.3–23.5) tested positive for NSP antibodies. The seroprevalence of FMD in each district is indicated in Table 1. The highest seroprevalence was seen in the Seweyna district at 35.2% (95% CI: 26.8–44.7) and the lowest seroprevalence was in the Dinsho district, at 13.0% (95%CI: 21.4–35.7).

The seroprevalence of FMD virus infection in the lowest administration unit (Kebele) varied from 9.4 to 43.8% Homa to Boditi respectively as indicated in Table 2.

Out of the 182 herds analysed, 111 herds were identified as having at least one animal tested positive for FMD, resulting in a herd-level seroprevalence of 61.0% (95%CI: 53.5–68.1) Table 3.

Risk factors associated with FMD seroprevalence in the study area

The univariable logistic regression analysis for risk factors associated with the seroprevalence of FMD virus was presented in Table 4. The seroprevalence of FMD exhibited a significant association with age of cattle ($P<0.05$). The study revealed a noteworthy correlation between sex and FMD seropositivity ($P<0.05$). There was a significant association between the Seweyna district and the seroprevalence of FMD virus antibodies in cattle ($P<0.05$).

The multivariable logistic regression analysis for risk factors associated with the seroprevalence of FMD is indicated in Table 5. The seroprevalence of FMD was found to be significantly associated with the age of

Table 1 Seroprevalence of FMD virus antibodies in cattle in districts of Bale Zone, Ethiopia ($N=962$)

District	Samples tested	Samples positive	Positivity (%)	95% CI	
Dawe-Qachen	107	21	19.6	12.6	28.4
Dinsho	277	36	13.0	9.5	17.5
Ginir	150	42	28.0	21.4	35.7
Legehida	225	45	20.0	15.3	25.7
Rayitu	98	19	19.4	12.8	28.3
Seweyna	105	37	35.2	26.8	44.7
Total	962	200	20.8	18.3	23.5

Table 2 Seroprevalence of FMD virus antibodies in cattle in Kebeles of study districts in Bale Zone, Ethiopia ($N=962$)

District	Kebele	Number tested (%)	Number positive (%)	95% CI	
Dawe-Qachen	Sof umer	48	8 (16.7)	7.5	30.2
	Ade-Arage	27	6 (22.2)	8.6	42.3
	Kubi-Weldiya	32	7 (21.9)	9.3	40.0
Rayitu	Jara-torbi	98	19 (19.4)	12.1	28.6
Legehida	Hida-hunda	97	17 (17.5)	10.6	26.6
	Goro-Raya	34	6 (17.6)	6.8	34.5
	Luku	13	4 (30.8)	9.1	61.4
	Sema	81	16 (19.8)	11.7	30.1
Seweyna	Boditi	16	7 (43.8)	19.8	70.1
	Adele	89	30 (33.7)	24.0	44.5
Ginir	Pudu	29	9 (31.0)	15.3	50.8
	Melka oda	16	4 (25.0)	7.3	52.4
	Gamoduksi	105	29 (27.6)	19.3	37.2
Dinsho	Gojera	71	7 (9.9)	4.1	19.3
	Homa	85	8 (9.4)	4.2	17.7
	Miyo	74	11 (14.9)	7.7	25.0
	Abakora	47	10 (21.3)	10.7	35.7
Total		962	200 (20.8)	18.3	23.5

cattle ($P<0.05$). The study found no significant association between sex and FMD seropositivity ($P>0.05$). There was a significant association between district and FMD virus antibodies in cattle ($P<0.05$). The odds of being FMD seropositive in Sewyena was 2.11 times higher (95% CI: 0.125–1.397) than in the reference district (Dawe-Qachen).

Table 3 Seroprevalence of FMD virus antibodies in cattle herds in the study Kebeles of Bale Zone, Ethiopia ($N=182$)

Districts	Kebele	Number of herds	Number of herds positive (%)	95% CI	
Dawe-Qachen	Sof umer	9	5 (55.6)	21.2	86.3
	Ade-Arage	6	4 (66.7)	22.3	95.7
	Kubi-Weldiya	7	4 (57.1)	18.4	90.1
Rayitu	Jara-torbi	20	10 (50.0)	27.2	72.8
Legehida	Hida-hunda	19	10 (52.6)	28.9	75.6
	Goro-Raya	7	4 (57.1)	18.4	90.1
	Luku	3	3 (100.0)	29.2	100.0
	Sema	17	12 (70.6)	44.0	89.7
Seweyna	Boditi	3	3 (100.0)	29.2	100.0
	Adele	19	11 (57.9)	33.5	79.7
Ginir	Pudu	7	6 (85.7)	42.1	99.6
	Melkaa oda	4	2 (50.0)	6.8	93.2
	Gamoduksi	19	12 (63.2)	43.4	87.4
Dinsho	Gojera	11	5 (45.5)	16.7	76.6
	Homa	4	2 (50.0)	6.8	93.2
	Miyo	15	8 (53.3)	44.9	92.2
	Abakora	12	6 (50.0)	21.1	78.9
Total		182	111 (61.0)	53.5	68.1

Seroprevalence of FMD serotypes in the study area

The seroprevalence of FMD virus serotype present in the study area was determined by initially screening samples for non-structural antibodies (NSP). Subsequently, 200 samples that were positive for NSP antibody underwent additional testing at a 1:10 dilution to detect serotype-specific antibodies. Serotype-specific antibody detection for serotypes O, A, SAT 1, and SAT 2 are indicated in Table 6. The result showed that 71% of the samples had serotype SAT1, 43% had serotype O, 38% had serotype SAT2 and 30% had serotype A. Antibody for serotype SAT1 was the most commonly detected, with the highest

Table 4 Univariable logistic regression to assess the association of risk factors and FMD seroprevalence in cattle in study districts of Bale Zone, Ethiopia ($N=962$)

Risk factors	Categories	Sample tested	Positive (%)	Odds ratio	95% CI		*P*-value
Districts	Dawe-Qachen	107	21 (19.6)	1	NA	NA	
	Dinsho	277	36 (13.0)	0.611	0.338	1.105	0.103
	Ginir	150	42 (28.0)	1.592	0.877	2.888	0.125
	Legehida	225	45 (20.0)	1.023	0.574	1.825	0.936
	Rayitu	98	19 (19.4)	0.984	0.493	1.966	0.965
	Seweyna	105	37 (35.2)	2.228	1.195	4.153	0.011
Age	Adult	412	114 (27.7)	1	NA	NA	
	Young	550	86 (15.6)	0.484	0.353	0.664	0.001
Sex	Female	610	140 (23.0)	1	NA	NA	
	Male	352	60 (17.0)	0.689	0.493	0.965	0.030

NA: Not available

Table 5 Multivariable logistic regression to assess the association of risk factors with the seroprevalence of FMD in districts of Bale Zone, Ethiopia ($N=962$)

Risk factors	Categories	Number of Sample	Positive (%)	Odds ratio	95% CI		*P*-value
Districts	Dawe-Qachen	107	21 (19.6)	1	NA	NA	
	Dinsho	277	36 (13.0)	0.810	0.422	1.553	0.525
	Ginir	150	42 (28.0)	1.489	0.812	2.729	0.197
	Legehida	225	45 (20.0)	0.936	0.518	1.693	0.828
	Rayitu	98	19 (19.4)	0.886	0.437	1.797	0.738
	Seweyna	105	37 (35.2)	2.11	1.124	3.994	0.020
Age	Adult	412	114 (27.7)	1	NA	NA	
	Young	550	86 (15.6)	0.598	0.409	0.876	0.008
Sex	Female	610	140 (23.0)	1	NA	NA	
	Male	352	60 (17.0)	0.7608	0.534	1.082	0.129

The Hosmer-Lemeshow goodness-of-fit test $\chi2=3.5711$; $p=0.7345$ [21]

NA: Not available

Table 6 Serotype-specific antibodies to the FMD virus in districts of Bale Zone, Ethiopia

Districts	Samples positive for FMD NSP	Samples positive per serotype (%)			
		O	A	SAT1	SAT2
Dawe-Qachen	21	13 (61.9)	4 (19)	14 (66.7)	8 (38.1)
Dinsho	36	8 (22.2)	10 (27.8)	11 (30.6)	8 (22.2)
Ginir	42	12 (28.6)	27 (64.3)	36 (85.7)	20 (47.6)
Legehida	45	22 (48.9)	1 (2.2)	37 (85.7)	14 (31.1)
Rayitu	19	12 (63.2)	2 (10.5)	15 (78.9)	6 (31.6)
Seweyna	37	18 (48.6)	15 (40.5)	29 (78.4)	19 (51.4)
Total	200	85 (43)	59 (30)	142 (71)	75 (38)

percentage 71%. Antibody for serotype A was the least detected serotype.

All studied districts showed evidence of the presence of more than one serotype. Multiple FMD serotypes were observed in 15–37.5% (30–75) of animals in each district while 8.5% [18] animals showed all serotypes shown in Table 7.

The univariable logistic regression to assess the association of risk factors and FMD serotype A, O, SAT 1 and SAT2 virus antibodies are presented in Table 8. There was a significant association between seroprevalence of FMD serotype A and district. Ginir and Legehida districts showed a significant association with FMD serotype A ($P<0.05$). In Ginir, the odds ratio was 7.65 (95% CI: 0.217–26.934), indicating a significantly higher likelihood of FMD serotype A. In contrast, in Legehida, only 1 out of 45 NSP positive sera 2.2% (95% CI: 0.01–0.927) was positive for serotype A.

There was no significant association between the age of cattle and the seroprevalence of FMD serotype A ($P>0.05$). The odds ratio was 0.791 (95%CI: 0.425–1.469), indicating a lower likelihood of serotype A in young compared with adults (reference category). Out of 86 NSP positive sera from young animals, 23 (26.7%) tested positive for serotype A. There was no significant association between the sex of cattle and the FMD serotype A ($P>0.05$). The odds ratio is 0.565 (95%CI: 0.278–1.476), suggesting a lower likelihood of serotype A compared with that of female cattle. Out of 60 NSP positive sera from male cattle, 13 (21.7%) tested positive for serotype A. There was a significant association between seroprevalence of FMD serotype O and districts. Animals from the Dinsho and Ginir districts were less likely to be infected with FMD serotype O compared to the reference district ($P<0.05$). There was no statistically significant association between age or sex and seroprevalence of FMD serotype O ($P>0.05$).

Table 7 Detection of multiple FMD serotype-specific antibodies in NSP-positive samples in districts of Bale Zone, Ethiopia ($N=200$)

Districts	Number of positive per serotype (%)						
	O and A	O and SAT1	O and SAT2	A and SAT1	A and SAT2	SAT1 and SAT2	Positive for Serotype (O, A, SAT1 &SAT2)
Dawe-Qachen	4 (19.0)	10 (47.6)	4 (19.0)	4 (19.0)	2 (9.5)	6 (28.6)	2 (9.5)
Dinsho	3 (8.3)	6 (16.7)	3 (8.3)	7 (19.4)	5 (13.9)	6 (16.7)	2 (5.6)
Ginir	11 (26.2)	12 (28.6)	8 (19.0)	26 (61.9)	17 (40.5)	19 (45.2)	7 (16.7)
Legehida	1 (2.2)	20 (44.4)	10 (22.2)	1 (2.2)	0 (0.0)	13 (28.9)	0 (0.0)
Rayitu	1 (5.3)	10 (52.6)	4 (21.1)	2 (10.5)	0 (0.0)	6 (31.6)	0 (0.0)
Seweyna	10 (27.0)	17 (45.9)	11 (29.7)	15 (40.5)	9 (24.3)	18 (48.6)	6 (16.2)
Total	30 (15.0)	75 (37.5)	40 (20)	55 (27.5)	33 (16.5)	68 (34.0)	17 (8.5)

Table 8 Univariable logistic regression risk factors associated with FMD serotype O, A, SAT 1 and SAT2 virus in the study districts of Bale Zone, Ethiopia

FMD virus serotype	variables	District						Age		Sex	
		Dawe-Qachen	Dinsho	Ginir	Legehida	Rayitu	Seweyna	Adult	Young	Female	Male
	Number positive for NSP	21	36	42	45	19	37	114	86	140	60
Serotype A	**Positive (%)**	**4 (19.05)**	**10(27.8)**	**27 (64.3)**	**1 (2.2)**	**2 (10.2)**	**15 (40.5)**	**36 (31.6)**	**23 (26.7)**	**46 (32.9)**	**13 (21.7)**
	Odds ratio	1	1.634	7.650	0.097	0.500	2.897	1	0.791	1	0.565
	95% CI	NA	0.440–6.063	2.17-26.939	0.010–0.927	0.081–3.103	0.812–10.335	NA	0.425–1.469	NA	0.278–1.147
	P-value		0.462	0.001***	0.042*	0.456	0.101		0.458		0.114
Serotype O	**Positive (%)**	**13 (61.9)**	**8 (22.2)**	**12 (28.6)**	**22 (48.9)**	**12 (63.2)**	**18 (48.6)**	**53 (44.5)**	**32 (37.2)**	**64 (45.7)**	**21 (35.0)**
	Odds ratio	1	0.176	0.246	0.588	1.055	0.582	1	0.682	1	0.639
	95% CI	NA	0.054–0.572	0.081–0.744	0.204–1.693	0.293–3.803	0.195–1.737	NA	0.385–1.207	NA	0.341–1.195
	P-value	0.280	0.004**	0.013*	0.326	0.934	0.333		0.189		0.162
SAT1	**Positive (%)**	**14 (66.7)**	**11 (30.6)**	**36 (85.7)**	**37 (82.2)**	**15 (78.9)**	**29 (78.4)**	**95 (83.3)**	**48 (55.8)**	**106 (75.6)**	**37 (61.7)**
	Odds ratio	1	0.220	3.000	2.315	1.875	1.813	1	0.2687	1	0.536
	95% CI	NA	0.069–0.695	0.856–10.503	0.706–7.573	0.449–7.820	0.547–6.007	NA	0.141–0.511	NA	0.281–1.023
	P-value	0.134	0.009*	0.086	0.166	0.388	0.331		0.001**		0.058
SAT2	**Positive (%)**	**8 (38.1)**	**8 (22.2)**	**20 (47.6)**	**14 (31.1)**	**6 (31.6)**	**19 (51.4)**	**56 (13.6)**	**19 (3.5)**	**55 (9.0)**	**20 (5.7)**
	Odds ratio	1	0.464	1.477	0.734	0.750	1.715	1	0.293	1	0.772
	95% CI	NA	0.142–1.511	0.507–4.301	0.248–2.168	0.202–2.774	0.576–5.109	NA	0.156–0.550	NA	0.410–1.458
	P-value	0.280	0.203	0.474	0.576	0.666	0.333		0.001**		0.426

Significant codes:-0.'***'0.001.'**'0.01'**0.05'* '.''0.1''1

NA: Not available

There was a significant association between seroprevalence of FMD serotype SAT1 and district. Dinsho district showed a statistically significant association with seroprevalence of FMD serotype SAT1 ($P<0.05$). Cattle in Dinsho were 0.22 times less likely to be positive for seroprevalence of FMD serotype SAT1 than a reference group. There was a significant association between age and seroprevalence of FMD serotype SAT1 ($P<0.05$). No significant association was found between sex and seroprevalence of FMD SAT1 ($P>0.05$).

The univariable analysis of risk factors linked to seroprevalence of FMD serotype SAT2 revealed that district and sex did not exhibit a significant association ($p>0.05$). Whereas, the age of cattle was significantly associated with seroprevalence of FMD SAT2 ($p<0.05$). Young animals were less affected than adult animals with the SAT2 FMD virus serotype.

Multivariable logistic regression to assess the association of risk factors and seroprevalence of FMD serotype A, O, SAT 1 and SAT2 virus antibodies are presented in Table 9. A significant association was observed between FMD serotype A and the district. Ginir and Legehida districts were significantly associated with seroprevalence of FMD serotype A ($P<0.05$). Cattle in Ginir had 7.65 (95% CI: 0.217–26.934) times higher likelihood of FMD serotype A. Animals from Ginir and Legehida districts were less likely to be infected with FMD serotype O compared to the reference district ($P<0.05$). There was no statistically significant association between age and sex and FMD serotype O ($P>0.05$). In the multivariable logistic regression analysis, FMD virus SAT1 had no significant association with district, age and sex of animals. There was a statistically significant association between age and seroprevalence of FMD SAT2. Young animals were less likely to be affected by FMD SAT2 ($P<0.05$).

Discussion

FMD poses a significant threat to the livestock industry due to its rapid spread and economic impact. Among the 962 cattle sera tested for NSP antibodies against the FMD virus infection, 20.8% were positive (95% CI: 18.3–23.5). There was a heterogeneity of FMD virus seroprevalence within the study districts. The FMD virus seroprevalence was highest in the lowland areas of Seweyna and Ginir

Table 9 Multivariable logistic regression risk factors associated with FMD serotype A, O, SAT 1 and SAT2 virus in the study districts of Bale Zone, Ethiopia

FMD virus serotype	variables	District						Age		Sex	
		Dawe-Qachen	Dinsho	Ginir	Legehida	Rayitu	Seweyna	Adult	Young	Female	Male
Serotype A	Odds ratio	1	1.463	7.017	0.065	0.400	2.237	1	1.071	1	0.358
	95% CI	NA	0.326–6.551	1.937–25.410	0.006–0.648	0.062–2.565	0.605–8.264	NA	0.431–2.66	NA	0.158–0.808
	P-value		0.618	0.003**	0.019*	0.334	0.227		0.881		0.013*
Serotype O	Odds ratio	1	0.158	0.222	0.485	0.947	0.504	1	1.064	1	0.645
	95% CI	NA	0.042–0.594	0.072–0.687	0.159–01.476	0.254–3.523	0.164–1.554	NA	0.523–2.161	NA	0.319–1.305
	P-value	0.280	0.006**	0.009**	0.202	0.965	0.233		0.863		0.223
SAT1	Odds ratio	1	0.311	2.932	2.193	2.046	1.708	1	0.606	1	0.757
	95% CI	NA	0.083–1.162	0.824–10.424	0.633–7.602	0.470–8.904	0.499–5.840	NA	0.265–1.388	NA	0.355–1.613
	P-value		0.082	0.096	0.215	0.340	0.392		0.236		0.471
SAT2	Odds ratio	1	1.1934	1.534	0.782	0.950	1.806	1	0.303	1	0.945
	95% CI	NA	0.302–4.704	0.506–4.651	0246-2.488	0.239–3.773	0.572–5.694	NA	0.138–0.665	NA	0.463–1.926
	P-value		0.800	0.449	0.677	0.942	0.312		0.002**		0.877

Significant codes:-0.'***'0.001.'**'0.01'**0.05'* '.''0.1''1

NA: Not available

districts, 35.2% (95% CI: 26.8–44.7) and 28.0% (95% CI: 21.4–35.7), respectively. Dinsho district, located at the Highland Massif of Bale Mountains, had the lowest seroprevalence, 13.0% (95% CI: 9.5–17.5).

Many studies recorded similar reports to our findings in different areas of Ethiopia [20, 22–24] in Bale, 21% [24] in Borana and 19.8% from Afar [19]. On the other hand, lower seroprevalence (ranging from 8.18 to 12.08%) compared to our findings were reported from different parts of Ethiopia [25–31]. In contrast to our study, higher seroprevalence were reported as 72.1% by Awel et al. [32] in central Ethiopia, 40.4% by Ahmed et al. [33] in West Shoa, 41.5% in Tigray [34], and 42.7% [35] and 53.6% [36] in Borana. Higher seroprevalence was also reported in other countries: 39% in Eritrea [37], 52.5% in Kenya [38], 55.9% in Nigeria's sedentary cattle [39] and 76.1% in Nigeria [40]. Variations in the seroprevalence of FMD across different regions are linked to ecological differences, disease dynamics, timing of sampling during outbreaks, and variations in the livestock production systems in various localities [41].

High FMD seroprevalence in Seweyna and Ginir showed that pastoral settings were affected more. Likewise, Megersa et al. [42] reported a 16 times higher likelihood of disease occurrence in pastoral areas of southern Ethiopia. The magnitude of seroprevalence decreases when agroecology changes from lowland to highland [43] and animals found in midlands and highlands were less likely to be infected by FMD infection. This was supported by low seroprevalence in the Dinsho district. The FMD virus transmission is influenced by geographic location, livestock management practices, and diverse animal populations. Extensive cattle movement in lowland areas for grazing and water increases the likelihood of contact with other cattle and wildlife reservoirs [24].

In our study, seroprevalence of FMD was found to be significantly associated with the age of cattle (27.7% in adults and 15.6% in young). The young animals were 0.484 (95% CI: 0.353–0.664) times less affected than the adult cattle. The age-specific seroprevalence study showed an increasing prevalence with age consistent with Gelaye et al. [26] and Molla et al. [30]. Woldemryiam et al. [23] reported a statistically significant association between seropositivity in cattle with age. A study in Nigeria showed a higher seroprevalence of FMD in adults (40.24%), followed by young animals (26.55%) [39]. Lower seroprevalence in younger animals may show infrequent exposure to risk factors. Adult animals might have acquired the infection from multiple serotypes and could produce antibodies against all serotypes of FMD. According to a study in Gamo Gofa, adult animals were 9.01 times more likely to be positive for FMD than young animals [43]. Adult animals had a high chance of freely moving for grazing and watering points where infection could occur when they come in contact with other animals. The low seroprevalence in younger animals may be due to less exposure to risk factors and limited contact with other herds, as most farmers keep their calves near the household during grazing [43].

In our study seroprevalence of FMD virus antibodies was 23% in females and 17% in males. In a comparable study in Nigeria, a higher seroprevalence was observed in

both females (58.2%) and males (50.62%) [39]. In the multivariable analysis, the odds ratio indicated that male cattle had 0.760 (95% CI: 0.534–1.082) times lower odds of FMD infection compared to females. However, no significant differences in FMD seroprevalence were observed between males and females ($P>0.05$). Our findings differ from previous studies in Ethiopia, where sex showed a significant association with the seroprevalence of FMD [24, 26, 42]. Our findings differ from previous studies in Ethiopia, where sex showed a significant association with the seroprevalence of FMD. Female cattle have a higher risk of FMD infection due to longer exposure than male animals, which are typically sold or removed from the herd shortly.

The serotype-specific antibody screening conducted at a dilution of 1:10 in NSP-positive cattle brought valuable insights into the serotype-specific immune responses against the FMD virus. In our study, serotypes SAT1, O, SAT2, and A were found to be 71%, 43%, 38%, and 30% of the samples, respectively. Serotype A was the least common serotype in the study population. Most of the districts had evidence of 15–37.5% multiple serotypes. The highest cross-reactivity tends towards serotypes SAT1 (27.5%, 34%, 37.5% with serotype A, SAT2 and Serotype O, respectively) and to some extent serotype O, and few with serotype A and SAT2. These findings provide an understanding of the serotype distributions of the FDM virus in the cattle population in the study areas.

Studies of serotype-specific FMD were scant or none in Ethiopia. Rufael et al. [24] reported a higher prevalence of serotype O (99.2%), A (95.8%) and SAT 2 (80%) in the Borana pastoral area compared to the findings of our study. Our findings are consistent with earlier research indicating that serotype O, A, SAT1, and SAT2 FMD viruses are currently circulating in Ethiopia [10, 17, 19, 44]. FMDV serotypes O, A, and SAT 2 were the cause of most of the outbreaks reported in domestic livestock in Ethiopia [11]. Similar to the findings of our study, 44% of NSP-positive samples were positive for serotype O by serotype-specific SPCE ELISA in Pakistan [45]. Unlike our results, the low seroprevalence of serotype A (15.4%) and serotype SAT2 (3.4%) were reported in Sudan [46]. Our findings showed different levels of antibodies against serotypes O, A, SAT1, and SAT2 in cattle. In pastoral production systems where animals travel significant distances to find water and pasture, having a mixture of different age groups in the herd, along with the lack of regular vaccination against FMD, may result in varying levels of exposure to FMD virus antibodies in our study.

Due to cross-reactivity between serotypes occurring in these assays, the higher finding of SAT1 in this study may not be conclusive. The occurrence of serotypes SAT1 was detected mostly through serological assay in the country. However, multiple FMD virus serotype combinations were observed in the SPCE. The antigenic relationships between the serotypes could lead to cross-reactivity or heterotypic immune responses to previous exposures from one or more infections [3].

The multivariable logistic regression analysis revealed the presence of a sex-specific association with serotype A antibody prevalence ($P<0.05$). Dinsho and Ginir districts exhibited statistically significant associations with FMD serotype O antibody presence (p-<0.05). Previous studies from the outbreak in Ethiopia showed Serotype O was the widely distributed serotype [11]. There was no significant association between FMD serotype O seroprevalence and the age and sex of the cattle ($P>0.05$). Multiple serotype infection was also observed in a study carried out in Uganda indicating that serotype-specific antibodies in Solid phase Blocking ELISA (SPBE)s were 61%, 33%, 67%, 37% and 12% of the investigated samples for serotypes O, A, SAT1, SAT2 and SAT3, respectively [3]. Multivariable logistic regression analysis showed that serotype SAT1 had no significant association with district, age and Sex of animals ($P<0.05$). The SAT serotype is more likely associated with the wildlife [47, 48]. Dinsho is adjacent to wildlife conservation of Bale Mountain National Parks where different hoof-cloven wild animals dwell. Unlike our study, a lower seroprevalence of 37% of SAT1 was reported in Uganda [3]. There was a significant association between the age of cattle and the presence of FMD serotype SAT2 antibodies ($P<0.05$). However, sex had no significant association with SAT2 antibody presence ($P>0.05$).

Understanding the distribution and prevalence of different FMD serotypes is crucial for effective control and prevention strategies, as vaccines and control measures may need to be targeted to specific serotypes based on their prevalence in a given region. In Ethiopia, regular vaccination for FMD is not common, and the vaccine utilized included Serotypes A, O, and SAT2, excluding SAT1. This study is limited to indicating FMD in wildlife which might require elucidating the epidemiological link of FMD virus antibody to outbreaks from wild animals in the study area, and transmission of the virus from livestock to wild animals or wild animals to livestock in the case of Dinsho district being near the national wildlife reservoir.

Conclusion

The result of NSP 3ABC ELISA showed an overall FMD antibody seroprevalence of 20.8%, due to exposure to active or previous viral infection. Serotype-specific antibodies against the FMD virus indicate the occurrence and distribution of four serotype-specific immune responses. Multiple serotypes in the study area might complicate the control of FMD in the export-livestock sourcing areas. Our findings highlight the need for continuous

surveillance to monitor the spread of the FMD virus in pastoral and sedentary areas.

Our study contributes to understanding the FMD virus serotype prevalence and associated risk factors to aid in developing targeted control and prevention strategies, including serotype-specific vaccination campaigns.

Methods

Study area and study design

A cross-sectional study was conducted in six districts of export livestock sourcing areas of Bale zone of Oromia Regional State, Southeast Ethiopia from January to March 2022 to estimate the seroprevalence and identify associated risk factors of FMD. Risk factors of FMD such as geographic location, age, sex and interaction with wildlife and pastoral production systems were considered. There were no FMD vaccination practices in the selected districts. The study areas were chosen based on the lack of reported FMD outbreaks in the last two years. The national strategic control plan for FMD in Ethiopia [49] identifies key export livestock sourcing areas in southern Ethiopia: Borana, Guji, Bale, Liban zone of Somali region and South Omo and feedlots along the trade route to Djibouti, where live animals for export are collected for export market.

Dinsho, one of the study districts, is situated near the Bale National Park. The Bale Mountains are part of the Bale-Arsi massif, forming the western section of the south-eastern Ethiopian highlands. Within the Dinsho district, four kebeles were chosen specifically from areas that intersect with the Bale Mountain National Park, where wild ungulates coexist or come into contact with domestic livestock. A selection of Dinsho as a study site allows for an investigation into the potential transmission of FMD between wild and domestic animals. The other districts which include Dawae Kechen, Ginir, Rayitu, Legahida, and Seweyna located within the pastoral agro-ecology of Bale were included in the study (Fig. 1). These districts are characterized by an agro-pastoralist way of life, where livestock rearing is the primary occupation.

Study animals

A total of 962 cattle samples were collected. Study animals consisted of indigenous cattle breeds raised in a pastoral and mixed crop-livestock system. Cattle six months of age or older from both sexes were chosen for

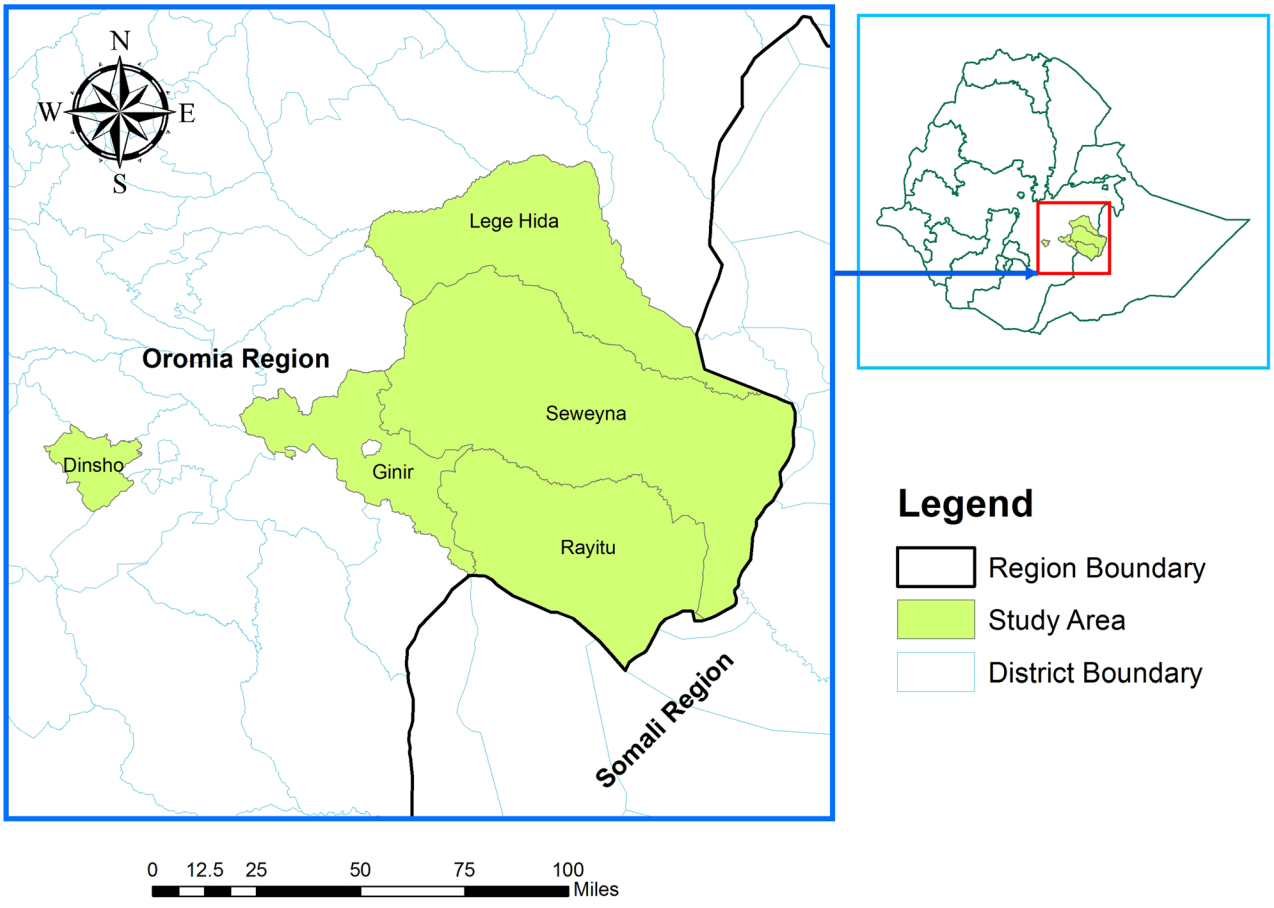

Fig. 1 Foot and Mouth Disease (FMD) seroprevalence and serotype-specific study district in Bale zone, Ethiopia

sampling. The age categories were defined as young (less than or equal to 3 years) and adults (greater than 3 years) [50]. Among the sampled cattle, 352 were males and 610 were females with ages ranging from 1 to 8 years and a mean age of 3.05 years. The median age for both males and females was 2 years.

Sampling methods

A multistage sampling approach was employed in the study. Districts were selected purposefully based on their accessibility. Within each selected district, 1 to 4 kebeles (the lowest administration unit in Ethiopia) were included based on cattle population and animals in the herds were sampled.

Associated risk factors

-Age, sex, and the production location of the animals (districts) were taken as important risk factors associated with FMD. This information was recorded in the prepared data sheet for each animal. The sample size was determined using simple random sampling and adjusted for the clustering effects. It was calculated using a formula that implies the normal approximation to the binomial distribution [51].

$$\text{Sample size} = (1.96/d)^2 \times \text{P}(\text{q})$$

$$(1.96/0.05)^2\, 0.2159\, (1 - 0.2159) = 260\,\text{cattle}$$

Where p is prevalence and q is the (1 – p), d is the precision of the tolerance around the prevalence for the 95% confidence limits, i.e. the desired maximum size of confidence, the expected positivity of 21.59% [22] in Senana and Goba districts in Bale. Due to the highly transmissible nature of FMD, a significant clustering of cases was anticipated. The initial sample size was calculated for simple random sampling and subsequently quadrupled to reach a total of 962 animals [21].

Sample collection storage and transportation

approximately 7 ml of blood samples were collected from the jugular veins of each animal using a plain vacutainer tube. The sera were separated from the blood, transferred to sterile cryovials, and stored at -20 °C freezer until testing.

Laboratory test

All sera were screened for antibodies against FMD virus antibody non-structural proteins (NSP) using the FMD virus 3ABC ELISA ID Screen competition kit (ID-VET, Grabels, France) to detect specific antibodies against the (NSP) of FMD virus regardless of serotypes as per the manufacturer instructions. Pre-coated 96-well microtiter plates with 3ABC antigen to detect FMD virus were used. Test sera, positive and negative control sera were added to the wells, followed by incubation and the washing step. Anti-ruminant antibody conjugate and tetramethyl benzidine substrate were added, and the plates were incubated for colour development. The reaction was stopped with sulphuric acid, and the optical density was measured at 450 nm using an ELISA reader. Results were expressed as an index based on absorbance values, and samples with percent inhibition (PI) less than or equal to 50% were considered positive, while samples with PI greater than 50% were classified as negative.

Serum samples positive for NSP of FMD were additionally examined for serotype-specific FMD virus antibodies using a solid-phase competitive ELISA (SPCE) as per the protocol outlined by the IZSLER Biotechnology Laboratory (Italy). The FMD virus serotype SAT3 was omitted from our SPCE test as this particular serotype has not been identified in Ethiopia. While the other FMD virus serotypes O, A, SAT1 and SAT2 were included in the test. The SPCE assay used 96-well plates coated with specific FMD virus antigens for serotypes O, A, SAT 1, and SAT 2, along with monoclonal antibodies for antigen capture. Serum samples were diluted and added to the wells, then the addition of a peroxidase conjugate. After washing, a substrate solution was added, and the plates were incubated in the dark. The reaction was stopped, and the plates were read at 450 nm. The percent inhibition (PI) value below 70% was interpreted as negative, while a PI value of 70% or above indicates positivity for serotypes O, A, and SAT2. However, for serotype SAT1, a PI greater than or equal to 60% was considered positive, while less than 60% PI was considered negative. The test demonstrated high specificity (99%) and sensitivity (100%) based on OIE guidelines [52].

Data analysis

The data obtained from laboratory investigations and the associated risk factors were recorded and coded in a Microsoft Excel spreadsheet (Microsoft Corporation) for analysis using R software (version 4.2.3 Copyright (C) 2023 The R Foundation for Statistical Computing Platform). Descriptive statistics were utilised to present results and compute the proportion of FMD-related risk factors. The seroprevalence was determined by dividing the positive ELISA results by the total number of samples tested. The association between seroprevalence and independent risk factors was evaluated through univariable and multivariable logistic regression analyses using R Software. The odds ratio, p-value, and 95% confidence interval were determined. The Hosmer-Lemeshow test was employed to evaluate the goodness of fit of the final model. Odds ratios (OR) were calculated to determine the degree of association between each risk factor and FMD seropositivity. A 95% confidence Interval (CI) was

calculated, and $p<0.05$ was considered statistically significant. In both univariable and multivariable logistic regression analyses, Dawe-Qachen was used as the reference district because of its position between pastoral and sedentary livestock production systems, along with its average seroprevalence.

Abbreviations

FMD	Foot and mouth disease
NSP	Non-structural protein
ELISA	Enzyme-Linked immuno sorbent Assay
SPCE	Sold phase competitive ELISA
CI	Confident Interval
SAT	South African Territories
PI	Per cent inhibition
Nm	Nanometer
SNNPR	South Nation, Nationalities and People Regional State
N	Number
df	Degree of freedom
AHI	Animal Health Institute
OR	Odds Ratio
SPBE	Sold phase Blocking ELISA
DG	Daniel Gizaw
TK	Tesfu Kassa
DN	Demessa Negessu
ML	Mengstitu Legesse
AF	Aynalem Fentie
HA	Hagos Ashanafi
HAS	Hagos Asgedom
AM	Ayelech Muluneh
CG	Chala Guyassa

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Author contributions
DG: Contributed to the conception of the research idea, data analysis and interpretation of data, writing, and editing of the manuscript. TK, and ML: Contributed to the conception of the research idea, designing, interpretation of data, and editing of the manuscript. HA and WT: Contributed to the conception of the research idea and interpretation of data, editing or reviewing of the manuscript. HAS, CG, and DN: contribute to sample collection and laboratory testing. AF and AM: Contributed to laboratory work and test analysis. All authors read and approved the final manuscript.

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Data availability
The datasets used and analysed during the current study are available from the corresponding author on request.

Declarations

Ethics approval and consent to participate
Ethical approval and consent obtained from the Addis Ababa University Aklilu Lemma Institute of Pathobiology, Institutional Review Board (ALIPB-IRB) Ethical clearance certificate ALIPB-IRB/82/2014/22. Verbal consent was obtained from village leaders and elders to incorporate their village/Kebele as study participants. Informed consent was obtained from all participating livestock owners to collect blood samples from their animals and used for testing foot and mouth disease.

Consent for publication
The consent of publication is not applicable.

Competing interests
The authors declare no competing interests.

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