



Seroprevalence, characterization, and risk factors of brucellosis in cattle, sheep, goats, and camels in the Oromia region, Borena Zone, Ethiopia

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ABSTRACT

Brucellosis is a significant issue in the Borena Zone of Ethiopia, causing economic losses due to decreased milk production, abortions, infertility, and weak offspring. However, it is underreported and adequately addressed in the region. A cross-sectional study was conducted in the Gomole and Dhas districts of the Borena Zone in Ethiopia between November 2022 and June 2023 to estimate the seroprevalence of brucellosis in livestock. The study included cattle, camels, sheep, and goats, with random sampling at the individual and herd levels. A total of 490 cattle, 160 camels, and 330 each of sheep and goats were tested for seroprevalence. Random sampling was done on farms within the peasant association to test livestock at the individual and herd levels. Blood samples were collected from 490 cattle, 160 camels, and 330 each from sheep and goats. Samples were screened with the rose Bengal test (RBT) confirmed with the indirect enzyme-linked immunosorbent assay (iELISA). Polymerase chain reaction (PCR) detected *Brucella* species in blood clot samples collected from seropositive animals. The highest prevalence was observed in goats, with individual and herd-level rates of 10.0 % and 56.7 %, respectively. In contact, cattle had the lowest prevalence, 1.4 % at the individual level and 23.0 % at the herd level. Both *B. abortus* and *B. melitensis* were detected in cattle, sheep, and camels, while *B. melitensis* was the most common species found in goats. Statistically significant differences in brucellosis prevalence were observed among species, with the highest rates in the Gomole district compared to Dhas. Female animals had a higher prevalence than males, especially those with a history of abortion. Logistic regression showed that district and host species were associated with *Brucella* infection, with Gomole herds at higher risk. *Brucella melitensis* infections were common in sheep, camels, and goats, while *B. abortus* infections were mainly in cattle, with mixed infections in all species except sheep. *Brucella melitensis* infections were common in sheep, camels, and goats in the Oromia pastoral community, while *B. abortus* infections were mostly seen in cattle, with mixed infections in all species except sheep.

1. Introduction

Brucellosis remains a neglected but important zoonotic disease that continues to challenge the health and livelihoods of both humans and animals, especially in low- and middle-income countries (WHO, 2016). It is caused by *Brucella* species, small, Gram-negative, non-spore-forming bacteria that can survive and replicate inside host cells, making them particularly difficult to eliminate (Godfroid et al., 2011; Seleem et al.,

2010). Each *Brucella* species tends to favor specific animal hosts: *B. abortus* typically affects cattle, *B. melitensis* targets sheep and goats, *B. suis* infects pigs, and *B. ovis*, though not zoonotic, causes reproductive disorders in rams. Still, under conditions of close contact and shared environments, these bacteria can cross species boundaries, posing risks to a wider range of animals and people (Godfroid et al., 2010, 2014).

Humans generally contract brucellosis through consuming unpasteurized milk and dairy products or by coming into contact with infected

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animals or their tissues, especially during handling of aborted fetuses or placental material (OIE, 2000). Those who live and work closely with animals, such as pastoralists, veterinarians, abattoir workers, and laboratory personnel, are particularly vulnerable (McDermott et al., 2013). In livestock, brucellosis typically presents as a reproductive disorder, causing abortions, infertility, and decreased milk production. These effects not only reduce productivity but also lead to significant economic losses and limit access to national and international markets (Bernués et al., 1997).

In Ethiopia, brucellosis remains endemic, especially in pastoral regions where about 30 % of the country's livestock population is found (WHO, 2016). Serological surveys have repeatedly confirmed the presence of *Brucella* antibodies in cattle, camels, sheep, and goats (Tadesse, 2016). While *B. abortus* and *B. melitensis* are thought to be the dominant strains affecting cattle and small ruminants, respectively, serological tests alone cannot differentiate between these smooth *Brucella* species. Recent molecular studies have confirmed *B. abortus* in cattle from central Ethiopia (Geresu et al., 2016; Edao et al., 2023) and *B. melitensis* in goats from the Afar Region (Tekle et al., 2019), reinforcing the importance of combining serology with molecular diagnostics to understand the local epidemiology.

Brucellosis prevalence varies significantly across regions and species in Ethiopia. For example, Terefe et al. (2017) reported a 7.1 % seroprevalence in cattle in Oromia's pastoral areas, while Megersa et al. (2011) found 6.2 % in cattle and 1.8 % in small ruminants in Afar. These differences are often linked to varying husbandry practices, herd mobility, and levels of biosecurity. Certain animal-related factors such as herd size, age, and reproductive status have also been associated with increased risk of infection, with adult females of reproductive age often being the most susceptible (Alemu et al., 2014). Despite these studies clearly portraying brucellosis as endemic in Ethiopia, there is no official control strategy in place (Edeo et al., 2023; Megersa et al., 2011; Tadesse, 2016). The absence of effective control strategies, such as routine vaccination and systematic testing schemes, allows this zoonotic disease to continue spreading and remain endemic in Ethiopia. Without these key interventions, infected animals remain undetected and serve as ongoing sources of infection to both livestock and humans. The Borena Zone in southern Ethiopia is among the regions most affected. TeshomeYimer et al. (2021) reported a seroprevalence of 17.4 % in goats from the Elewaya, Moyale, and Yabello districts. Similarly, Edeo et al. (2023) identified seroprevalence rates of 2.4 % in cattle, 3.2 % in sheep and goats, and 2.6 % in humans in districts such as Elewaya, Gomole, Dubuluk, and Miylo, with Elewaya having the highest burden. These findings highlight the interconnected nature of animal and human health in pastoralist settings, where zoonotic transmission is facilitated by close contact with livestock.

Pastoral communities in Ethiopia cover over 60 % of the country's landmass and rely heavily on livestock for food, income, and cultural identity (Abduselam, 2019). In these areas, brucellosis continues to pose a serious threat to both animal productivity and public health. Limited access to diagnostic services, weak disease surveillance systems, and the absence of targeted control programs make it difficult to manage the disease effectively (Edeo et al., 2023). While several studies have addressed brucellosis prevalence, few have explored *Brucella* species identification using molecular tools or assessed context-specific risk factors in Ethiopia's southern pastoral regions.

In endemic regions, disease awareness, poor coordination amongst stakeholders, uncontrolled animal trade, infrastructural weaknesses, and the lack of or uncritical adoption of control and eradication strategies are persistent challenges in controlling brucellosis (Hikal et al., 2023; Moriyón et al., 2023). This situation is not unique to Ethiopia, as most East African countries have limited surveillance systems. The prevalence of brucellosis, which is higher in most pastoral communities in East African countries, including Kenya, Eritrea, South Sudan, Rwanda, Tanzania, and Somalia (Madut et al., 2018; Mohamud et al., 2021; Djangwani et al., 2021; Moriyón et al., 2023; Hikal et al., 2023),

further reflects the absence of effective control and eradication strategies, allowing the disease to continue to spread and remain endemic across the region. The present study aimed to estimate the seroprevalence of brucellosis in cattle, camels, sheep, and goats in the Gomole and Dhas districts of the Borena Zone, southern Ethiopia. It also sought to identify the circulating *Brucella* species using AMOS-PCR and IS711-based real-time PCR and to examine animal and herd-level risk factors through univariate and multivariate logistic regression analyses.

2. Materials and methods

2.1. Description of study areas

The study was conducted in the Gomole and Dhas districts in the Oromia region of the Borena zone, in Ethiopia (Fig. 1). The research focused on the Xilo, Dhas, Buya, Xile, and Harbor peasant associations of pastoralist communities from November 2022 to January 2023. The area is located at 05° 12' 17" N latitude and 038° 18' 00" E longitude, in the southern part of Ethiopia, bordering Kenya in the south, the Somali regional state in the east, and the Southern regional state in the north. The region experiences a bimodal rainfall pattern with the longest wet season from March to May, contributing 65 % of the annual rainfall. The shorter rainy season occurs from October to November, providing the remaining 35 % of the annual precipitation. The landscape is characterized by savanna grassland and varies in elevation from 1270 to 1630 m above sea level. Traditionally pastoral, the community primarily raised cattle and small ruminants. However, in recent decades, camels have become a significant part of the livestock due to recurrent droughts and livelihood challenges (Mitiku et al., 2022).

In the Gomole and Dhas districts of the Oromia region in the Borena zone, the dominant livestock species are cattle, sheep, and goats, with cattle as the primary livestock raised in this area. Cattle herd varies from small (10–50 head) to large (over 100 head), depending on the wealth and resources of the owner. Sheep and goats are also commonly kept, with herd sizes generally ranging from 20 to 60 heads per owner. Camels, while less numerous compared to other species, have become increasingly significant due to recurrent droughts and livelihood challenges, with herds typically ranging from 10 to 30 head. Animal husbandry practices in the study area involve communal grazing and watering points. Cattle from different owners are kept together on common pastures and share watering points, although they are housed separately in individual stables. Camels are generally kept apart from other species but may interact with other flocks at watering points. Sheep and goats graze together and share watering points with other livestock but are also housed separately by each owner. Currently, there are no brucellosis control interventions, such as vaccination programs, in Ethiopia (Megersa et al., 2011).

2.2. Study design

A cross-sectional study was conducted from November 2022 to January 2023 to study seroprevalence, characterize *Brucella* species using PCR assays in seropositive animals, and determine the risk factors of brucellosis in cattle, camel, sheep, and goats.

2.2.1. Sampling technique and sample size determination

The following formula has been used to determine the sample size to create reliable estimates in each area and account for unknown variability.

$$N = 2 * 1.96^2 * p(1 - p)/d^2$$

Where: N = sample size for one study site, p = expected prevalence, d = absolute precision

Utilizing the above formula, an expected prevalence of 14 % as reported by (Teferi, 2010) was used for the sample size calculation,

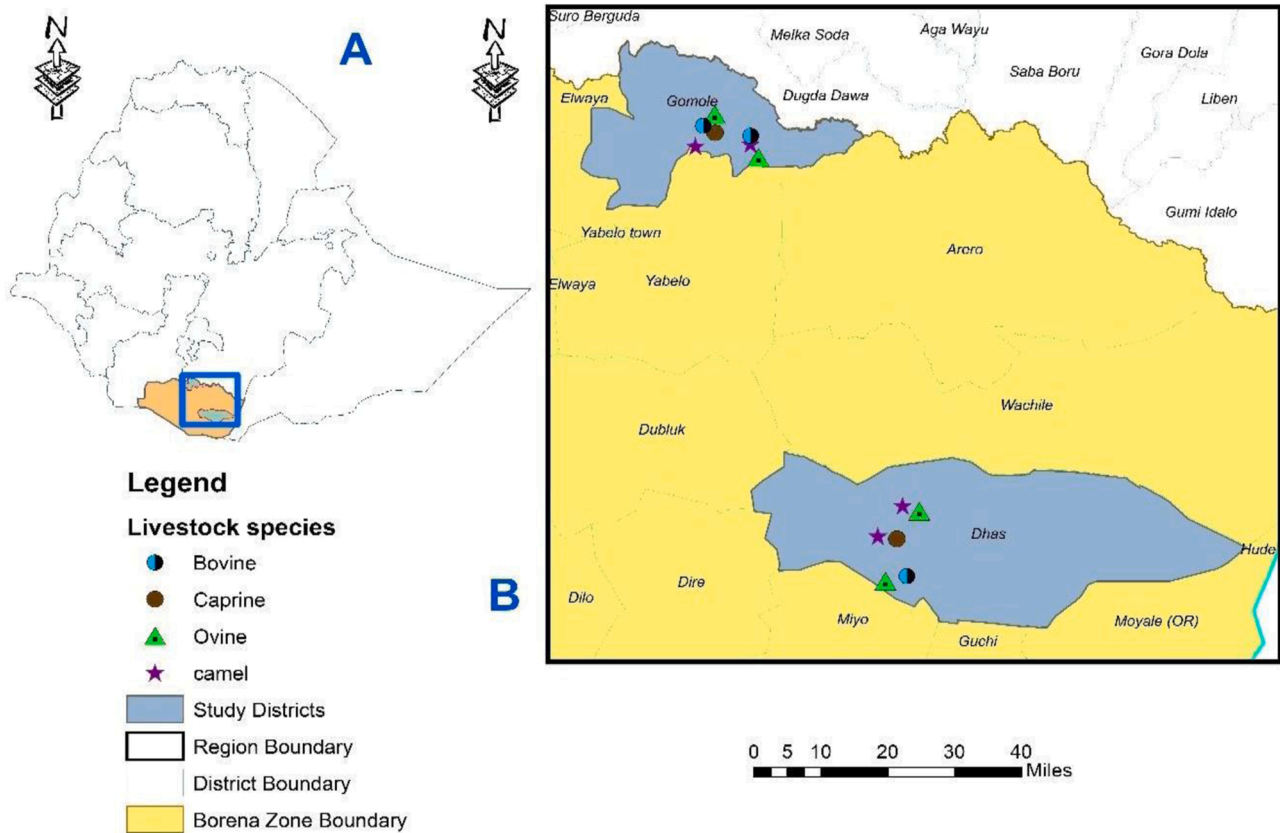


Fig. 1. A. The map of Ethiopia with the Borena zone is highlighted in orange with the study area within the blue block that borders Kenya to the south and Somalia to the east. B. The Gomole and Dhas districts (in blue) with the neighboring districts in the Borena zone (yellow) in the southern part of Ethiopia. Legend indicates the different livestock species sampled.

resulting in a required sample size of 186 cattle, which was further optimized with the design effect of Gumm (1997) and adjusted sample size formula indicated below.

$$p = \frac{D - 1}{n - 1}$$

The design effect (D) is the intra-cluster correlation coefficient (ICC) with n = average cluster size with $n = 9$, $\rho = 0.2$ as indicated by Otte and Gumm (1997) with a design effect, $D = 2.6$. A total of 490 cattle need to be samples as calculated using the formula:

$$N_{adj} = N_{unadj} \times D$$

Where N_{adj} is the adjusted sample size, N_{unadj} = Unadjusted sample size and D is = design effect.

A proportional allocation method was used based on the livestock population distribution within the districts of the Borena zone using the sample calculations above and the seroprevalence of the specific host. This method ensured that the sample sizes were representative of the animal populations in the respective districts. Using the seroprevalence of brucellosis previously reported for camels (Bekele, 2011) was assumed for sheep and goats resulting in 160 camels, 490 cattle, 330 sheep, and 330 goats being sampled with a 95 % confidence level. Herds were selected using a random sampling method from this list. Once a herd was selected, individual animals within the herd or flock were chosen using systematic random sampling. To facilitate the sampling process, a list of herds was prepared for each district in collaboration with local animal health workers. Using a herds sampling frame random sampling was made for the herd and animals were picked using systematic random sampling from a selected herd or flock.

2.3. Sample collection

2.3.1. Blood sample

All animals were appropriately restrained to minimize movement. Blood samples were collected from the jugular vein: 8 ml from cattle and camels, and 5 ml from sheep and goats. The blood was collected in pre-labelled vacutainer tubes, which were inverted five times to ensure proper mixing. The vacutainer tubes were kept at room temperature for 10–60 min to allow clot formation, after which they were centrifuged at 1100–1300 rpm for 10–15 min to separate the serum from the blood clot. The separated sera were stored at -20°C for subsequent testing, including the RBPT and iELISA, conducted at the Animal Health Institute (AHI) serology laboratory in Ethiopia. DNA was extracted from the blood clots to detect *Brucella* DNA using PCR assays, performed at the molecular laboratory of the Animal Health Institute (AHI).

2.4. Rose Bengal test (RBT)

All serum samples collected from cattle, camel, sheep, and goats were screened using RBT based on the procedures described by OIE (2003) using the manufacturer's instructions. Rose Bengal antigen (Onderstepoort Biological Products, South Africa) was used in the volume of 30 μl of serum and 30 μl of *Brucella* antigen mixed on a ceramic plate and agitated for 4 min. After four minutes, visible agglutination was recorded as positive. Agglutinations were recorded as 0, +, ++, and +++, according to the degree of agglutination, with a score of 0 indicating the absence of agglutination; + indicates weak agglutination; ++ indicates medium agglutination; and +++ indicates strong agglutination. The presence of agglutination was considered a positive reaction, while the absence of agglutination was considered negative. *Brucella* positive and negative control sera (Onderstepoort Biological

Products, South Africa) were also tested along with the test sera to validate the result reading (Alton et al., 1988).

2.5. Indirect enzyme-linked immunoassay (iELISA) test

An IDVET iELISA brucellosis serum indirect multi-species kit (ID Screen, France) was used to test sera samples according to the manufacturer's instructions, which included positive and negative controls. Result interpretation according to the manufacturer's instruction, S/P reading < 110, is considered as negative, S/P > 110 and < 120 is doubtful, S/P > or = 120 is concluded as a positive result.

2.6. Molecular detection test

2.6.1. DNA extraction

DNA was extracted from about 200 µL of blood clot samples taken from individuals who tested positive for brucellosis using both the RBT and iELISA tests. The extraction was carried out using the QIAGEN DNA extraction kit (Germany), following the manufacturer's instructions. Once extracted, the DNA samples were stored at -20 °C until they were ready for further analysis. Each seropositive sample was measured using a fluorometer, and all were found to contain more than 10 ng of DNA, an amount sufficient for amplification using both qPCR and AMOS-PCR methods.

2.7. *Brucella* IS711 real-time PCR (qPCR)

The genus-specific IS711 qPCR described by Hinic et al. (2008) was used, followed by *B. melitensis* and *B. abortus* specific qPCR, each containing 5 µL of template DNA in a 25 µL PCR reaction with TaqMan® Universal PCR Master mix, no AmpErase® UNG (Applied Biosystems, USA), and 0.3 µM forward and reverse primers and 0.2 µM probe (Microsynth, Germany) (Table 1) with *B. abortus* S19 and *B. melitensis* Rev1 vaccine strains as positive controls. With thermal cycling conditions at 95 °C for 10 min of denaturation, amplification with 95 °C for 15 s and 65 °C for 1 min for 45 cycles on the Applied Biosystem 7500 real-time PCR system. A qPCR result was considered positive with an amplification curve with a C_t (threshold cycle) value lower than 38 considered to be positive.

2.8. Conventional AMOS-PCR assay

AMOS-PCR that identifies and differentiates *B. abortus* bv 1, 2, and 4, *B. melitensis* bv 1, 2 and 3, *B. ovis*, and *B. suis* bv. 1 was conducted (Bricker and Halling, 1994; Bricker et al., 2003) using DNA extracted from blood clots from seropositive animals as a template. Four

species-specific forward primers were used at a final concentration of 0.1 µM with 0.2 µM reverse primer IS711 (Table 1) with 1 × MyTaq™ Red PCR Mix (Bioline, South Africa) and 2 µL of template DNA in 25 µL PCR reaction. PCR cycling condition was initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, 55.5 °C for 2 min, 72 °C for 2 min, and a final extension step at 72 °C for 10 min. PCR products were analyzed by electrophoresis using a 2 % agarose gel stained with ethidium bromide and viewed under UV light.

2.9. Ethical consideration and certificate

The study received ethical clearance from the University of Pretoria's research, animal, and human ethics committees (REC 043–22, HUM008/1123) and the Oromia Health Bureau Research and Ethics Department in Ethiopia. Before conducting the research with the pastoral community, the importance, procedure, and confidentiality of the owner's data were explained. The community members understood and approved the consent by signing before the research was carried out.

2.10. Data management and analysis

The disease risk factor and questionnaire data were assessed using STATA software version 14.0 (Stata Corp, 4905 Lake Way Drive, College Station, Texas 77845 USA) to analyze the data and compute the effects of risk factors on the seroprevalence of *Brucella* infection. Univariable analysis using descriptive statistics, such as a chi-squared test, was used to analyze the effect of individual factors on the prevalence of infection. Multivariable logistic regression was employed to compute the associations between the putative risk factors and prevalence. The variables were manually fitted to the logistic regression model to check for confounding. The Hosmer-Lemeshow and Pearson methods were used to evaluate the fitness of the logistic regression model. A P value of < 0.05 and a 95 % confidence interval were significant in this study. The study findings were analyzed using ODDs ratio logistic regression analysis to determine the relationship between seropositivity and possible risk factors. Based on their age, sex, species, previous abortion history, districts, and grazing of animals with other species. The prevalence is estimated on an individual and herd level of prevalence.

3. Results

3.1. Seroprevalence

Out of 490 randomly selected cattle, 12 (2.5 %) tested positive for bovine brucellosis using RBT, and 7 (1.4 %) were iELISA positive at the

Table 1

Sequences and characteristics of primers used for *Brucella* real-time PCR and different *Brucella* species in AMOS PCR assays.

PCR IS711	Primer Amplification	Sequence (5'-3')	DNA Target	Amplification(bp)	Concentration (µM)
	Forward	GCTTGAAGCTTGC GGACAGT	IS711	N/A	0.2
	Reverse	GGCCTACCGCTGCGAAT			0.2
	Probe	FAM-AAGCCAACACCCGGCCATTATA-BHQ-1			0.2
<i>B. abortus</i>	Forward	GCACACTCACCTTCCACAACAA	BruAb2_0168		0.2
	Reverse	CCCCGTTCTGCACCCAGACT			0.2
	Probe	FAM-TGGAACGACCTTTCAGGCGAGATC-BHQ-1			0.2
<i>B. melitensis</i>	Forward	TCGCATCGGGAGTTTCAA	BME110466		0.2
	Reverse	CCAGCTTTTGGCCCTTTTCC			0.2
	Probe	FAM-CCTCGGCATGGCCCCGAA-BHQ-1			0.2
AMOS-PCR					
<i>B. abortus</i>	Forward	GAC GAA CGG AAT TTT TCC AAT CCC		498	0.1
<i>B. melitensis</i>	Forward	AAA TCG CGT CCT TGC TGG TCT GA		731	0.1
<i>B. ovis</i>	Forward	CGG GTT CTG GCA CCA TCG TCG GG		976	0.1
<i>B. suis</i>	Forward	GCG CGG TTT TCT GAA GGT GGT TCA		285	0.1
IS711	Reverse	TGC CGA TCA CTT AAG GGC CTT CAT		N/A	0.2

individual animal level, with a herd prevalence of 13.5 %. Among 160 camels, 7 (4.4 %) were RBT positive and 6 (3.8 %) were iELISA positive at the individual level, with a herd prevalence of 33.3 %. For 330 sheep, 7 (2.2 %) were RBT positive, 5 (1.5 %) were iELISA positive, and the herd prevalence was 15.5 %. Among 330 goats, 36 (10.9 %) were RBT positive, 33 (10.0 %) were iELISA positive, and the herd prevalence was 57.6 % (Table 2B).

3.2. Molecular characterization

Of the 12 seropositive cattle, the IS711 qPCR and AMOS-PCR detected 5 (41.7 %) as PCR positive for *Brucella* DNA, with 2 cattle infected with *B. abortus* and 3 with mixed infections of *B. abortus* and *B. melitensis* (Table 2B). All PCR positives were from different herds, 2 from Gomole and 3 from Dhas districts. Out of 7 seropositive sheep samples, 3 (42.9 %) were detected with AMOS-PCR and IS711 qPCR as *B. melitensis*, with all samples from different herds in the Dhas district. Of the 7 seropositive camels, 5 (71.4 %) were positive with AMOS-PCR and IS711 qPCR, with 3 having mixed infections and 2 with *B. melitensis*. Two herds had 2 positives of *B. melitensis*, while the rest had single positives from each herd in the Dhas district. Of the 36 seropositive

Table 2

Brucellosis prevalence amounts animal species at A. individual level and B. herd level with the serological tests and the *Brucella* PCR results of sero-positive animals in the Borena Zone, Oromia region, Ethiopia.

Type of tests	A. Individual species prevalence (Individual prevalence %)				Total
	Cattle (13.5 %)	Camel (33.3 %)	Goats (54.5 %)	Sheep (15.5 %)	
RBT					
Negative	478	153	294	323	1248
Positive	12 (2.45 %)	7 (4.4 %)	36 (10.9 %)	7 (2.2 %)	62
Total	490	160	330	330	1310
RBT and ELISA					
Negative	483	154	291	320	1250
Positive	7 (1.4 %)	6 (2.8 %)	33 (10.0 %)	5 (1.5 %)	51
Total	490	160	330	330	1310
AMOS-PCR					
Negative	7	2	6	4	19
Positive	5 (41.7 %)	5 (71.4 %)	30 (83.3 %)	3 (42.9 %)	43
Total	12	7	36	7	62
<i>Brucella</i> IS711 qPCR					
<i>B. abortus</i>	2	0	0	0	2
<i>B. melitensis</i>	0	2	14	3	19
<i>B. abortus</i> and <i>B. melitensis</i>	3	3	16	0	22
Sum	5	5	30	3	43
B. Herd level prevalence (herd prevalence%)					
RBT	Cattle	Camel	Goats	Sheep	Sum
Negative	40	11	13	26	90
Positive	12 (23.0 %)	7 (61.1 %)	20 (61.0 %)	7 (21.2 %)	46
ELISA and RBT	Cattle	Camel	Goats	Sheep	Sum
Negative	45	12	14	28	99
Positive	7 (13.5 %)	6 (33.3 %)	19 (57.6 %)	5 (15.2 %)	37
PCR (% of seropositive animals)	Cattle	Camel	Goats	Sheep	Sum
Negative	47	14	15	30	106
Positive	5 (9.6 %)	4 (22.2 %)	18 (54.5 %)	3 (9.1 %)	30

* Herd prevalence based on RBT and iELISA results. RBT- Rose Bengal test; qPCR = real-time PCR.

goats, 30 (83.3 %) (Table 2B) were PCR positive, with 14 having *B. melitensis* and 16 having mixed infections of *B. abortus* and *B. melitensis*.

3.3. Risk factors: mono and multivariate analysis

3.3.1. Univariable analysis

The difference observed in the prevalence of brucellosis among host species was statistically significant ($\chi^2 = 45.8$; $p < 0.001$). Gomole district had a higher prevalence than Dhas ($\chi^2 = 13.8$; $p < 0.001$). A significantly higher prevalence ($\chi^2 = 5.2$; $p = 0.023$) was observed in female animals than in male animals. Female animals with a history of abortion yielded a higher prevalence ($\chi^2 = 127.1$; $p < 0.001$) (Table 3).

3.4. Multivariable analysis

Five potential risk factors were selected and their association with the occurrence of brucellosis was analyzed using multivariable logistic

Table 3

Results of univariable analysis using chi-squared test on the association between seropositivity and putative risk factors.

Variable	N ₀ tested	N ₀ positive	percent	95 % CI	χ^2	P
District					13.8	< 0.001
Dhas	922	24	2.6	1.7 – 3.8		
Gomole	388	27	6.9	4.6 – 9.9		
Peasant Association					19.9	0.001
Buya	303	24	7.9	5.1 – 11.6		
Dhas	753	19	2.5	1.5 – 3.9		
Harbor	85	3	3.5	0.7 – 9.9		
Xile	169	5	2.9	0.9 – 6.8		
Locality					20.6	0.002
Buya	34	1	2.9	0.07 – 15.3		
Dhaka	636	16	2.5	1.4 – 4.1		
Dima	87	3	3.4	0.7 – 9.7		
Eela	30	0	0.0	0.0 – 9.5		
Harbor	85	3	3.5	0.7 – 9.9		
Haro Daye	169	5	2.9	0.9 – 6.8		
Chalalaka	269	23	8.6	5.5 – 12.6		
Species					45.8	< 0.001
Cattle	490	7	1.4	0.6 – 2.9		
Camel	163	6	3.7	1.4 – 7.8		
Goat	330	33	10.0	6.9 – 13.7		
Sheep	327	5	1.5	0.5 – 3.5		
Sex					5.2	0.023
Female	1076	48	4.5	3.3 – 5.9		
Male	234	3	1.3	0.3 – 3.7		
Abortion history					127.1	< 0.001
Yes	22	11	50.0	28.22 – 71.78		
No	1288	40	3.1	2.2 – 4.2		
Overall	1310	51	3.9	2.9 – 5.1		

regression. The results revealed that animals from the Gomole district were twice at risk of infection with *Brucella* spp. than those animals originated from Dhas district (OR = 2.00; 95 % CI: 1.071–3.743; $p = 0.030$). Goats had higher odds (risk) of infection than cattle (OR = 7.23; 95 % CI: 3.113–16.791; $p = 0.000$). Similarly, female animals were nearly three times more at risk of acquiring infection than male animals. However, herd size and age of animals were not associated with serological evidence of infection with *Brucella* species (Table 4). The association between sero-positivity and history of abortion was analyzed separately for female animals using logistic regression. The result showed that the risk of sero-positivity was 27 times higher in animals having a history of abortion than those without abortion (OR = 27.49; 95 % CI: 11.19–67.46; $p = 0.000$). The logistic regression analysis results showed that district and host species were statistically significantly associated with the serological evidence of *Brucella* infection. Herds from Gomole were 4 times more at risk of infection than herds from Dhas (OR = 4.342; (5 % CI: 3.195–5.901; $p < 0.001$). Significantly higher seroprevalence was observed with the highest in camel herds (OR = 8.169; 95 % CI: 5.092–13.105; $p < 0.001$) followed by sheep flocks (OR = 3.804; 95 % CI: 2.769–5.224; $p < 0.001$) and goat herds (OR = 3.102; 95 % CI: 2.258–4.263; $p < 0.001$) relative to cattle herds (Table 5). Sex and age were not significant predictors of infection (Table 5).

4. Discussion

The study revealed the highest prevalence was in goats (10.0 %; 95 % CI: 6.9–13.7), with the lowest prevalence in cattle (1.4 %; 95 % CI: 0.6–2.9) in the Gomole and Dhas districts in the Borena zone in the Oromia region of Ethiopia. TeshomeYimer et al. (2021) similarly reported a high prevalence in goats (17.4 %) in the Borena zone, while Edeo et al. (2023) found a brucellosis prevalence of 2.4 % in cattle and 3.2 % in sheep and goats in the same zone. The low prevalence reported by Edeo et al. (2023) in sheep and goats compared to the current study and TeshomeYimer et al. (2021) suggests that geographical differences and /or management practices might play a significant role in influencing the prevalence of brucellosis across species. This is further emphasized by the statistically

significant difference in brucellosis prevalence observed among species ($\chi^2 = 45.8$; $p < 0.001$), with higher rates in Gomole than in Dhas ($\chi^2 = 13.8$; $p < 0.001$). Logistic regression analysis showed that the Gomole district and host species (goats) were significantly associated with infection, with herds in Gomole being four times more at risk than those in Dhas (OR = 4.342; 95 % CI: 3.195–5.901; $p < 0.001$). Interestingly, Gomole, identified as a higher-risk area in this study, was not sampled in the studies by TeshomeYimer et al. (2021) and Edeo et al. (2023). This area borders the Elewayo district, where the highest prevalence was also found in these previous studies. Furthermore, females, particularly those with a history of abortion, showed a

Table 4

Results of multivariable logistic regression analysis on the association between Sero-positivity and putative risk factors.

Variable	OR	SE	Z	P	95 % CI
District					
Dhas	Ref				
Gomole	2.00	0.639	2.17	0.030	1.071–3.743
Species					
Cattle	Ref				
Camel	2.92	1.713	1.83	0.068	0.925–9.220
Goat	7.23	3.108	4.60	0.000	3.113–16.791
Sheep	1.305	0.786	0.44	0.658	0.401–4.250
Sex					
Female	Ref				
Male	0.299	0.183	–1.98	0.048	0.090–0.991
Age	1.038	0.134	0.29	0.774	0.806–1.336
Herd size	0.978	0.018	–1.22	0.222	0.943–1.014
Constant	0.017	0.015	–4.65	0.000	0.003–0.096

Table 5

The results of the logistic regression analysis of the effects of potential risk factors on the serological evidence of *Brucella* infection in the Borana pastoral area.

Variable	OR	SE	Z	p	95 % CI
District					
Dhas	Ref				
Gomole	4.342	0.679	9.38	0.000	3.195–5.901
Sex					
Female	Ref				
Male	0.981	0.162	–0.12	0.905	0.709–1.354
Species					
Cattle	Ref				
Goats	3.102	0.503	6.98	0.000	2.258–4.263
Camels	8.169	1.969	8.71	0.000	5.092–13.105
Sheep	3.804	0.616	8.25	0.000	2.769–5.224
Age	1.044	0.050	0.90	0.368	0.950–1.148
Constant	0.441	0.105	–3.45	0.001	0.277–0.702

significantly higher prevalence ($\chi^2 = 127.1$; $p < 0.001$) while AMOS-PCR and qPCR assays detected *B. melitensis* predominantly in sheep, camels, and goats although *B. abortus* was detected in cattle, with mixed infections observed across all species except sheep. This finding aligns with the isolation of *B. abortus* from cattle in central Ethiopia (Geresu et al., 2016; Edeo et al., 2023), which borders the Oromia region, though not specifically the Borena zone, and *B. melitensis* from pastoral goats in the Afar region (Tekle et al., 2019). These results also support the higher seroprevalence observed in goats (OR = 3.102; 95 % CI: 2.258–4.263), camels (OR = 8.169; 95 % CI: 5.092–13.105), and sheep (OR = 3.804; 95 % CI: 2.769–5.224) compared to cattle reported in this study.

The findings of the current study on camel brucellosis in Ethiopia show a prevalence of 3.1 %, which aligns with similar rates observed in other regions, such as the Amibara District in Afar (Wegi et al., 2021) and the Yabello District in Borena Zone (Admasu et al., 2017), both of which reported 3.2 % and 3.1 % prevalence, respectively. However, slightly lower and higher rates of 2.0 % (Waktole et al., 2022) and 4.9 % were recorded in Dire Dawa and Fafan Zone (Lakew et al., 2019), respectively. The current study also identified both *B. melitensis* and mixed infections of *B. abortus* and *B. melitensis* in camels, in contrast to the findings of non-peer-reviewed Legesse et al. (2024), which detected only *B. abortus* in slaughtered camel tissues using PCR in the Akaki abattoir in central Ethiopia. These results are consistent with reports from Kenya (Akoko et al., 2021), where *B. abortus* was detected in multiple animal species, including camels, and *B. melitensis* was found in camels, goats, and sheep, with mixed infections observed in sheep. This underscores the ongoing challenge of brucellosis in camels and other livestock, highlighting the need for continued surveillance and effective control measures across the region. In most camel-rearing societies, Camel milk is mainly consumed in its raw state without being subjected to any processing treatment. Consumption of raw camel milk should be of major concern from a public health point of view (Sisay and Awoke, 2015). Brucellosis in camels represents an animal health issue and a critical public health challenge, necessitating improved surveillance, education on safe milk and meat consumption practices, and implementation of control strategies to reduce human and animal exposure to the pathogen.

The present study reports a prevalence of brucellosis in sheep of 1.5 %. This is lower than the prevalence observed in a previous study by Sorsa et al. (2022), which found a prevalence of 6.8 %, 5.98 %, and 5.4 % using RBPT, C-ELISA, and CFT tests, respectively, in the South Omo Zone of Southern Ethiopia. In contrast, Geletu et al. (2021) reported a much lower prevalence of 0.18 % in the West Hararghe Zone. The findings suggest that brucellosis in sheep is less prevalent in the Borena Zone compared to other regions of Ethiopia. Additionally, the current study identifies *B. melitensis* as the causative agent of brucellosis in sheep in the Borena Zone, as detected by PCR.

Goats are at higher risk of acquiring *Brucella* infection than sheep. This may be due to the greater susceptibility of goats to *Brucella* infection. It could also be partly because goats excrete the organism for a long period, unlike sheep. This reduces the potential for disease spread among sheep flocks (Radostits et al., 2000). The receptivity of ewes to *B. melitensis* varies according to the breed. Milk-producing ewes are more receptive than sheep raised for slaughter (Corbel and Banai, n.d.).

The prevalence rate of bovine brucellosis in the pastoralist area of Gomole and Dhas district was 1.4 %, with even a lower prevalence of 0.05 % in the Arsi Zone in Oromia State (Degefa et al., 2011). In Ethiopia, *B. abortus* were isolated from cattle in central Ethiopia (Geresu et al., 2016; Edao et al., 2023) and *B. melitensis* from goats in the pastoral Afar region (Tekle et al., 2019). Terefe et al. (2017) reported a brucellosis prevalence rate of 7.1 % in cattle in the Oromia pastoral region, like Megersa et al. (2011), who found a prevalence of 6.2 % in cattle and 1.8 % in small ruminants in the Afar pastoral region. The variation in prevalence rates can be attributed to differences in husbandry practices, animal movement, and biosecurity measures identified factors such as herd size, age, and reproductive status were identified as significant determinants (Alemu et al., 2014).

In the laboratory diagnosis of human brucellosis, PCR has demonstrated superior sensitivity compared to blood culture and greater specificity than serological tests, both in acute and chronic stages of the disease. Moreover, PCR-based methods reduce the risk of laboratory-acquired infections associated with handling highly infectious live cultures. Hinić et al. (2008) used the IS711 qPCR to detect *Brucella* spp. in wild boars. Their study found that 11.1 % of wild boars that were serologically negative by RBT tested positive via qPCR. In contrast, serological methods detected 15.6 % positive by RBT, 7.5 % by cELISA, and 5.5 % by iELISA, with an overall 2.0 % positivity across all serological tests. When compared to *Brucella* isolation, PCR showed a higher detection rate, identifying 26 % of animals as positive, while *Brucella* spp. was isolated from only 9.4 % of animals. In this study, PCR was employed to detect *Brucella* species in seropositive blood clots using IS711 qPCR and AMOS-PCR. This approach could be considered a novel method for confirming the species of *Brucella* via PCR based on the approach of Hinić et al. (2008), which reported a PCR-based technique for the identification and differentiation of *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae*. Our study also demonstrated the utility of PCR assays for the identification of *Brucella* spp from clot blood in seropositive animals. This method is suitable for both conventional and real-time PCR systems.

The study on bacterial isolation and molecular detection of brucellosis in small ruminants in the Afar region identified *B. melitensis* in both sheep and goats, with a mixed infection of *B. abortus* and *B. melitensis* found in goats. This finding aligns with the work of Tekle et al. (2019), who isolated *B. melitensis* from vaginal swabs and milk samples taken from goats in the Afar region of Ethiopia. Similarly, in Ethiopia isolated vaginal swabs and milk samples from goats in the Afar region, Ethiopia. In the previous study (Wainaina, 2020) conducted a study in pastoralist areas of Kenya using qPCR to detect *B. abortus* and *B. melitensis* in goats and sheep. Moreover, our analysis of clot blood samples from camels in pastoralist areas using both qPCR and AMOS-PCR revealed mixed *Brucella* infections, including the identification of *B. melitensis*. This finding aligns with a study from Egypt, where camel milk samples tested positive for both *B. abortus* and *B. melitensis* (Hussein et al., 2025). Another study reported the possibility of spillover from the principal host to other animals due to shared grazing and watering areas, with *B. abortus* detected in sheep and goats in Egypt (Wareth et al., 2015). The results of this study are consistent with our current findings, suggesting that the pastoralist husbandry system, which involves common grazing and watering of animals, facilitates the potential spillover of *Brucella* bacteria from cattle to small ruminants. In the Borena zone, the pastoralist community exhibits a dietary preference for consuming raw milk from cows, camels, and goats, often under unhygienic conditions. This practice increases the risk of bacterial transmission from animals to humans,

with the possibility of further transmission to children. According to a study by Tulu (2022), this unsanitary handling of milk contributes significantly to the transmission of brucellosis, highlighting the importance of improving hygiene and management practices to mitigate the spread of the disease.

The current study identifies several significant potential risk factors for brucellosis in livestock, including age, previous abortion history, sex, and the geographical area where the animals were kept. These factors were found to be statistically significant. A similar study by Efrem et al. (2023) also highlighted previous abortion history and sex as potential risk factors, with females being more affected than males. Furthermore, a report on the risk factors for brucellosis in the southwestern part of Ethiopia by TeshomeYimer et al. (2021) indicated that animals with a history of previous abortions, as well as those in contact with goats and sheep, had a higher prevalence of brucellosis, with these factors being statistically significant. This supports the findings of the current study, emphasizing the importance of these risk factors in the epidemiology of brucellosis in livestock.

5. Conclusion

This study shows a difference in the magnitude of *Brucella* seroprevalence among livestock species considered. Perhaps this may partly help in identifying the target species for the control endeavor. Moreover, detecting the pathogen in the blood is conclusive evidence of *Brucella* infection and a clear indication of its zoonotic risk in the community due to prevailing animal product consumption habits and unsafe handling of aborted materials.

CRedit authorship contribution statement

Abebe Olani: Data curation. **Tesfaye Rufael:** Funding acquisition. **Teferi Benti:** Data curation. **Melaku Sombo:** Data curation. **Mekonnen Belete:** Data curation. **Duba Bule:** Data curation. **Massimo Scacchia:** Resources, Funding acquisition. **Fabrizio De Massis:** Supervision, Funding acquisition. **Giuliano Garofolo:** Funding acquisition, Data curation. **Henriette van Heerden:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Investigation, Formal analysis, Conceptualization. **Getachew Abichu:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Abede Aliyi:** Data curation.

Declaration of Competing Interest

The authors have declared no conflict of interest.

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