Journal of Animal Research: v.15 n.06 p. 219-231. December 2025

DOI: 10.30954/2277-940X.06.2025.3

Sero-Prevalence and Risk Factors of Small Ruminant Brucellosis in Selected Districts of West Omo and Kafa Zones, South Western Ethiopia

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Received: 26 Oct., 2025 **Revised:** 24 Nov., 2025 **Accepted:** 29 Nov., 2025

ABSTRACT

A cross-sectional study was conducted from December 2023 to July 2024 to estimate sero-prevalence and assess the risk factors of brucellosis in small ruminants in selected districts of West Omo and Kafa zone of southwestern Ethiopia. A total of 552 (341 goats and 211 sheep) serum samples were collected from 80 flocks by simple random sampling method and two serological testing was performed using a Rose Bengal plate test (RBPT) for screening and indirect enzyme-linked immunosorbent assay (i -ELISA) for confirmation of small ruminant brucellosis antibodies. The overall sero-prevalence of brucellosis was 4.17% (95% CI 2.8 to 6.2) at individual animal level and 20% (95% CI 12.7 to 30) at herd level. Multivariable regression analysis showed that the odd of seropositivity in larger herds were 4.69 times greater than that of seropositivity in small herds. Brucellosis sero-prevalence was also significantly associated with animals that had prior history of abortion (95% CI: 1.614-14.175 OR = 4.78, $P < 0.005$) which had 4.78 times higher risk of developing brucellosis. Those animal with retained fetal membrane were found to be 6.83 times more likely to be at higher risk of *Brucella* infection compared with no history of retained fetal membrane with (95% CI: 2.178-21.472 OR = 6.83, $P < 0.001$). Seroprevalence of brucellosis was also significantly associated with female animals those had prior history of still birth with 6.08 times more likely susceptible for *Brucella* infection than those animals without prior history of still birth (95% CI: 1.846-20.039, OR = 6.08, $P = 0.003$). This study confirms the moderate infection of small ruminant brucellosis in the study area.

HIGHLIGHTS

- The overall sero-prevalence of brucellosis among small ruminants was within the range commonly reported in Ethiopia.
- Goats showed a higher seroprevalnce than sheep, suggesting greater susceptibility.

Keywords: Brucellosis, Ethiopia, keffa and West Omo zone, Sero-prevalence, Small ruminants

Brucellosis is a widespread zoonosis, transmitted from cattle, sheep, goats, pigs, and camels by direct contact with blood, fetal placental, or uterine secretions, or by consumption of contaminated raw animal products (especially unpasteurized milk and soft cheeses) in endemic areas (Baldi and Giambartolomei, 2024). It is caused by infection with gram-negative cocci of the genus *Brucella* (Adabi *et al.,* 2023). In goats and sheep, *B. melitensis* causes the disease, but in rams, *B. abortus* can cause clinical brucellosis and *B. ovis* cause epididymitis.

The disease was characterized by abortion in late pregnancy, stillbirth and the birth of weak offspring, acute orchitis and infertility (Zange and Scholz, 2023). *Brucella melitensis*is a serious disease characterized by fever (undulent and Malta fever), weakness, fatigue and osteomyelitis, which is common in humans and is the most prevalent species,owing in part to difficulties in immunizing free-ranging goats and sheep.

By understanding the sero-prevalence of brucellosis, we

How to cite this article: Habte, M.A. (2025). Sero-Prevalence and Risk Factors of Small Ruminant Brucellosis in Selected Districts of West Omo and Kafa Zones, South Western Ethiopia. *J. Anim. Res.,* **15**(06): 219-231.

Source of Support: None; **Conflict of Interest:** None

can better allocate resources to implement surveillance and monitoring programs and develop control measures to prevent the spread of the disease (Akoko *et al.,* 2023). Therefore, this study will play an important role in guiding future interventions and efforts towards the prevention and control measures of small ruminant brucellosis in the study area by providing essential data on the extent of the disease and its potential impact on small ruminant populations.

The sero-prevalence of brucellosis in small ruminants and the associated risk factors in Ethiopia have been the subject of several studies. However, no previous research has been done on this topic on the sero-prevalence of Brucellosis in small ruminants in the study area. Therefore, it is essential to conduct a sero-prevalence study in order to determine the extent of Brucellosis in small ruminant's in the study area and the potential impact it may have on small ruminant populations. The reports from various regions of Ethiopia are showing that the occurance of brucellosis in small ruminant is increasing. Research on the prevalence of brucellosis has been conducted in several areas of Ethiopia by different researchers. Desalegn Dossa and his colleagues (Dosa *et al.,* 2023) reported 3.3% prevalence in two selected districts of southern region, Ethiopia.

However, there is no previous reports on the prevalence of small ruminant beucelosis to quantify and document the actual prevalence of small ruminant brucellosis in South Western Ethiopia of kaffa and West Omo zone, therefore, this study was designed to know the status of small ruminant brucellosis and to fill this gap in the study area with the following objectives:

- To estimate the Sero-Prevalence and Risk Factors of Small Ruminant Brucellosis in Tello and GoriGesha districts of South-western region,Ethiopia.
- To estimate the sero-prevalence of Brucellosis in a defined population of small ruminants in a specific study area.
- To assess risk factors associated with brucellosis seropositivity in the study area.

MATERIALS AND METHODS

Description of the study area

The study was conducted in two purposively selected districts namely Tello and GoriGesha district of South Western Ethiopia regional state from December 2023 to July 2024. The districts of Tello and GoriGesha are about 500 and 681 km south West of Addis Ababa, the capital city of Ethiopia respectively.

Geographically, Tello district lies between 7º07'09.245 latitude and 36º28'06.180 longitudes, whereas GoriGesha district lies between 6º32'40.755 latitude and 35º32'47.152 longitudes. In Tello district, the altitude ranges from 2436 to 2451 meter above sea level (Mustefa *et al.,* 2024), whereas in GoriGesha district, the altitude ranges from 1100 to 2050 meters above sea level. The annual average rainfall in the area was reported as 1278 mm at Tello and 850 mm in GoriGesha, according to the districts socioeconomic data, while the average annual temperature in Tello district was 17 to 25º C (Mustefa *et al.*, 2022), and the temperature of GoriGesha district ranges between 18 to 29ºC.

Tello district surrounded by Adiyo district in the North, Decha district in the west, Konta zone in the east and Cheta district in the south. The administrative town of Tello district is Odda, GoriGesha district surrounded by Debub bench district in the North, Guraferda district in the West, MenitShasha district in the East and Bero and Maji districts in the South. The adminstrative town of GoriGesha district is Kuju.

According to the districts agricultural and livestock sector office data, pastoralism in GoriGesha and agropastoralism or mixed crop-livestock farming in Tello district are the main economic activities.

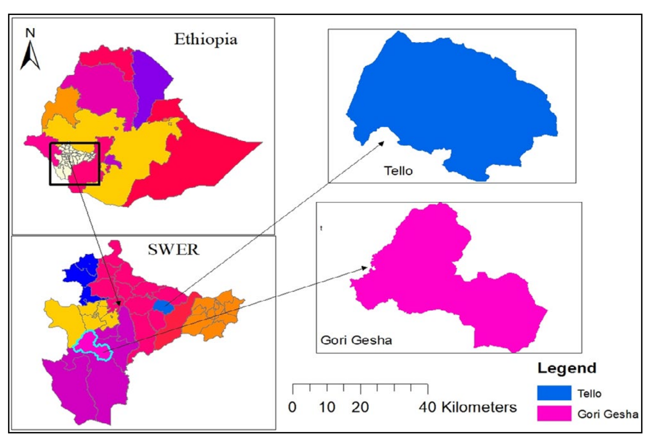

Fig. 1: Map of Tello and GoriGesha districts (***Source:*** Designed by Arc GIS)

Study animals

The study animals were composed of sheep and goats found in Tello and GoriGesha districts. According to each district Agricultural and livestock sector office data, Tello and GoriGesha districts have 211,128 sheep and 71,633 goat and 65,657 sheep and 122,250 goat population respectively. The study included a total of 211 sheep and 341 goats, classified based on their respective species. Out of all the study animals, 252 were under agro pastoral production management system, while 300 were under pastoral production management system. Based on age groups, 159 individuals were classified as young (under one years old), while 393 were categorized as adults (above one years old) (Geletu *et al.,* 2021).

Study design

A cross-sectional study design was employed in the study area from December 2023 to July 2024 G.C. to estimate the sero-prevalence of brucellosis in sheep and goats and to assess potential risk factors associated with seropositivity. The design of sero-prevalence studies for small ruminant brucellosis is crucial for understanding the epidemiology of the disease and informing control measures.

Sampling strategy

A multistage cluster sampling technique was carried out to select the study animals. Tello district from Kaffa zone and GoriGesha district from West Omo zone were selected purposively based on the complaints on small ruminant diseases from both district agricultural and live stock office to Mizan regional veterinary laboratory center, higher numbers of sheep and goats population and accessibility during sample collection. The total number of peasant associations in the district was 25 for Tello and 13 for GoriGesha.

Accordingly, four PAs from GoriGesha district (Bout, Tuyie, Shewajibabu and Gelie), and four PAs from Tello district (Odda, Dacha, Yama and Washa) were selected randomly. The numbers of animals sampled from each peasant associations were employed by the proportion of the small ruminant population existing in each peasant associations using simple random sampling technique. Households or flockswere selected using systematic random sampling method.

Several risk factors were reviewed from previous epidemiological studies that were believed to be a potential risk factors at animal level such as herd size, production system, species, age, sex, reproductive status, parity, history of occurrence of reproductive problems and risk factors at herd level such as herd size, management system, new animal introduction and history of abortion was included (Muhidin *et al.,* 2021). The age of animals was estimated based on information obtained from the owner and also by looking the dentition pattern of the animal (Annex 2). Small ruminants with all temporary incisor teeth were considered as young while small ruminant with one or more permanent incisors teeth were considered as adults (Kohut, 2022).

Sample size determination

The sero-prevalence of small ruminant brucellosis was estimated at 21% using previously reported results in South Omo zone which is adjusent to the study districts by (Gemeda, 2020) to calculate the sample size. The sample size was calculated according to the approach suggested by (Thrusfield, 2018) as follows:

$$N = \frac{1.96^2 P_{\text{exp}}\left(1 - P_{\text{exp}}\right)}{d^2}$$

Where: N = required sample size

P_{exp} = expected prevalence.

d = desired absolute precision

Thus, 254 sheep and goats were chosen as the overall sample size. Then, it was recalculated to achieve a closer accuracy with that of simple random sampling by taking into account the design effect (cluster effect). The design effect was determined by using the intra-cluster correlation coefficient (rho = ρ), which measures the correlation of observations within a cluster.

$$\textit{Design effect} = 1 + \rho\,(m - 1);$$

Where "*m*" represents the number of individuals sampled per cluster, and a flock of sheep/goats in one village was considered as a cluster, with approximately 7 animals sampled from each selected cluster. An intra-cluster correlation coefficient (Rho) value for zoonotic diseases (and infectious diseases in general) typically ranges from 0.05 to 0. 2 and rarely goes beyond 0. 3 in cases of highly contagious viral infections (Bennett *et al.*, 1991). Therefore, a Rho value of 0. 2 was used for the calculation of the sample size. As a result, the approximate sample size of 552 animals were collected.

Sample collection procedures

In a sero-prevalence study of small ruminant brucellosis, the sample collection procedure is crucial for obtaining accurate and reliable data. Approximately 5ml of blood samples were collected directly from jugular vein of sheep and goats by venipuncture using plain vacutainer tubes and sterile needles (Fereig *et al.*, 2022). Then, each samples were Clearly labeled with important information, including the animal ID, date of collection, and location. The blood samples were allowed to stand in slant position overnight at room temperature to allow serum separation. Clear straw colored serum was decanted into 2ml cryovials and labelled accordingly and kept in ice box until arrival to Mizan Regional Veterinary Laboratory and stored at -20°C deep freeze. Serum samples were analysed using Rose Bengal plate test and indirect enzyme linked immuno-sorbent assay (i-ELISA) (collectively produced by ID. vet innovative diagnostics, France) according to instructions of the manufacturer.

Questionnaire data collection

Relevant information on animal management and disease history was collected from owners using semi structured questionnaire. During the study, data on risk factors related to herd size, production system, species, age, sex, reproductive status, parity, history of occurrence of reproductive problems were collected through face-to-face interview using a semi-structured questionnaire (Annex 1).

Laboratory procedures

Rose bengal plate test

All serum samples collected were screened for *Brucella* antibodies using the Rose Bengal Plate Test at Mizan Regional Veterinary Laboratory center to detect the presence of positive agglutinin sera. For the RBPT the procedure described by (Corbel, 2020, Oie, 2008) was followed. Before performing test; antigen and sera are brought to room temperature. Then 30µl of each serum sample was placed on a clean white tile and mixed with an equal volume of antigen. Subsequently, an equal volume of antigen was placed near each serum spot. The serum and antigen were mixed thoroughly using a clean tooth pick to produce a circle approximately 2 cm in diameter and the mixture was agitated gently for 4 min. at ambient temperature and the result was noted based on the presence or the absence of agglutination. For each reagent, test validation was done by using known positive and negative controls as a reference. The interpretation was performed as follows: 0 = no agglutination, + = barely perceptible, ++ = fine agglutination, some clearing, +++ = coarse clumping, definite clearing. Those samples identified with no agglutination were recorded as negative and those with +, ++, +++ were recorded as positive (Annex 3).

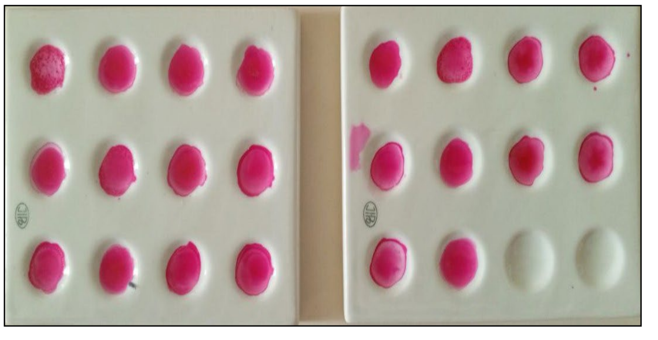

Fig. 2: Result of rose bengal plate test (RBPT)

Indirect Enzyme Linked Immunosorbent Assay

All serum samples detected positive for RBPT were subjected to further confirmation using i-ELISA at Mizan Regional Veterinary Laboratory center. The i-ELISA technique followed the manufacturer's instructions (ID Screen Brucellosis serum Indirect Multi-species ID.vet, 310, rues Louis Pasteur-Grabels-FRANCE). The kit detects antibodies to various species of smooth lipopolysaccharide (S-LPS) expressing *Brucella*, such as *B. abortus, B. mellitensis*, and *B. suis*. The sensitivity and specificity of this test were 96.8 and 96.3%, respectively, according to the Bayesian estimation approach (Getachew *et al.*, 2016). The procedure for i-ELISA were: 190 µl of dilution buffer

2 was added to all wells and 10 µl of negative control was added to wells A1 and B1 and 10 µl of positive control was added to wells C1 and D1 and 10 µl of sample serum was added to the remaining wells then the plate was incubated for 45 minutes at 21 degrees centigrade then each well was washed three times with 300 µl of the wash solution. 100 µl of conjugate was added to each well and the plate was incubated for 30 minutes at 21 degrees centigrade. Each well was washed three times with 300 µl of the wash solution. 100 µl of the substrate solution was added to each well, and the plate was incubated for 15 minutes in the dark area. 100 µl of the stop solution was added to each well to stop the reaction then the optical density (OD) at 450 nm was read and recorded for each sample.

For each sample, the percentage of inhibition (S/P %) was calculated according to below:

$$\text{S/P \%} = \frac{OD\ sample - ODnc \times 100}{ODpc - ODnc}$$

The results were interpreted as follows: If the percentage of inhibition was 110 or lower, the outcome was categorized as negative. In cases where the inhibition percentage fell between 110 and 120, it was regarded as uncertain or doubtful. If the inhibition percentage was 120 or greater, the result was recorded as positive, in accordance with the manufacturer's guidelines (Annex 4).

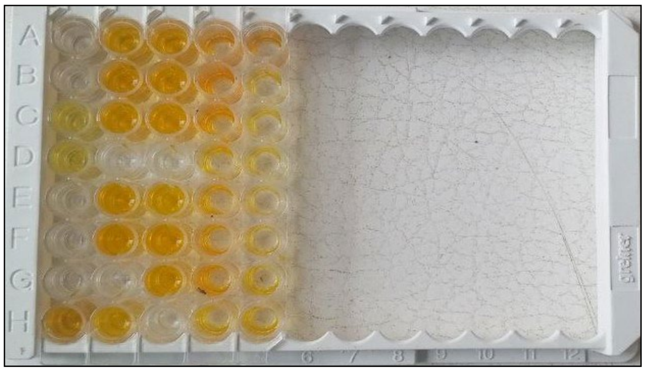

Fig. 3: Result of indirect Enzyme Linked Immuno sorbent Assaye (i-ELISA)

Ethical considerations

Ethical considerations are crucial in the sampling process. The study was carried out in a laboratory mandate area for disease surveillance and outbreak investigation, approval was obtained from the Mizan Regional Veterinary Laboratory Center with a reference number ML26/763/2016 and date 22/06/2016 E.C, (Annex 8). Owners of small ruminants taking part in the research were briefed about the study's objectives prior to gathering data. After obtaining verbal consent, serum samples were collected under aseptic conditions and appropriate precautions.

Data management and analysis

Data from laboratory results and questioner surveys were entered and stored in a Microsoft (MS) Excel 2010 spreadsheet. Descriptive statistical analysis of different risk factors were calculated using Statistical Software Stata/IC 14.2. Animals were considered positive if they were seropositive by both RBPT and i-ELISA tests. The prevalence of individual animals was calculated by dividing the number of positive animals by the total number of animals tested and multiplying by hundred. Similarly, the herd-level prevalence was calculated by dividing the number of positive flocks with one or more seropositive animals in the flock by the total number of flocks examined and multiplying by hundred.

Univariate logistic regression analysis was used as a preliminary step before conducting more complex multivariable analyses and Identifying significant variables through univariable analysis for the selection of variables for inclusion in multivariable models. For all risk factors with $p < 0.25$ were further checked for multicollinearity using the variance inflation factor (VIF) and tolerance factor (TF) before multivariable logistic regression analysis. Variance inflation factor values of greater than 10 and tolerance less than 0.1 were considered the cut-off points for the multicollinearity diagnostics. Then, multivariate logistic regression analysis was performed to determine associated risk factors.

The odds ratio (OR) was used to indicate the degree of association between disease occurrence and risk factors. A *p*-value of less than 0.05 ($p<0.05$) was considered as statistically significant association.

RESULTS

Socio-demographic Characteristics

Of the 80 respondents who were selected by systematic random sampling method, about 35 respondants and 45 respondants were from the Tello and GoriGesha districts, respectively. From the total households interviewed, most of them were illiterate, while some of them were able to write and read and a few individuals were attended formal education. In terms of sex, the majority of the respondents were male and the rest were female (Table 1).

Table 1: Socio-Demographic Characteristics of Respondantes

Variables	Category	Frequency in %	Percentage
Districts	Tello	35	43.8
	GoriGesha	45	56.3
Peasant association	Odda	10	12.5
	Dacha	10	12.5
	Yama	10	12.5
	Washa	5	6.3
	Boute	15	18.8
	Tuyie	15	18.8
	Shewajibabu	10	12.5
	gelie	5	6.3
Age of respondent	Young (<20 years)	12	15.0
	Adult (20-50 years)	55	68.8
	Old (>50 years)	13	16.2
Sex of respondent	Male	59	73.8
	Female	21	26.2

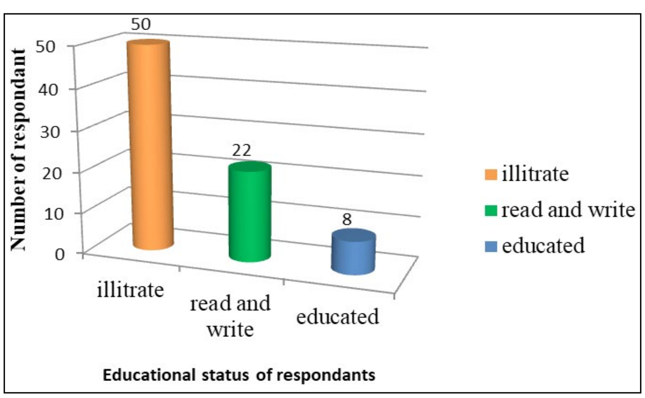

Fig. 4: Educational status of the respondants

Overall sero-prevalence of brucellosis on study districts

A total of 552 animals, including 341 goats (61.8%) and 211 sheep (38.2%) aged above 6 months were sampled and tested for *Brucella* antibodies. The present study revealed that there was non-significant difference in sero-prevalence of brucellosis among the two study districts. Based on the test results, the highest sero-prevalence of 9.52% was recorded in the Washa peasant association of Tello district, followed by 6.67% in the Boute peasant association of GoriGesha district and the lowest sero-prevalence of 1.33% and 1.54% was recorded in Odda and Dacha peasant association of Tello district, respectively.

Species-specific Sero-prevalence of 4.4% (15 out of 341) and 3.8 % (8 out of 211) were observed in goats and sheep respectively using the i-ELISA test. However, the difference between the two species was not statistically significant ($p > 0.05$).

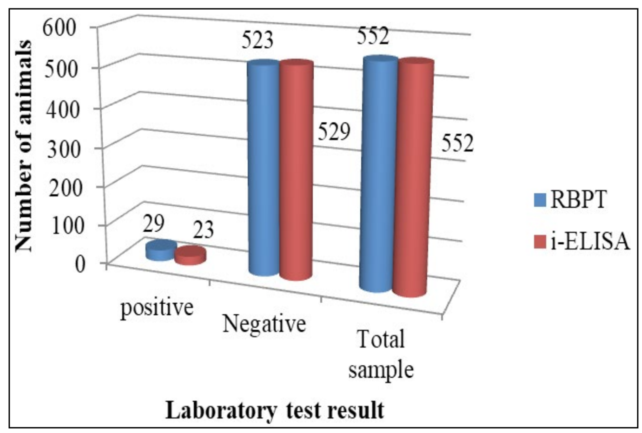

Fig. 5: Serological test results using RBPT and i-ELISA

Serum samples that tested positive for both RBPT and i-ELISA were considered as seropositive, resulting in an overall small ruminant brucellosis sero-prevalence in the study area of 4.17%. The individual level small ruminant brucellosis in the two districts of the southwestern region ranged from 3.2% to 5.0% (Table 2).

Table 2: Overall sero-prevalence of brucellosis on study districts

District	Peasant Association	Total sampled	Positive		Prevalence
			RBPT	***i-ELISA***	
Tello	Odda	75	2	1	1.33%
	Dacha	65	2	1	1.54%
	Yama	70	3	2	2.85%
	Washa	42	4	4	9.52%

GoriGesha	Boute	75	6	5	6.67%
	Tuyie	75	5	4	5.33%
	Shewajibabu	75	4	4	5.33%
	Gelie	75	3	2	2.67%
Total		**552**	**29**	**23**	**4.17%**

The apparent prevalence (Ap) was adjusted for the sensitivity (Se) and specificity (Sp) of i-ELISA (96.1% and 97.5%, respectively) (ROGAN and GLADEN, 1978) to get the true prevalence by the formula:

$$\text{True Prevalence} = \frac{AP + Sp - 1}{Se + Sp - 1}$$

Risk factors for small animal *Brucella* serostatus

Univariate logistic regression analysis examines the relationship between a single independent variable and a binary outcome and assess the effect of one predictor on the likelihood of the outcome occurring (Table 3). After conducting univariate logistic regression analysis, it was observed that factors such as herd size, age, history of abortion, history of retained fetal membrane and female animals with history of still birth exhibited significant associations with small ruminant brucellosis ($p<0.05$) (Table 3). Accordingly, these significant factors were chosen for subsequent analysis using multivariate logistic regression to provide more reliable estimates of the relationships between independent variables and *Brucella* seropositivity.

Multivariable logistic regression is a valuable statistical tool used to analyze the sero-prevalence of brucellosis in small ruminants, allowing to identify significant risk factors associated with the disease. Multivariable logistic regression analysis revealed significant risk factors for *Brucella* seropositivity (Table 4). Risk factors with a p value ≤ 0.25 in the univariate logistic regression model were entered into the multivariate logistic regression model. Therefore, herd size, age, reproductive status, parity, abortion history, fetal membrane retention history, and history of stillbirth were a candidate variable for entering the final logistic regression model.

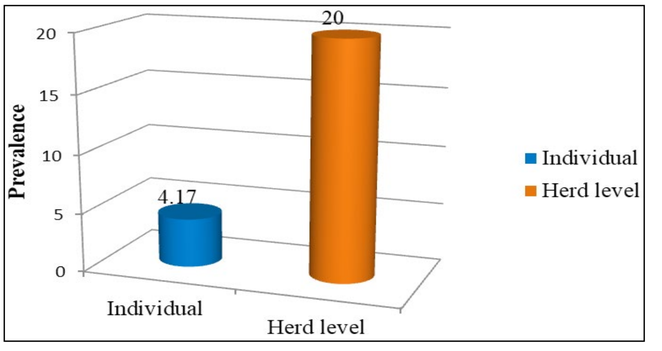

Fig. 6: Individual and flock level sero-prevalence of brucellosis

In this study, among the candidate variable which is entered to the final multivariable logistic regression model, age, reproductive status of the small ruminant and party status were not significantly associated with *Brucella* infection. Of all these variables, animal seropositivity in the final analysis was most influenced by herd size, abortion history, fetal membrane retention history, and history of stillbirth.

Overall flock level sero-prevalence of small ruminant brucellosis

The sero-prevalence of small ruminants in the study area at flock level was 20% (95% CI: 12.7 to 30). By using RBPT and i-ELISA, five herds from Tello (14.3%) and 11 herds from GoriGesha (24.4%) were seropositive, and with at least one animal from each herd was seropositive. At the district level, there was no statistical difference between herds ($p>0.05$), but the highest sero-prevalence was in the GoriGesha district than in the Tello district. Because of small sample size the herd level analysis was conducted using exact logistic regression tool. Herd size and history

Table 3: Overall sero-prevalence of individual animal and flock-level brucellosis

District	Number of animal	Individual prevalence		Number of flock	Flock prevalence	
		RBPT	i-ELISA		RBPT	i-ELISA
Tello	252	11	8	35	8	5
GoriGesha	300	18	15	45	13	11
Total	552	29	23	80	21	16

Table 4: Univariate logistic regression analysis of risk factors

Variables	Category	Tested samples	Number Positive	COR	95% CI	P-value
Districts	Tello	252	8			
	GoriGesha	300	15	1.61	0.669-3.851	0.289
Herd size	(Small)≤ 10	230	2			
	(Large)≥ 10	322	21	7.95	1.846-34.266	0.005
Production system	Agro past	252	8			
	Pastoral	300	15	1.61	0.669-3.851	0.289
Species	Caprine	341	15	0.86	0.357-2.056	0.729
	Ovine	211	8			
Age	Yn(≤ 1year)	159	1			
	Ad(≥ 1year)	393	22	9.36	1.252-70.111	0.029
Sex	Male	76	3			
	Female	476	20	1.07	0.309-3.682	0.918
Reproductive status	Heifer	135	0			
	Pregnant	219	16	1.56	0.837-2.915	0.161
	Lactating	122	4			
Parity	No parity	136	0			
	Primiparous	68	8			
	Pluriparous	272	12	1.64	0.889-3.037	0.113
Abortion	No	400	11			
	Yes	76	9	4.75	1.896-11.899	0.001
Retained fetal m/m	No	431	13			
	yes	45	7	5.92	2.229-15.734	0.000
Still birth	No	436	14			
	Yes	40	6	5.31	1.921-14.725	0.001

Yn = young Ad = adult.

Table 5: Multivariate logistic regression analyzes of potential risk factors

Variables	Category	Tested samples	Positives	AOR	95% CI	P-value
Herd size	≤ 10	230	2			
	≥ 10	322	21	4.69	1.031-21.367	0.046
Abortion	No	400	11			
	Yes	76	9	4.78	1.615-14.175	0.005
Retained fetal m/m	No	431	13			
	yes	45	7	6.83	2.178-21.471	0.001
Still birth	No	436	14			
	Yes	40	6	6.08	1.846-20.039	0.003

Table 6: Risk factors for seropositivity at herd level based on exact logistic regression

Variables	Category	Tested samples	Positives	OR	95% CI	P-value
District	Tello	35	5			
	GoriGesha	45	11	0.98	0.152-6.194	1.000
Herd size	Small	48	3			
	Large	32	13	6.20	1.089-55.277	0.037
Management system	Semi intensive	16	3			
	Extensive	64	13	0.63	0.073-6.162	0.957
New introduction	No	49	3			
	Yes	31	13	4.39	0.700-37.694	0.136
Abortion	No	56	4			
	Yes	24	12	8.69	1.807-52.825	0.004

of abortion in the herd were significantly associated with herd seropositivity for Brucella infection in the study area ($p < 0.05$).

Table 6 shows the results of the exact logistic regression analysis showing the main risk factors for Brucella seropositivity in the herd. Therefore, herd size and history of abortion in the herd fit the exact logistic regression analysis and were significantly associated with Brucella seropositivity ($p<0.05$). Exact logistic regression analysis depicts that large herd size were 6.20 times more likely to become Brucella seropositive compared to that of small herd size. Larger herds have more animals in close proximity, facilitating the spread of Brucella through direct contact or contaminated environments.

The study also confirmed that, those animals with prior history of abortion in the herd were 8.69 times more likely to become seropositive for brucellosis than those animals without history of abortion in the herd. Flocks with a history of abortions may harbor infected animals that can act as reservoirs for the bacteria, maintaining Brucella within the herd.

Both herd size and the incidence of abortions are critical factors influencing *Brucella* seropositiveness in small ruminants. Larger herds tend to have higher transmission rates due to increased animal density and management challenges, while abortions serve as a clear indicator of infection, contributing to the overall seropositivity within the flock.

DISCUSSION

Two serological tests were employed in this study to estimate the sero-prevalence in small ruminants using serum samples. This approach was also utilized by (Getachew *et al.*, 2023, Awais *et al*., 2024) who showed that the combination of RBPT for screening and i-ELISA for confirmation of antibodies were considered to be suitable and effective diagnostic tool for large-scale serological testing of brucellosis. RBPT is prone to cross-reactivity with other Gram-negative bacteria, such as *Yersinia enterocolitica* O:9 and *Eschrichia coli* O:157 and Some species of Salmonella which can produce false positive results (Dosa *et al.*, 2023). However, both methods are useful for detecting *Brucella* antibodies in small ruminants. According to the study, the overall prevalence of small ruminant brucellosis by RBPT and iELISA among small ruminants in the study area was 4.17%.

The prevalence detected by I-ELISA in small ruminant brucellosis in the present study was almost similar to the findings of some authors in different regions of Ethiopia. 3.3% by (Dosa *et al.,* 2023) in southern region, 3.7 % by (Koto and Boru, 2023)in Arero District of Borana Zone, 4.6% by (Deddefo *et al.*, 2015) in selected districts of Arsi and East Shoa zones, Oromia region and 4.1% by (Aloto *et al.*, 2022) in southern region. However, a relatively lower prevalence of 1.7% (Ahad, 2021) in South Eastern Somali Region, 2.6% (Edao *et al.,* 2020) in Borena zone, Southern Ethiopia, 1.23 % by (Hussen *et al.,* 2023) in the Korahey zone, Somali regional state, and 0.24% by (Geletu *et al.*, 2021)in West Hararghe Zone of Oromia Regional State, were reported.

Higher prevalence of small ruminant brucellosis than the present study was reported as 26.5% in Afar region by (Tekle *et al.*, 2023), 21% in south omo zone by

(Dima, 2022), 36.7% in semi arid region of India by (Vakamalla *et al.,* 2023). These differences in sero-prevalence estimates in small ruminants are due to differences in animal species and production systems, the different agroecological regions in which the studies were conducted and the sampling methods and diagnostic tests used. Another important issue is the difference in sensitivity and specificity of the serological tests used to diagnose brucellosis in small ruminants. The RBPT used for screening individual animals in this study is a cheap, rapid and highly sensitive test (Legesse *et al.,* 2023). However, its specificity is low because the smooth lipopolysaccharides of the Brucella antigen can cross-react with antibodies from closely related Gram-negative bacteria such as *Yersinia enterocolitica*, *Escherichia coli, Salmonella* spps and *Sternotrophomonas maltophilia* as well as antibodies produced by B. abortus S19 vaccine (Baldi and Giambartolomei, 2024).

Univariate or bivariate logistic regression analysis identified that herd size, age, reproductive status, parity, history of abortion, history of retained fetal membrane and stillbirths were a candidate variable for entering the final logistic regression model ($P \leq 0.25$). Accordingly, multivariate logistic regression analysis of the effect of risk factors on seropositivity to *Brucella* antibodies in small ruminants showed that herd size, history of abortion, history of retained fetal membrane and stillbirth were significantly associated with seropositivity to Brucella antibodies in this study.

Although this study showed that the sero-prevalence detected by RBPT and I-ELISA was relatively higher in goats (4.4%) than in sheep (3.8%), this was not statistically significant ($P>0.05$), with the odd of the disease, as identified by univariable logistic regression analysis, being 0.86 times higher in goats than in sheep. This difference in sero-prevalence between sheep and goats could be associated with the difference in proportion of sheep and goats in the herd or flock, which were included in the present study. Similarly, Because of the higher preference to goats than sheep and the excretion time of the organism is longer than that of sheep.

The seropositivity of Brucella infection between sex was not found as a statistically significant difference as previously described by (Dosa *et al.,* 2023) in southern region. The lack of statistically significant difference between sexes could be due to the smaller sample size of males, and the habit of keeping males in the herd for a shorter period may decrease their exposure to the disease. Yet, (Bile *et al.*, 2024) have also reported that males are less susceptible to Brucella infection due to the absence of erythritol. Erythritol is a sugar alcohol present in relatively high concentrations in the placenta and fetal fluids of pregnant animals, providing a comfortable environment for the growth and multiplication of Brucella organisms. A higher concentration of erythritol sugar is found in females than in males, increasing the susceptibility of female animals to brucellosis (Bile *et al.*, 2024).

The study found that larger herd size were significantly associated with *Brucella* seropositivity in small ruminants. Multivariate logistic regression analysis showed that the odd of seropositivity in larger herds were 4.69 times greater than that of seropositivity in small herds. This finding is consistent with previous studies that associated brucellosis with large herds (Hussen *et al.,* 2023); (Dosa *et al.,* 2023) ; (Shirwany *et al.*, 2024); (Ahad *et al.,* 2024); (Geletu *et al.,* 2021). It is generally accepted that an increase in herd size is usually accompanied by an increase in animal density and an increase in the risk of infection, especially after abortions. Herd size is an important factor in the potential for transmission between susceptible and infected animals (Shirwany *et al.,* 2024).

In female animals, a history of abortion was statistically significantly associated with seropositivity for brucellosis, which is consistent with the results of many previous studies. This suggests that Brucella infection can cause abortion in pregnant animals, although no clinical cases of brucellosis were observed during the study period. In agreement with this study previous authors such as (Hussen *et al.*, 2023); (Sorsa *et al.*, 2022), (Getachew *et al.*, 2023) and (Vakamalla *et al.*, 2023) who reported statistically significant association between brucellosis and abortion. Abortion is documented in the literature as a typical consequence of brucellosis infections, because *Brucella* prefers the pregnant uterus, where erythritol stimulates the growth of these bacteria and increases the content in the placenta and fetal fluid during pregnancy. (Rossetti *et al.,* 2022).

In this study, small ruminants with history of retained fetal membranes were significantly associated with seropositivity ($p \leq 0.05$). The seropositivity was higher in animals with retained fetal membranes (15.5%) compared to animals without retained fetal membranes (3%) with the odds of approximately 6.83 times more in animals with history of retained fetal membrane than the counter part. Associations between sero-prevalence and the presence of retained fetal membranes have also been reported by (Hussen *et al.,* 2023) in the Korahey zone, Somali regional state, eastern Ethiopia; (Dosa *et al.,* 2023) in two selected districts of southern region, Ethiopia; (Ahad *et al*., 2024) in Somali pastoralists in Eastern Ethiopia; (Efrem *et al.,* 2024) the first report from Eritrea; (Shirwany *et al.,* 2024) in small ruminants of district Khanewal, Pakistan.

This study found history of stillbirth in small ruminants significantly associated with *Brucella* seropositivity ($p<0.05$) (Dosa *et al.,* 2023) with the odds of 6.08 times more in animals with history of still birth than the counter part. This is because dogs, carnivores, birds and other potentially contaminated materials can spread the *Brucella* organism into the environment, which can indirectly contaminate food and drinking places to spread the disease (Hussen *et al.,* 2023).

The overall herd-level sero-prevalence in small ruminants was 20% in the study area, which is almost similar with the findings of (Adem *et al.,* 2021) who reported sero-prevalence of 22% in Dallo-Manna and Haranna-Bulluk Districts of Bale Zone, Oromia regional state ,Ethiopia and (Muhidin *et al.,* 2021) who reported sero-prevalence of 17.5% in Berbere district of Bale Zone southeast Ethiopia**.** However, this result was higher than the previous report of (Hussen *et al.,* 2023) with 6.5% in the Korahey zone, Somali regional state, eastern Ethiopia. On the other hand the finding was lower than the finding reported by (Tegegn *et al.*, 2016) with 57.8% in two Districts of Afar Region, Ethiopia and (Deddefo *et al.,* 2015) 26% in selected districts of Arsi and East Shoa zones, Oromia region, (Teklue *et al.,* 2013) 28.3% from Southern Zone of Tigray Region, Northern Ethiopia.

In this study, herd size in the flock was significantly associated with seropositivity for small ruminant brucellosis. This finding was consistent with the reports of (Dosa *et al*., 2023) in two selected districts of southern region, Ethiopia; (Muhidin *et al.*, 2021) in Berbere district of Bale Zone southeast Ethiopia; (Ahad *et al*., 2024) in Somali pastoralists in Eastern Ethiopia: (Geletu *et al.*, 2021) in West Hararghe Zone of Oromia Regional State, Eastern Ethiopiaand (Aloto *et al.,* 2022) in southern Ethiopia. An increase in herd size is usually accompanied by an increase in animal density and an increase in the risk of infection. Stocking density is an important factor in the possibility of transmission between susceptible and infected animals (Edao *et al.,* 2020). It is also indisputable that the spread of the disease from one herd to another and from one area to another is almost always due to the movement of an infected animal from an infected herd to a non infected herd. Therefore, brucellosis should never be considered a disease of individual animals, but should be considered in the context of herd and also the animal population of the study area.

It was found that the presence of aborted females in the herd is significantly related to the herd seropositivity. Abortion in small ruminants is the main complaint caused by *Brucella* infection (Hussen *et al.,* 2023; Adem *et al.*, 2021; Muhidin *et al*., 2021; Tegegn *et al*., 2016). Infected females can excrete large amounts of the organism in their milk, placental membranes and aborted fetuses. Such females have been reported to continue to spread the organisms for several months (Bayu, 2018). This leads to environmental contamination and, as a result, the risk of transmission of pathogens between animals in the same herd and in free mixing between other herds in grazing and watering places.

In summary, both herd size and the incidence of abortions are critical factors influencing *Brucella* seropositiveness in the herd of small ruminants. Larger herds tend to have higher transmission rates due to increased animal density and management challenges, while abortions serve as a clear indicator of infection, contributing to the overall seropositivity within the flock.

CONCLUSION AND RECOMMENDATIONS

Based on the above conclusion, the following recommendations are forwarded to minimize the spread of the disease to animals and humans. The government, public health officers and veterinarians have to work together in accordance with one health approach to reduce the economic and zoonotic impact of brucellosis. Creating awareness for the community about economic importance of Brucella infections, modes of transmission and prevention methods in the study area. Encourage farmers to adopt strict biosecurity measures, including sanitation, safe disposal of aborted foetus and retained foetal membrane and proper handling of animal products. A strong disease surveillance system and regular screening should be employed by regional laboratories to prevent small ruminant brucellosis in the study area.

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