

Seroprevalence, Risk Factors Analysis and Outbreak Investigation of Foot and Mouth Disease in Selected Areas of Central and Southern Parts of Ethiopia

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SEROPREVALENCE, RISK FACTORS ANALYSIS AND OUTBREAK INVESTIGATION OF FOOT AND MOUTH DISEASE IN SELECTED AREAS OF CENTRAL AND SOUTHERN PARTS OF ETHIOPIA.

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ABSTRACT

Background: Foot and mouth disease (FM) is a highly contagious Transboundary animal disease that causes significant economic losses in cattle. The study objectives were to estimate the seroprevalence of FMD, identifying circulating serotypes in the areas with and without active outbreak report.

Methods: A cross sectional study and outbreak investigation was performed from October 2023 to May 2025. Semi-structured questionnaire survey was used to assess the community's KAP toward FMD. The serum samples were tested by using 3ABC-ELISA and circulating serotypes were identified by SPC-ELISA. Tissue sample from active Outbreak area were Cultivated on BHK 21 Cell line and virus genome detected using RT-PCR. The circulating serotypes were identified by Antigen Captured ELISA (IZSLER, Brescia, Italy).

Result: The overall seroprevalence from 314 cattle was 80.89%. From 254 seropositive serum samples 22 strong seropositives were selected and tested for serotyping. The serotyping result showed concurrently 4 serotypes circulating in South Omo Zone namely, the O, A, SAT2, and SAT1. There was statistically significant difference in seroprevalence in South Omo Zone districts. The district and herd size were identified as significant risk factors associated with FMDV seropositivity. Outbreak investigation relies on 26 epithelial tissue and 2 swab samples collected from 54 cattle showing active clinical sign of FMD. Totally 28 samples were cultivated on BHK21 cell lines for virus isolation and 35.7% (10/28) of

35 the samples were positive for both BHK-21cell monolayer and RT-PCR. For
36 serotyping 10 positives samples for FMDV CPE were used and two circulating
37 serotypes were identified namely, 50.0% (5/10) of serotype O, 30.0% (3/10) of
38 serotypes A, and 20.0% (2/10) of mixed serotypes O/A. KAP survey analysis showed
39 that 95% of respondents do not vaccinate their animal against FMD and do not
40 quarantined their diseased animal.

41 **Conclusion and recommendation:** FMD is highly important economical disease
42 affecting the animal population in the study area. It was recommended to implement
43 biosecurity principles including quarantine and isolation of the diseased and
44 vaccination of at risk animals to reduce the incidence of disease in the study area.

45 **Key words:** *Foot and Mouth disease, isolation, molecular detection, outbreak*
46 *investigation, risk factors analysis, sero-prevalence.*

47

48 INTRODUCTION

49

50 Ethiopia possesses Africa's largest livestock population, with over 65 million cattle
51 ranking of fifth among the total world cattle population[1]. This sector underpins
52 livelihoods for 80% of the rural population and contributes 45% to agricultural
53 GDP[2]. Foot and Mouth Disease (FMD) is a highly contagious transboundary
54 disease affecting cloven-hoofed animals including cattle, sheep, goats, pigs, and
55 wildlife [3].

56

57 The causative agent, *Foot and mouth disease virus (FMDV)* belongs to the genus
58 *Aphthovirus* within the *Picornaviridae* family. *FMDV* exhibits high genetic diversity
59 comprising seven immunologically distinct serotypes: O, A, C, Asia 1, SAT1, SAT2,
60 and SAT3 [4]. Global distribution of these serotypes shows significant heterogeneity.
61 Serotypes O and A demonstrate pandemic distribution circulating widely across
62 Africa, Asia, and South America[5].

63

64 Ethiopia has documented FMD as endemic since its first official report in 1957 [6].
65 Likewise, FMD remains one of the top five economically significant endemic viral
66 diseases affecting cattle with four endemic serotypes including the serotype O, A,
67 SAT 1, and SAT 2, and the Serotype O is the most prevalent and widespread serotype
68 of *FMDV* [7, 8]. FMD affects approximately 77% of cattle in the Horn of Africa

69 region including Ethiopia, with estimated annual losses reaching USD 1.5 billion
70 through combined productivity deficits and missed trade opportunities [7, 9].

71

72 According to the seroprevalence and molecular confirmation study conducted so far
73 in different regions of Ethiopia, the prevalence in cattle ranged from 4.8% to 72.1%
74 [10–28]. Hyperendemic zones with particularly high prevalence include pastoral
75 systems such as Borana (seroprevalence 23.4–41.8%), South Omo (32.6–68.2%), and
76 Afar (27.3%) [29, 30]. Highland regions including Arsi experience annual outbreak
77 incidence rates of 17.8%, with significant disease pressure also documented in Gamo
78 zone [23]. Western and northern regions such as Benishangul-Gumuz and Amhara
79 similarly report persistent outbreaks [19, 21].

80 Despite this well-established endemic status, critical knowledge gaps impede effective
81 control. Serotype dynamics remain poorly understood due to insufficient concurrent
82 surveillance across outbreak and non-outbreak areas. Diagnostic limitations persist
83 through overreliance on serology without complementary virus isolation during
84 outbreak investigations. Control programs face challenges with national vaccination
85 coverage remaining below 15% [20].

86 This study addresses these evidence gaps through implementation of integrated
87 diagnostics combining serology, virus isolation, and real time RT-PCR across the
88 study areas. By simultaneously investigating areas with active outbreaks and regions
89 without reported cases, this research generates comprehensive data on serotype
90 distribution and transmission dynamics.

91

92 **MATERIALS AND METHODS**

93

94 **Study area**

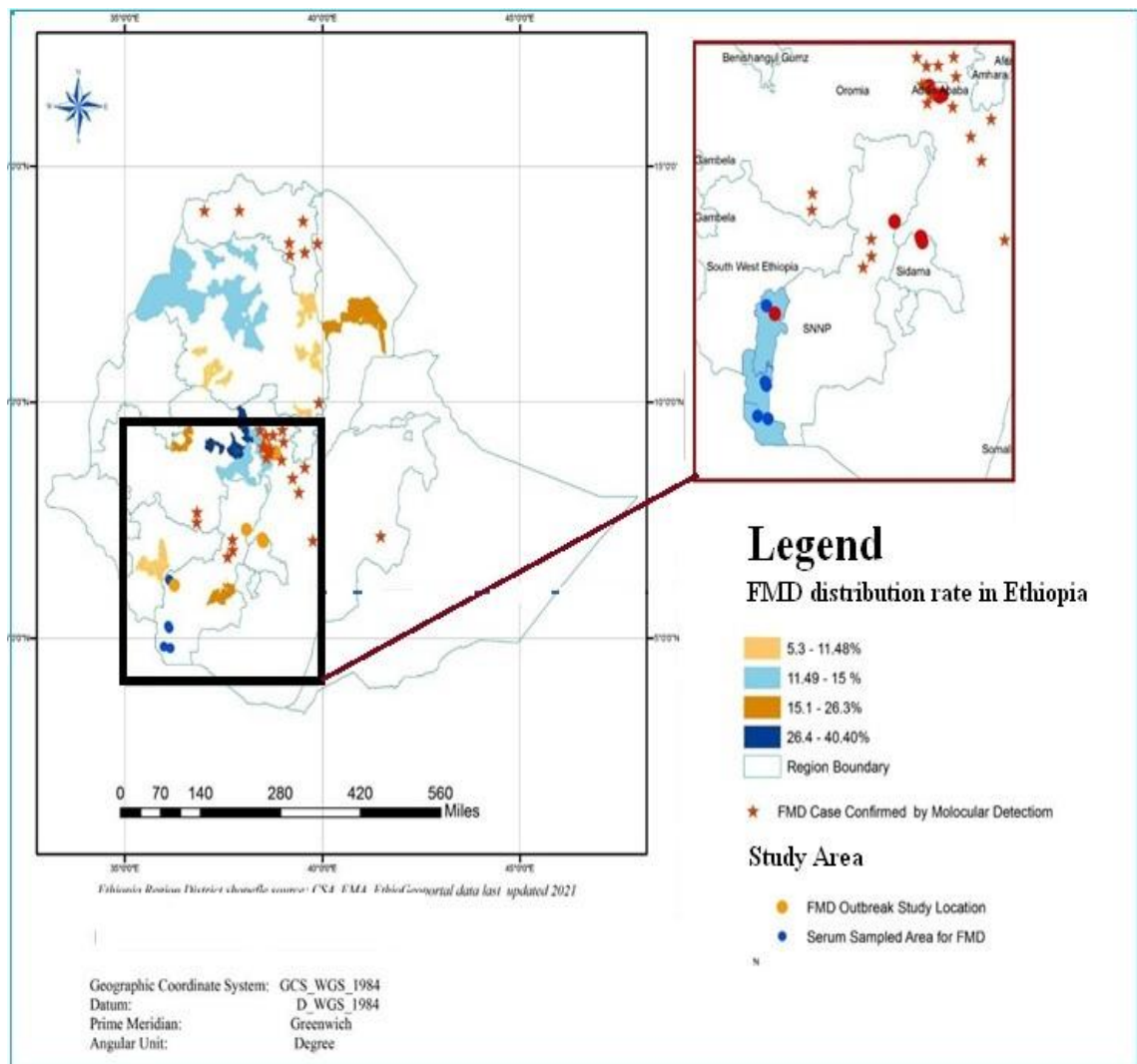
95

96 The study encompassed five ecologically diverse regions in Ethiopia, characterized by
97 distinct topographies and climatic conditions. The Sidama Region (Hawassa) features
98 a tropical climate (15–19.9°C) with elevations of 1500–2500 meters and annual
99 rainfall of 1200–1599 mm. Akaki Kality is one of the Addis Ababa sub-city that lies
100 at ~2300 meters, with a temperate climate (9.89–24.64°C) and high annual rainfall
101 (~1874 mm). In the Oromia Region, Burayu/Shegar City's highland terrain (2712 m
102 elevation) experiences cool temperatures (14.7°C mean), while Habas District (1918

103 m elevation) has a bimodal rainfall pattern (1500–2000 mm/year) and temperatures
104 ranging from 12–28°C.

105

106 Central Ethiopia’s Halaba Kulito (1554–2149m elevation) exhibits moderate
107 temperatures (12.8–26.8°C), contrasting sharply with South Omo Zone’s arid
108 lowlands (769 m elevation), characterized by semi-arid conditions, sporadic rainfall
109 (390 mm/year), and temperatures of 18–32°C. These regions collectively highlight
110 Ethiopia’s climatic and topographic diversity, spanning highland plateaus, temperate
111 urban zones, and arid lowland ecosystems as shown on the Figure 1 below.



112 **Figure 1:** Map of the study area.

113

114

115

116

117 **Study Population**

118
119 All cattle in the study area currently manifested the clinical signs of fresh FMD
120 lesions were included in the study subjects for outbreak investigation. All breeds,
121 Age, sex and management systems (the intensive, semi-intensive and extensive
122 management) were included in the study. All animal's ages were determined by
123 dental eruption age determination method [31] and therefore, animals were
124 categorized as young (<2 years), adults (2–5 years), and old (> 5 years). For
125 seroprevalence determination in the South Omo zone, only asymptomatic cattle and
126 unvaccinated animals against FMD were selected while in active Outbreak
127 investigation areas only cattle with active clinical sign were included.

128 129 **Study Design**

130
131 A cross sectional study supported by questionnaire survey and outbreak investigation
132 was performed from October 2023 to May 2025.

133 134 **Sample Size Determination and Sampling Method**

135
136 The sample size for the cross sectional study was determined using the Thrusfield
137 formula [32] based on Molla *et al.* [30] previous study of the disease's 8.18%
138 seroprevalence in cattle in the South Omo zone with a 95% confidence level and 5%
139 absolute precision. Hence, 115 animals were sampled; however, the sample size has
140 been increased by 2.73 to account for potential losses, resulting in a final sample size
141 of 314. The sample size required for the study was calculated based on the following
142 formula:

$$143 \quad n = ([1.96]^2 \times 0.08 \times (1 - 0.08)) / [0.05]^2 = 115 * 2.73 = 314$$

144 Where n = sample size, P_{exp} = expected prevalence, and d = absolute precision.

145 Then, proportionate numbers of animals were sampled from the study area based on
146 the cattle population size.

147
148 The epithelial tissue and swab samples were collected purposively from animals
149 manifesting FMD clinical sign and serum samples were collected from cattle without
150 showing FMD clinical sign.

Sample collection and Transportation

Blood Sample Collection: From each sampled animal, 10 milliliters of blood were collected from the jugular vein using a needle and plain Vacutainer tubes under aseptic environment. The type of farm, the sampling location, the date, and code of identification were all listed on the label of each vacutainer tube. Following that, the serum samples were decanted into 2 milliliter sterile cryovials after being kept upright at room temperature over a night. Following that, the serum was transported to the Animal Health Institute (AHI), Sebeta, Ethiopia, using an icebox containing icepacks. Until being processed, the samples were kept at temperature of -20 °C.

Tissue Sample collection: Epithelial tissue samples were obtained from intact and freshly burst vesicles and placed in 0.04 M phosphate buffer with 50% glycerol. Therefore, based on the availability of active cases of FMD, tissue samples were gathered and preserved in virus-transported media (VTM) by placing the sample in the cooling icebox. Following labeling and preserved in icebox, the samples were transported to Animal Health Institute, Sebeta, where the samples were stored in a cold chain and held at -80 °C until processing.

Laboratory Technique

Multiple stage laboratory tests were applied to assess seroprevalence and antibody based serotyping and moreover, serotyping by using the *FMDV* antigen in outbreak investigation by using various samples such as serum, tissue and Swab samples.

FMD Seroprevalence Study

Competitive 3ABC-ELISA: The serum sample tested using competitive 3ABC ELISA (ID Screen® FMD NSP Competition, ID Vet, Grabels, France) in the Animal Health Institute in Sebeta, Ethiopia. The tests were carried out following the manufacturer's instructions, which were designated for detection of antibodies against *FMDV* nonstructural Protein (NSP) in Serum. A spectrophotometer was used to measure the optical density (OD) at 450 nm. The test was considered validated if the mean value of the negative control OD was greater than 0.7 and the mean value of the

186 positive control OD was less than 30% of the ODNC. Based on the competition
187 percentage (S/N %) for each sample, the interpretation was determined as follows:
188 $S/N\% = OD \text{ sample}/NC \times 100$. If S/N% value was less than or equal to 50% samples
189 were defined as Positive, while if the S/N% value greater than 50% samples was
190 negative to the virus.

191

192 **Solid Phase Competitive-ELISA:** Strong positive serum resulted against *FMDV* by
193 Using 3ABC ELISA were serotyped by using Solid Phase Competitive-ELISA
194 (ISZLER: Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia
195 Romagna Brescia, Italy) to identify specific Serotype found in each sample. The tests
196 were carried out following the manufacturer's instructions and a spectrophotometer
197 was used to measure the optical density (OD) at 450 nm. The test was considered
198 validated if the mean value of the negative control OD was greater than 0.8 and the
199 positive control serum is expected to give $\geq 90\%$ inhibition at 1/10 dilution and $>50\%$
200 inhibition at the second dilution (1/30). This assay uses selected neutralizing anti-
201 *FMDV* monoclonal antibodies (Mab) specific for *FMDV* serotypes to measure
202 specific antibodies against the particular serotypes.

203

204 *FMD Outbreak investigation*

205

206 Tissue and swab specimens were collected from clinically suspected Foot and Mouth
207 Disease (FMD) cases in selected outbreak areas of Ethiopia. Individual tissue samples
208 were obtained from Akaki Kality districts 07 and 10 (Addis Ababa) and Burayu Town
209 (Sheger City), while pooled tissues samples were collected from Habas District,
210 Hawassa City, and Halaba Kulito Town. Pooled swab samples were collected from
211 Salamago District in the South Omo Zone. The specimens were inoculated onto
212 BHK-21 cell monolayers for virus isolation. Isolates showing characteristic cytopathic
213 effects (CPE) were confirmed by real-time RT-PCR, and the RT-PCR confirmed
214 isolates were subsequently serotyped using an antigen capture sandwich ELISA to
215 identify the circulating *FMDV* serotypes.

216

217 **FMD Virus Isolation:** Totally 28 epithelial tissue and swab samples collected from
218 FMD active outbreak areas were cultivated on baby Hamster Kidney21 cell lines
219 following the manufacturer's instructions, that 1 ml of filtered tissue suspensions were

220 inoculated on confluent baby hamster kidney (BHK-21) monolayer cells. The cells
221 were then incubated at 37 °C for 1 hour to allow the virus to absorb into the cells [33].
222 Then, for three consecutive days, the CPE was regularly evaluated three times a day at
223 intervals of twenty to 24 hours.

224

225 **RNA Extraction and Detection of *FMDV* by real time RT-PCR:** Following the
226 manufacturer's instructions, the total RNA was extracted from supernatants of the
227 homogenized epithelial tissue sample using the QIAamp RNA Mini kit (Qiagen,
228 Hilden, Germany) and the laboratory procedure protocol for RNA extraction and
229 FMDV genome detection by RTPCR was described in detail under Appendix 4. The
230 extracted RNA samples were tested by real time RT-PCR technique, which targeted
231 the 3D regions of the FMD virus genome to determine the presence of viral RNA.

232 For this RNA amplification purpose, the forward primer (5'-ACT GGG TTT TAC
233 AAA CCT GTGA-3'), reverse primer (5'-GCG AGT CCT GCC ACG GA-3'), and the
234 3D probe (5'-6-FAM-TCC TTT GCA CGC CGT GGG AC TAMRA-3') were used
235 [34]. After the PCR amplification was performed based on the instrument software
236 (Applied Biosystems 7500 Real-Time PCR System, Software), the successfully
237 amplified target presented an amplification curve and the cycle threshold (Ct) at
238 which the target amplicon first appeared above the background fluorescence levels.
239 The mean Ct value and standard deviation were given for each RT-PCR, which was
240 carried out minimally in duplicate [35].

241 **Antigen Detection Captured Sandwich ELISA:** The tissue and swab samples
242 resulted CPE positive after inoculated to confluent monolayer BHK21 cell lines were
243 further serotyped to identify *FMDV* serotypes by using *FMDV* Antigen Detection and
244 Serotyping ELISA (ISZLER, Brescia, Italy). The tests were carried out following the
245 manufacturer's instructions as described Laboratory protocol procedure is indicated
246 under Appendix 5 and the validation Criteria for antigen detection by sandwich
247 ELISA result was interpreted based on manufacture manual optical density (OD)
248 value.

249 **Questionnaire Survey**

250

251 The sample size for Questionnaire survey was determined using the formula ($n =$
252 $0.25/SE^2$) as per Arsham at the standard error (SE) of 0.05 with 95% confidence
253 interval [36]. Accordingly, 100 interviews were conducted with respondents with
254 South Omo zone with and without outbreak reported area, and 45 respondents were
255 from the outbreak reported area. The semi- structure questionnaire developed
256 originally in English then translated to local languages Me'en language, Daasanach
257 language, Nyangatom language, Afaan Oromo and Amharic language were used to
258 collect community knowledge, Attitude and Practice toward to FMD assisted with
259 Kobo Collect tool.

260

261 **Data Management and Analysis**

262

263 Data describing FMD information gathered both from field sample and laboratory
264 studies were appropriately coded, cleaned and entered in to Microsoft Excel® 2013.
265 The descriptive statistical method was used to compile the data. The data was then
266 loaded into STATA statistical software version 14.0. The significant difference and
267 association between the risk factors and FMD seropositivity was analyzed by p-value
268 that when the 95% confidence interval's p-value was less than 0.05, the results were
269 likely to be statistically significant. The questionnaire survey data collected from
270 respondents' to assess the knowledge, attitude and practices was determined by
271 descriptive statistics.

272

273 **Ethical Approval**

274

275 This ethical permission for this project was granted by both Addis Ababa University
276 College of Veterinary Medicine Animal Research Ethics Committee and Animal
277 Health Institute Research Ethics Committee by specifying reference numbers
278 VM/ERC/02/61/16/2025 and ARSERC/EC/025/27/05/2024, respectively. Thus, all
279 processes were conducted by qualified experts in accordance with the guidelines and
280 standards established by the university's ethical committee. The well-being and proper
281 care of the animals throughout the entire investigation were ensured during the
282 research was conducted. All owners of dairy farm and pastoral animals' owners
283 provided their verbal consent prior to the start of the study for the questionnaire
284 interview, as well as for the collection of their animals' serum or blood, epithelial
285 tissues, and swab samples.

RESULTS

Seroprevalence of *FMDV*

The seroprevalence of serum sample Antibodies against *FMDV* detected were 80.89% (254/314) of cattle tested. Seroprevalence varied significantly by district ($p < 0.001$) and herd size ($p < 0.001$), but not age ($p = 0.769$), sex ($p = 0.603$), or body condition ($p = 0.188$). Daasanech district had the highest seroprevalence (95%), exceeding Nyangatom (70.5%) and Salamago (74.4%). Prevalence increased with herd size: small herds (51.7%), medium (89.1%), and large herds (94.1%). No significant differences occurred among age groups: young (<2 years) (80.0%), adults (2-5 years) (77.9%), and older cattle (>5 years) (83.5%). Seroprevalence was comparable between males (82.2%) and females (79.9%). Body condition showed a non-significant trend toward higher prevalence in poor-condition animals (85.7%) versus medium (80.6%) or good condition (76.7%) (Table 1 below).

No significant association was observed between body condition score and *FMDV* seroprevalence ($p = 0.188$). Nevertheless, seropositivity was numerically higher in cattle with poor body condition (85.7%; 54/63) compared to medium (81.0%; 154/191) and good body condition score (76.7%; 46/60). Similarly, age groups showed comparable seroprevalence patterns: young (<2 years) 80.0% (88/110), adult (2-5 years) 77.9% (95/122) and old (>5 years) 83.5% (71/85), with no statistical significance ($p = 0.769$).

Table 1. The overall seroprevalence of antibodies against Foot and Mouth Disease Virus and the statistical analysis of the associated putative risk factors.

Risk factors	Variable	Total number of animals/Herd	Number of animals tested	Number of positive animals (%)	P-value
District	Dasenech	2039/33	120	114 (95%)	0.000
	Nyangatom	2235/33	112	79 (70.53%)	
	Salamago	2003/27	82	61 (74.39%)	
Age	Young (≤ 2)		110	88 (80%)	0.769
	Adult (2-5)		122	95 (77.86%)	
	Old (≥ 5)		85	71 (83.52%)	
Sex	Male		135	111 (82.22%)	0.603
	Female		179	143 (79.88%)	
Body Condition Score	Poor		63	54 (85.7%)	0.188
	Medium		191	154 (81%)	
	Good		60	46 (76.66%)	
Herd Size	Small		87	45 (51.72%)	0.000
	Medium		92	82 (89.1%)	
	Large		135	127 (94%)	
	Total	6277	314	254 (80.89%)	

Logistic regression analysis

Univariable logistic regression analysis

Table 2 below shows that univariable and multivariable logistic regression revealed the existence of risk factors that influence the seroprevalence of antibodies against *FMDV*. As a result, the risk factors that most likely did not affect the cattle seropositivity against FMD antibodies in possible risk factors like age ($p = 0.769$) and sex ($p = 0.603$) were among the variables removed from the model using univariable

logistic regression analysis, with a p-value of 0.25. Univariable logistic regression analysis revealed that risk factors for FMD seropositivity in the study location were significantly associated ($p < 0.05$) with study area ($p = 0.000$), body condition ($p = 0.188$), and herd sizes ($p = 0.001$).

Table 2. Univariable logistic regression analysis of risk factors associated with *FMDV* occurrence at individual animal's level.

Explanatory variable	Categories	No. tested	No. positive	Preval. (%)	OR	95% CI	P-value
Study area	Salamago	82	61	74.39%	1.148458	0.5784605-2.280114	0.692
	Dasenech	120	114	95%	7.997147	3.121234-20.49009	0.000
	Nyngatom	112	79	70.53%	Ref.		
Age	Young	110	88	80%	Ref.		
	Adult	119	95	79.83%	.9801786	0.485625 - 1.978379	0.955
	Old	85	71	83.52%	1.270685	0.5730451-2.817648	0.555
Sex	Male	135	111	82.22%	1.00414	0.5364146-1.879698	0.990
	Female	179	143	79.88%	Ref.		
Body condition	Poor	62	54	87.1%	1.340087	0.4873296 - 3.68505	0.571
	Medium	190	154	81%	.9740956	0.4689249 - 2.023485	0.944
	Good	62	46	74.1%	Ref.		
Herd size	Small	90	73	81.11%	2.14103	1.007241-4.551057	0.048
	Medium	141	125	88.65%	4.100488	1.962898-8.565908	0.000
	Large	83	56	67.46%	Ref.		

OR = Odd ratio, Ref. = References, CI = Confidence interval, Young = age less than 2 years, Adult = age between 2 to 5 years and Old = age ≥ 5 .

Multivariable logistic regression analysis of the putative risk factors

The multivariable logistic regression analysis identified district and herd size as significant risk factors associated with *FMDV* seropositivity (Table 3). After adjusting for the effects of other variables in the model, cattle from Daasanech district had significant higher odds of being seropositive for *FMDV* antibodies compared to cattle

from Nyangatom district (OR = 7.99; 95% CI: 3.12-20.5; $p < 0.001$). This indicates that animals in Daasanech were approximately eight times more likely to have been exposed to *FMDV* than those in Nyangatom. In contrast, the odds of seropositivity in Salamago district were not significantly different from those in Nyangatom district (OR = 1.14; 95%CI: 0.57-2.28; $p > 0.05$), as the confident interval included one.

351

Herd sizes were also strongly associated with *FMDV* seropositivity. Animals originating from large herds had significantly greater odds of seropositivity compared with those from small herds (OR = 15.00; 95% CI: 6.46-33.94; $P < 0.001$). Likewise, cattle from medium sized herds were significantly more likely to be seropositive than those from small herds (OR = 7.65; 95% CI: 3.50-16.68; $p < 0.001$). The magnitude of the odds ratios suggests a strong positive association between increasing herd size and *FMDV* exposure, with the highest risk observed in large herds.

Table 3. Multivariable logistic regression analysis of potential risk factors with *FMDV* seropositivity.

Risk factors	Categories	OR	(95% CI)	p - value
Study area	Salamago	1.148458	0.5784605 - 2.280114	0.692
	Dasenech	7.997147	3.121234 - 20.49009	0.000
	Nyangatom	Ref.		
Body condition	Poor	1.340087	0.4873296 - 3.68505	0.571
	Medium	0.9740956	0.4689249 - 2.023485	0.944
	Good	Ref.		
Herd size	Small	Ref.		
	Medium	7.65	3.5 – 16.68	0.000
	Large	14.8	6.46 – 33.94	0.000

361

363 Among the 254 serum samples that tested positive for antibodies against the *Foot and*
 364 *Mouth Disease Virus (FMDV)* by nonstructural protein 3ABC, 22 samples with high
 365 antibody titers were further analyzed using the Solid-Phase Competitive Enzyme
 366 Linked Immunosorbent Assay (SPCE-ELISA) for serotype identification. The
 367 analysis revealed the circulation of four *FMDV* serotypes of the SAT2, O, SAT1, and
 368 A. Serotype SAT2 was detected in 81.8% (18/22) of the examined samples, followed
 369 by serotype O in 72.7% (16/22), SAT1 in 59.1% (13/22), and serotype A in 45.5%
 370 (10/22). These findings suggest that four *FMDV* serotypes co-circulate in the study
 371 area and the Serotype SAT2 was the most frequently detected.

372 The distribution of serotypes varied across districts that all four serotypes were found
 373 in the Daasanech district, with SAT2 being the most prevalent (45.5%; 10/22),
 374 followed by O (40.9%; 9/22), SAT1 (27.3%; 6/22), and A (22.7%; 5/22). All four
 375 serotypes were also found in Nyangatom district, with SAT2 continuing to be the
 376 most common (31.8%; 7/22), followed by O (22.7%; 5/22), A (22.7%; 5/22), and
 377 SAT1 (18.2%; 4/22). On the other hand, only three serotypes with lower frequencies
 378 of occurrence SAT2, O, and SAT1 were found in the Salamago district. The
 379 prevalence of SAT2 in the districts of Daasanech and Nyangatom emphasizes its
 380 epidemiological significance and implies that it has had a significant role in the recent
 381 spread of *FMDV* in the studied area (as shown on Table 4 below).

382 Table 4. Serotyping of *FMDV* from South Omo Zone District

No.	District	Positive Sample (n)	Serotype O	Serotype A	Serotype SAT1	Serotype SAT2
1	Salamago	6	(2/22)	0	3	1
2	Daasanech	10	(9/22)	5	6	10
3	Nyangatom	6	(5/22)	5	4	7
	Total	22	72.72% (16/22)	45.45% (10/22)	59% (13/22)	81.81% (18/22)

383 **Outbreak Investigation Result**

384

385 **Virus Isolation:** Cytopathic effect (CPE), indicating successful isolation of live *Foot*
386 *and Mouth Disease Virus (FMDV)*, was observed in 10 (35.7%) of the 28 clinical
387 samples inoculated onto BHK21 cells monolayers. The appearance of characteristic
388 CPE confirmed the presence of viable infectious virus in these samples. The success
389 of virus isolation varied across the different geographical locations from which the
390 samples were collected. Based on the total number of clinical specimens collected
391 from each site, characteristic cytopathic effects (CPE) were observed in 30.8% (4/13)
392 of samples collected from Akaki kality, 50.0% (2/4) from Habas district, 40.0% (2/5)
393 from Halaba kulito Town, and 66.7% (2/3) from Burayu town. In contrast, none of the
394 specimens obtained from Hawassa City (0/1; 0%) and Salamago District (0/2; 0%)
395 developed CPE following inoculation onto BHK-21 cell monolayers, indicating
396 unsuccessful recovery of viable *FMDV* from these locations. These findings
397 demonstrate geographical variation in virus isolation success, which may be
398 associated with differences in viral load, stage of infection at the time of sampling,
399 specimen quality, or sample handling and transportation conditions.

400

401 **Real-Time RT-PCR:** among the total tissue (26) and swabs (2) specimens of
402 suspected FMDV collected, 10 CPE-positive virus isolates were subsequently tested
403 using universal *FMDV* primers and probes. Viral RNA was detected in 10 isolates
404 (35.7%), exhibiting Ct (Cycle threshold) values ranging from 19.8 to 32 (Figure 2).
405 Positives samples proportion from each sample locations were described that 4
406 positive samples from Akaki Kality Districts, 2 from Habas District, 2 from Halaba
407 Kulito Town, and 2 from Burayu Town.

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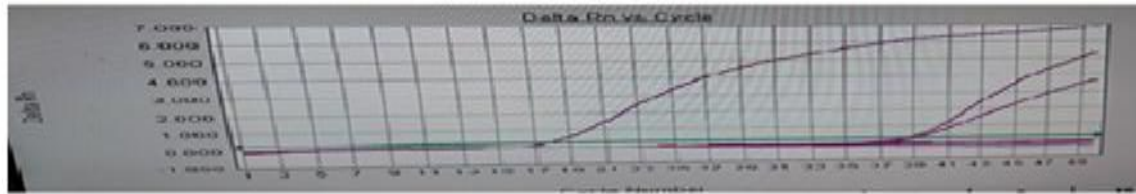


Figure 5A: Swab sample for FMD virus detection from Salamago district of South Omo zone Result

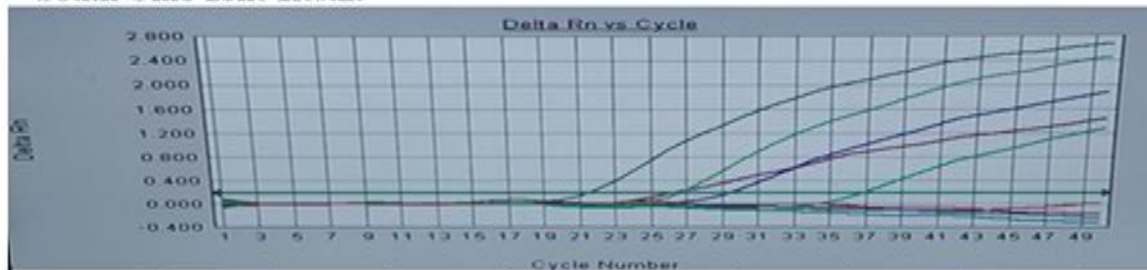
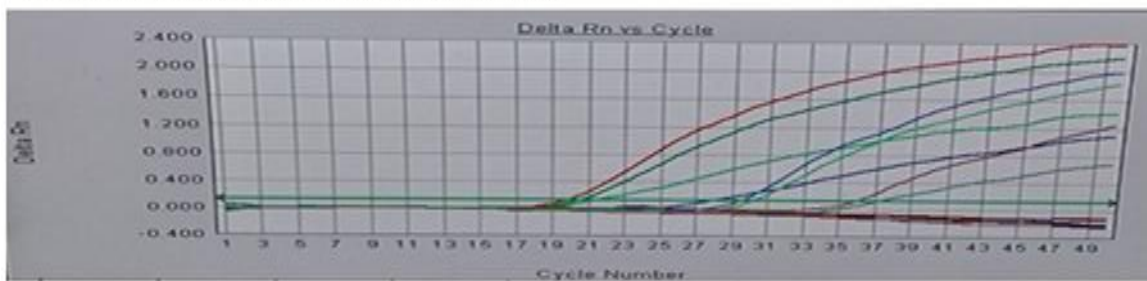


Figure 5B: Tissue sample for FMD virus detection from Hawassa city, Habas district and Halaba Kulito town Result



The Figure 5C: Tissue Sample for FMD virus detection from Burayu district, Addis Ababa Akaki Kalitworeda 07 and woreda 10 result

414 Figure 2: The RT-PCR Cycle threshold values

415 Following successful virus isolation in BHK-21 cell monolayers and molecular
 416 confirmation by real-time RT-PCR, the 10 confirmed *FMDV* isolates were serotyped
 417 using an antigen capture sandwich ELISA. Serotyping confirmed the co-circulation of
 418 *FMDV* serotypes O and A in the investigated outbreak areas. Serotype O was the
 419 predominant serotype, accounting for 50.0% (5/10) of the isolates, whereas serotype
 420 A was detected in 30.0% (3/10). Mixed infections involving both serotypes O and A
 421 were identified in the remaining 20.0% (2/10) of the isolates. Geographically,
 422 serotype O was detected in Akaki kalitworeda (2/10; 20.0%), Burayu Town (2/10; 20.0%),
 423 and Halaba Kulito Town (1/10; 10.0%). Serotype A was identified exclusively in
 424 Habas District (2/10; 20.0%) and Halaba Kulito Town (1/10; 10.0%). Mixed serotype
 425 O/A infections were detected only in Akaki kalitworeda District 07 (2/10; 20.0%). No
 426 *FMDV* isolates were recovered from samples collected in Hawassa City or Salamago
 427 District (Table 5).

Table 5. FMD outbreak investigation basis on virus isolation, serotyping & Molecular detection technique.

No.	Region	District	Sample Number	BHK21 CPE	Serotypes			RT-PCR (n=10)
					O	A	Mixed O&A	Sample
1	Addis Ababa	Akaki kality-07	13	4	2	0	2	4
2	Oromia	Habas	4	2	0	2	0	2
		Burayu	3	2	2	0	0	2
3	Central Ethiopia	Halaba kulito	5	2	1	1	0	2
4	Sidama	Hawassa city	1	0	0	0	0	0
5	South Ethiopia	Salamago	2	0	0	0	0	0
	Total		28	10	5	3	2	10

Questionnaire survey result

Respondent Demographics

A semi-structured questionnaire assessing knowledge, attitudes, and practices (KAP) related to FMD was administered to 145 respondents across eight districts in south Ethiopia's South Omo zone and other reference sites. The respondents were relatively well distributed among the major study districts, with the highest proportion originating from Nyangatom (24.1%; 35/145), followed by Daasanech (23.4%; 34/145) and Salamago (21.4%, 31/145). The remaining respondents were drawn from Addis Ababa akaki Kality (6.9%, 10/145), Habas (6.9%, 10/145), Halaba Kulito (9.0%, 13/145), Burayu (4.1, 6/145), and Hawassa Coty (4.1%, 6/145), providing a broad representation of both pastoral and mixed crop-livestock production system.

A demographic profile revealed a marked predominance of male respondents (96.6%; 140/145), while females accounted for only 3.4% (5/145). This imbalance likely

447 reflects the traditional gender roles prevalent in pastoral and livestock production
448 systems of Ethiopia, where livestock ownership, management, and decision making
449 are primarily undertaken by men. Similarly, the vast majority of respondents were
450 married (97.2%, 141/145), indicating that livestock husbandry and disease
451 management activities are largely practiced within established family households.

452

453 Age distribution showed that most respondents (81.4%, 118/145) were between 30
454 and 60 years of age, while 11.7% (17/145) were younger than 30 years and only 6.9%
455 (10/145) were older than 60 years. This predominance of economically active adults
456 suggests that the survey captured individuals with substantial involvement and
457 experiences in livestock production and animal health management. Comparable age
458 distributions have been reported in previous livestock and animal health studies
459 conducted in Ethiopia, where middle-aged household heads constitute the principal
460 livestock managers.

461

462 Occupationally, pastoralists represented the largest respondent group (43.4%;
463 63/145), followed by smallholder farmers (25.5%; 37/145), dairy farmers (23.4%;
464 34/145), and veterinary professionals (7.6%; 11/145). The predominance of
465 pastoralists is consistent with the livestock based livelihood systems of South Omo
466 Zone, where extensive pastoral and agro-pastoral production systems are common.
467 The inclusion of dairy farmers and veterinary professional's provided additional
468 perspectives on FMD awareness and management practices across different
469 production settings.

470

471 Educational attainment varied considerably among respondents. A substantial
472 proportion (60.7%; 88/145) had no formal education, while 24.1% (35/145) had
473 completed elementary education, 6.9% (10/145) had attained secondary education,
474 and only 8.3% (14/145) possessed a university degree as indicated in Table 6 below.
475 The high level of illiteracy observed among respondents is characteristic of many
476 pastoral communities in the study area of Ethiopia and may influence the
477 dissemination and adoption of animal health information, including FMD prevention
478 and control measures. Conversely, the relatively small proportion of respondents with
479 higher education may facilitate the transfer of technical knowledge and support
480 disease awareness initiatives within their communities.

481 Table 6. The overall Respondent demographic information during questionnaire
 482 survey.

Variables	Category	Response/Total	Percent (%)
Study area	Salamago	31	21.5%
	Daasanech	34	23.5%
	Nyangatom	35	24%
	Addis Ababa Akaki kality	10	7%
	Burayu	6	4%
	Habas	10	7%
	Halaba kulito	13	9%
	Hawassa city	6	4%
Gender	Male	140	96.5%
	Female	5	3.5%
Age	Age less than 30 years	17	12%
	Age between 30 – 60 years	118	81%
	Age above 60 years	10	7%
Marital status:	Married	141	97%
	Single	4	3%
Occupation	Small holder farmers	37	25.5%
	Employee	11	7.6%
	Pastorals	63/145	43.4%
	Dairy farmers	34/145	23.4%
Level of education:	Illiterate	88/145	60.%
	Elementary	35/145	24%
	High School	10/145	7%
	Degree	12/145	8.3%

483 **Farmer Knowledge of Foot and Mouth Disease**

484

485 Survey results revealed widespread awareness of FMD among respondents, with
486 81.4% (117/145) reporting familiarity with the disease, while 19.3% (28/145)
487 indicated no prior knowledge. However, critical gaps existed in biosecurity
488 understanding: only 4.8% (7/145) recognized the need for vaccination or quarantine
489 of infected animals, contrasting sharply with 95.2% (138/145) who acknowledged
490 neither practice. Symptom recognition was more prevalent, with respondents
491 identifying FMD primarily through excessive salivation (26.2%, 38/145), oral/udder
492 lesions (27.6%, 40/145), and lameness (32.4%, 47/145). Notably, 14.5% (21/145)
493 could not describe any clinical signs. Regarding outbreak timing, 17.2% (25/145)
494 reported occurrences within the preceding 6 months, while 67.6% (98/145) indicated
495 outbreaks beyond this period. Perceived transmission routes included animal markets
496 (38.6%, 56/145), wildlife contact (18.6%, 27/145), and neighboring herd exposure
497 (23.4%, 34/145), with minor contributions (1.4%, 2/145) attributed to other sources
498 (Table 7).

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519 Table 7. The Knowledge of the Respondents toward FMD.

Categories	Response	Frequenc y	Percent (%)
Knowledge FMD	YES	117/145	81%
	NO	28/145	19%
Vaccinating the animals	YES	(7/145)	4.8%
	NO	(138/145)	95%
The know- how animals quarantining	YES	(7/145)	4.8%
	NO	(128/145)	95%
Symptoms of FMD	Excessive salivation	38/145	26%
	Lesions and Ulcers on tongue, gums and udder	40/145	27.5%
	Lameness	47/145	32.5%
	NO	20/145	14%
Time of FMD occurrence.	Before six months	25/145	17%
	Less than six months	100/145	67%
	NO	20/145	14%
The means of FMD transmission	From market animals entered to herd	55/145	38%
	From neighboring animals	34/145	23%
	From wild life contact	26/145	18%
	NO	20/145	14%
	Others suspicion (contaminated feed, materials)	10/145	1%

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Community Practices and Attitudes toward Foot and Mouth Disease

Questionnaire Survey responses revealed that heterogeneous approaches to FMD management during outbreaks. While 34.5% (50/145) of respondents reported cases to veterinary professionals, most employed alternative strategies: 22.8% (33/145) treated animals directly, 13.8% (20/145) utilized traditional remedies, and 10.3% (15/145) undertook no intervention. Critical knowledge gaps persisted, with 13.8% (20/145) unable to recognize clinical signs. Notably, only 4.8% (7/145) implemented quarantine measures to separate infected animals. No respondents reported selling affected cattle to village butchers as a control strategy (Table 8).

Table 8. Community attitudes and practices toward FMD Control and Prevention.

Practice	Categories	Response/ Total	Percent (%)
What would you do if you suspected that your herd had Foot and Mouth Disease?	Quarantining the diseased animals	7/145	4.8%
	Report to veterinary Professionals	50/145	34%
	Treating the animals	33/145	23%
	Do nothing	15/145	10%
	Sell cattle to village butcher	0	0%
	NO	20/145	14%
	Others/traditional treatment	20/145	14%

DISCUSSION

This study investigated Foot and Mouth Disease (FMD) outbreaks and assessed FMD seroprevalence in cattle across selected districts of South Omo Zone, South Ethiopia. Using the 3ABC-NSP ELISA, we detected anti-*FMDV* antibodies in 80.89% (254/314) of purposively collected serum samples from Salamago, Daasanech, and Nyangatom districts. This finding represents a notably high level of infection, exceeding most previous seroprevalence reports from Ethiopia.

Specifically, our observed seroprevalence (80.89%) is higher than the 72.1% reported by Awel *et al.* [11] in central Ethiopia and significantly surpasses several other regional studies: Shurbe *et al.* [21] reported 26.82% in Gamo Zone; Ahmed *et al.* [16]

549 found 40.4% in West Shewa; Bandaw *et al.* [25] reported 46.88% in Wolaita Zone;
550 Seifu *et al.* [24] found 56% around Addis Ababa; and Megersa *et al.* [37] reported
551 only 9.5% in Southern Ethiopia. However, our result aligns closely with the 83.8%
552 seroprevalence reported by Nthiwa *et al.* [38] in the Maasai Mara, Kenya. This
553 similarity may reflect shared ecological and management factors, notably extensive
554 pastoral systems and significant wildlife-livestock interactions in both regions.

555

556 The co-circulation of multiple serotypes (O, A, SAT1, SAT2) resembles patterns
557 observed in Chad by Ouagal *et al.* [39] and Kenya by Nthiwa *et al.* [38], where similar
558 environmental conditions (high rainfall/humidity) and transboundary animal
559 movements in search of grazing were implicated. In contrast, Seifu *et al.* [24]
560 identified only serotypes A and O in dairy cattle around Addis Ababa and Sabeta.
561 The high overall seroprevalence observed was statistically significant across the
562 districts ($p = 0.000$), consistent with earlier findings by Molla *et al.* [30] in the South
563 Omo Zone. Key risk factors likely contributing to this elevated prevalence include
564 dynamic wildlife movements and frequent contact between wildlife and cattle,
565 particularly during shared grazing and watering.

566

567 District-level analysis revealed significant variation in seroprevalence: Daasanech had
568 the highest rate (95%), followed by Salamago (74.39%) and Nyangatom (70.53%).
569 The prevalence in Salamago and Nyangatom aligns with the 71.4% reported by Awel
570 *et al.* [11] for Holeta, while the exceptionally high prevalence in Daasanech mirrors
571 the 97% observed in Ada'a Barga by the same study.

572

573 Herd size emerged as a significant risk factor ($p = 0.001$). Prevalence increased
574 markedly with herd size, reaching 94% in large herds. This strong association is likely
575 driven by increased animal density and contact frequency, facilitating *FMDV*
576 transmission. This finding is consistent with studies by Tegegne *et al.* [27] and Batu *et al.*
577 [40] in Jimma Zone, Dubei and Negash [20] in the Afar region, and Woldesenbet
578 *et al.* [41] in Boset and Adama districts. In contrast, intrinsic factors such as sex and
579 age were not significantly associated with seroprevalence ($P > 0.05$). Seroprevalence
580 was similar between male (82.11%) and female (79.88%) cattle. This lack of
581 association concurs with previous findings by Woldesenbet *et al.* [41], Yahya *et al.*
582 [42], and Mohamoud *et al.* [43].

583

584 The odds of being seropositive of the cattle to *FMDV* is 7.9 times higher in Daasanech
585 districts than the Salamago district that it was due to the presence of uncontrollable
586 cattle movement around the Omo delta because of flood and for the search of seasonal
587 fodder for their cattle. These risk factors was actually strongly agreed with study
588 conducted by Molla *et al.*, [30], that the high seroprevalent of the animals with in
589 Dasenech and Nyangatom were due to the uncontrollable animals movements both by
590 crossing national boundary and the boundary of the Park and Sanctuaries which might
591 made great intermingling of wild animals and herds from different areas because for
592 the search of fodder. The odds of being seropositive of the cattle to *FMDV* are 1.3
593 times higher in cattle with poor body condition than the medium body condition. This
594 study disagreed with Awel *et al.*, [11] that the cattle with good body conditions were
595 highly exposed to the disease. The odds of being seropositive of the cattle to *FMDV*
596 are 4.1 times higher in medium cattle herd size than cattle with small herd size.

597

598 Solid Phase ELISA identified four concurrent *FMDV* serotypes circulating in the
599 study area: SAT2 (81.81%), O (72.72%), SAT1 (59%), and A (45.45%). This co-
600 circulation of multiple serotypes (O, A, SAT1, SAT2) resembles patterns observed in
601 Chad by Ouagal *et al.* [39] and Kenya by Nthiwa *et al.* [38], where similar
602 environmental conditions (high rainfall/humidity) and transboundary animal
603 movements in search of grazing were implicated. In contrast, Seifu *et al.* [24]
604 identified only serotypes A and O in dairy cattle around Addis Ababa and Sabeta.

605

606 Outbreak investigation using real-time RT-PCR and antigen capture sandwich ELISA
607 on tissue and swab samples confirmed the presence of *FMDV* genome and antigen.
608 Outbreak diagnostics specifically identified serotypes O and A, including mixed
609 infections: serotype O was detected in 50.0% of positive samples, serotype A in
610 30.0%, and mixed O/A infections in the remaining 20.0%. This finding confirms
611 previous reports of serotypes O and A circulating in Ethiopia. Serotype O was
612 detected by Awel *et al.* [11] in Addis Ababa and Ada'a Berga and Suleyman *et al.*
613 [14] in central Ethiopia. Serotype A was reported by Ayelet *et al.* [44] in Oromia,
614 Tigray, Dire Dawa, and SNNPR, and by Negessu *et al.* [45] in Dima Guranda and
615 Sabeta. Mixed O/A infections were observed by Negessu *et al.* [45] and Woldesenbet
616 *et al.* [41] in Boset and Adama.

617 Serotype O was the dominant strain in this outbreak (50.0%), aligning with several
618 Ethiopian studies reporting high prevalence: Tesfaye *et al.* [17]: 60% in Tigray;
619 Negessu *et al.* [45]: 87.5% in Central Ethiopia; Dubei and Amare [18]: 100% in Afar;
620 Awel *et al.* [11]: 90%; Seifu *et al.* [46]: 75.5% in Central Ethiopia. Tesfaye *et al.* [13]
621 also reported serotype O dominance in Northern and Central Ethiopia. The prevalence
622 of serotype A (30.0%) and mixed O/A infections (20.0%) in this study aligns partially
623 with prior work: Negessu *et al.* [45] reported 25% serotype A, while Tesfaye *et al.*
624 [47] reported 18% serotype O (lower than this study), 46% serotype A (higher), and
625 2.33% mixed O/A (lower).

626
627 The questionnaire survey revealed that 81% of respondents were familiar with Foot
628 and Mouth Disease (FMD), primarily identifying it through clinical signs. This aligns
629 with studies by Seifu *et al.* [24] (90.2%), Tegegne *et al.* [27] (86%), and Mesfine *et al.*
630 [15] (82.4%), confirming broad awareness among pastoral and dairy farmers.
631 Respondents accurately described clinical manifestations, including excessive
632 salivation, oral/hoof lesions, and lameness consistent with observations by Barre *et al.*
633 [48] in Somalia. Notably, FMD is recognized by diverse local terminologies:
634 "Maassaa" (Afaan Oromo), "Butlich" (Bodi/Me'en), "Ekire" (Nyangatom), and
635 "Wuluf" (Daasanach), underscoring its endemicity across cultural contexts.

636
637 Fewer respondents (38%) identified market-sourced infected animals as the primary
638 transmission route, a finding partially supported by Tegegne *et al.* [27] (82%) but
639 contrasting with Mesfine *et al.* [15] (22%). Additionally, 23% attributed transmission
640 to inter-herd contact during communal grazing, corroborating Mesfine *et al.* [15] (78%)
641 and Seifu *et al.* [24] (57%), though diverging from Tolawak *et al.* [23] (100%).
642 Secondary routes included wildlife contact (18%, especially in South Omo) and
643 contaminated feed/fomites (1%), the latter reinforcing Mesfine *et al.* [15] on sporadic
644 Amhara outbreaks linked to feed and contaminated objects.

645
646 Only 45% of respondents reported outbreaks to veterinary professionals. Disease
647 occurrence peaked within six-month intervals (67%), consistent with Nyaguthii *et al.*
648 [49] in Kenya, where 5.9% of farmers reported recent FMD cases. Despite this,
649 prevention measures were scarce: vaccination and quarantine coverage was minimal
650 (4.8%), starkly contrasting with Athambawa *et al.* [50] in Sri Lanka, where 49.2–

651 54.5% of farmers practiced vaccination. Furthermore, 35% notified animal health
652 professionals about infections, differing from Jemberu *et al.* [51], where 78% of
653 pastoralists reported annual outbreaks.

654

655 **Conclusion and Recommendations**

656

657 This study demonstrates that FMD remains hyperendemic in South Omo Zone,
658 Ethiopia, as evidenced by a high overall seroprevalence of 80.89%. District and herd
659 size were identified as significant risk factors associated with *FMDV* seropositivity,
660 indicating spatial variation in disease occurrence and increased transmission within
661 larger herds. The concurrent circulation of four *FMDV* serotypes (SAT2, O, SAT1,
662 and A) highlights the complex epidemiology of the disease and presents a substantial
663 challenge for effective prevention and control. Outbreak investigations revealed that
664 serotype O was the predominant cause of active outbreaks (50%), followed by
665 serotype A (30%) and mixed O/A infections (20%). Although respondents generally
666 demonstrated good knowledge of FMD, the adoption of essential disease control
667 measures was limited. Only 5% of respondents reported implementing vaccination or
668 quarantine practices, and fewer than 45% notified veterinary authorities of suspected
669 outbreaks, indicating important gaps in disease prevention and reporting.

670 Based on these findings, the following recommendations are proposed:

- 671 • **Strengthen vaccination strategies:** Implement routine vaccination programs
672 using multivalent vaccines containing serotypes O, A, and SAT2 that are
673 antigenically matched to the circulating field strains identified through
674 continuous surveillance.
- 675 • **Enhance surveillance and diagnostic capacity:** Strengthen integrated
676 surveillance systems by combining serological, virological, and molecular
677 diagnostic approaches for early outbreak detection and continuous monitoring
678 of circulating *FMDV* serotypes. Where appropriate, incorporate wildlife
679 surveillance to better understand the role of wildlife in virus maintenance and
680 transmission.
- 681 • **Improve community-based disease control:** Develop culturally appropriate
682 awareness and extension programs that promote vaccination, quarantine,

683 movement control, timely outbreak reporting, and practical biosecurity
684 measures among pastoral and agro-pastoral communities using local languages
685 and locally relevant communication approaches.

- 686 • **Support further research:** Conduct longitudinal molecular epidemiological
687 studies to characterize the genetic diversity and transmission dynamics of
688 circulating *FMDV* serotypes, investigate the role of wildlife reservoirs in
689 maintaining SAT serotypes, and evaluate the field effectiveness of currently
690 available vaccines against locally circulating strains.

691 Implementing these evidence based interventions will contribute to reducing FMD
692 transmission, improving disease surveillance and outbreak response, and supporting
693 the development of sustainable FMD control strategies in the Ethiopia.

694 LIST OF ABBREVIATIONS

695	3ABC-ELISA	3abc-Enzyme Linked Immune Sorbent Assay
696	AHI	Animal Health Institute
697	BHK21	Baby Hamster Kidney 21
698	BSL2	Biosafety level two
699	CPE	Cytopathic effect
700	ELISA	Enzyme Linked Immune Sorbent Assay
701	FMD	Foot and Mouth Disease
702	FMDV	Foot and Mouth Disease Virus
703	GDP	Gross Domestic Product
704	KAP	Knowledge Attitude and Practices
705	MAb	Monoclonal Antibody
706	NSP	Non-Structural Protein
707	OIE	Office of International Epizootics
708	PBS	Phosphate Buffer Saline
709	PCR	Polymerase Chain Reaction
710	RNA	Ribonucleic Acid
711	RT-PCR	Real Time Reverse Transcriptase Polymerase Chain Reaction
712	SAT	South African Territories
713	SPC-ELISA	Solid phase Competitive ELISA
714	SW-Ethiopia	Soft West Ethiopia

715 WRLFMD World Reference Laboratory Foot and Mouth Disease

716 **Ethics Approval**

717 This ethical permission for this project was granted by Addis Ababa University
718 College of Veterinary Medicine Animal Research Ethics Committee by specifying
719 reference numbers VM/ERC/02/61/16/2025. Thus, all processes were conducted by
720 qualified experts in accordance with the guidelines and standards established by the
721 university's ethical committee. The well-being and proper care of the animals
722 throughout the entire investigation were ensured during the research was conducted.

723 **Consent to Participate**

724 Informed consent was obtained from all respondents. Before obtaining their consent,
725 the purpose of the study was clearly explained to each respondent.

726 **Clinical trial number**

727 Not Applicable.

728 **Consent to publish the paper.**

729 Not applicable.

730 **Availability of Data and Materials.**

731 The data used to support the findings of this study can be received from the
732 corresponding author on request.

733 **Declaration of competing interests.**

734 The authors have declared that no competing interest exists.

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736 The authors received no specific funding for this work.

737 **Authors contributions.**

738 **Tekalegn Desta:** Writing original research paper, conceptualization, sample
739 collection, laboratory work processing and review the research paper project.

740 **Eyob Hirpa:** Supervising the paper work, give guidance, Edit the concept of
741 Original research idea, Review, Edition, Visualization and Validation.

742 **Tesfaye Rufael and Getnet Abie:** Supported and supervised the execution of the
743 research project from initiation through successful completion.

744 **Daniel Gizaw:** support and follow up the field sample collection and laboratory test
745 works.

746 **Ayelech Muluneh and Ayinalem Fantie:** technically support on serum antibody
 747 detection against FMD suspected specimen.

748 **Takele Worku:** Tissue sample collection and serum sample running to detect
 749 antibody against FMD specimen sample.

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753 **Tsion Blatta:** Provided technical support for cell culture procedures.

754 **Demesa Negesu:** Provided technical support for cell culture procedures and
 755 contributed to tissue sample collection during field and laboratory investigations.

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 757 laboratory analysis, including the detection of *Foot and Mouth Disease virus* in tissue
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