Animal and Veterinary Sciences
2025, Vol. 13, No. 4, pp. 114-118
https://doi.org/10.11648/j.avs.20251304.14

Research Article

Study on Prevalence of Small Ruminant Brucellosis and Associated Factors in Wemberima and North Mecha Districs of Amahara Region, Ethiopia

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Abstract

Brucellosis is one of the infectious disease that most affects sheep and goats productivity. Ethiopia is home to a significant number of animals, but it has not fully capitalized on the potential of small ruminant production. This underperformance is due to several factors, including poor genetic quality, inadequate management practices, and major challenges related to diseases. These issues pose significant barriers to achieving economic returns from small ruminant production. Additionally, farmers often develop a strong attachment to their animals, making it crucial to assess the status of zoonotic diseases, such as brucellosissis. A cross-sectional study was conducted to investigate the seroprevalence of brucellosis in small ruminants and its related risk factors from February to May 2023 in Wemberima and North Mecha districts of the Amhara Region. A total of 600 sera were collected from ten purposively selected villages of the two districts (Goat, n=355 and Ovine, n = 245) and screened using RBT for the evidence of antibodies against brucellosis and confirmed by Complement Fixation Test (CFT). The study revealed an overall 3% (95% CI: 1.48-26.69) sero-prevalence of ruminant brucellosis. Species were significantly associated risk factors with *Brucella* seropositivity ($P < 0.05$) in this study. Hence, the odds of being sero-positive to *Brucella* was found to be 6.11 (OR=6.11; 95% CI: 1.48- 26.69) times higher in Ovine than in Caprine. All other risk variables had no significant effect ($P > 0.05$).

Keywords

Brucellosis, Small Ruminant, Seroprevalence, West Gojam, Ethiopia

1. Introduction

Goats and sheep are major domestic animals in African tropical livestock production systems, accounting for 21% of the global small ruminant population [3]. Ethiopia is one of the sub-Saharan African countries, of the largest animal and second largest human population, with 31.30 million sheep and 32.74 million goats primarily raised in the country's lowland and pastoral regions [5]. These small ruminants due to their high short generation cycles and great ability to adapt to various environmental conditions make them valuable animal production and represents an important export commodity that significantly contributes to the livelihood and national economy of rural farmers as a source of food (milk and meat), wool, skins, source of income, and monetary asset, especially in pastoral and lowland areas of the country while

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Received: 2 June 2025; **Accepted:** 30 June 2025; **Published:** 7 August 2025

reproductive diseases like brucellosis inhibit their productivity [19]. Brucellosis is a zoonotic infectious disease caused by a bacterium of the genus *Brucella*, common to certain animals, both domestic and wild, and to humans. Historically, this disease is known as Malta fever or melitococcal disease. The causative agent was isolated in 1887 on the Island of Malta by David Bruce. Its extension is worldwide with predominance in developing countries [7, 8].

The main clinical signs of brucellosis in small ruminants are abortion, retained placenta, stillbirth, orchitis and arthritis. Infected animals, milk and dairy products are the sources of infection in humans. B. melitensis, B. abortus and B. suis are the most important species in terms of public health and economy. Pigs are the source of almost all human contaminations. B. melitensis, the species most frequently implicated in human pathology, is largely predominant in sheep and goats. This organism contains three biovars and all of them can cause disease in small ruminants [18, 4].

B. Melitensis and B. Ovis are the common infective agents of small ruminants characterized by reproductive problems, abortion, still breathing and infertility [15, 17]. Ethiopia is a large country with a huge livestock population, and previously published work has only described sero- prevalence for specific areas and not the whole country [3]. Furthermore, no published research work on small ruminant brucellosis was found in the current study area. Therefore, this study was initiated to investigate the seroprevalence and the associated risk factors of small ruminant brucellosis in Wemberima and North Mecha districts.

2. Materials and Methods

2.1. Description of the Study Areas

The study was undertaken in the Amara regional state in Wemberima and North Mecha districts from February to May 2023. The region located in the north Western and north central parts of Ethiopia, between 9 ℗20' and 14 ℗20' North and 36 ° 20' and 40 °20' east. The area coverage of the region is 170,752 km^2. It divided into 11 zones, and about 169 districts [2].

2.2. Study Populations

The indigenous sheep and goats raised in the extensive production system in the West Gojam zone of Wemberima and North Mech districts were the subject of this study. Sheep and goats six months of age and older were included in the study, along with both sexes from the chosen flock who had never received a brucellosis vaccination. Documentation was provided about potential risk factors, such as the animal's origins, species, sex category, parity, and reproductive health issues.

2.3. Study Design and Methods

Across-sectional study was conducted to estimate prevalence of small ruminants' brucellosis and to identify associated risk factors. Purposive sampling technique was used to select districts and peasant associations (Kebeles) based on small ruminant population densities and accessibility for transportation while collecting the required sera sample simple random sampling strategy was used. 60 animals from ten selected villages were included in this study therefore a total of 600 animals were sampled for this study purpose. Designed questionnaire targeted on different variables such as sex, age, parity, abortion history, herd size was presented to owners to assess associated risk factors.

2.4. Sample Size Determination

Using an internationally accepted standard formula, the total sample size was calculated [13]. To determine the maximum sample size, a 95% confidence level at 5% absolute precision and a 50% predicted occurrence were used in the sample size calculation.

$$n = [1.96^2 \times Pexp(1-Pexp)] / d^2$$

Where n = required sample size, d = desired absolute precision, P = expected prevalence (50%) by substituting the value, the minimal sample sizes of 384 small ruminants were found. A design effect of 2 was taken into consideration in order to account for intra-class correlation at the herd, village, and district levels. This resulted in a minimum sample size of 600 animals were calculated of which 355 were sheep while the rest 245 goats were sampled to investigate prevalence rate of small ruminant brucellosis.

2.5. Sample Collection and Laboratory Analysis

Approximately 5-7mL of blood was collected from the jugular vein of each animal using sterile needles and plain vacutainer tubes. The tubes were individually labeled, stored on ice in an icebox, and transported to the Animal Health Institute (AHI), Sebeta. To enable serum separation, the samples were left to stand for the overnight. After centrifugation for 10 minutes at 1500rpm, the sera were separated. Sera were harvested into Cryo-vials without mixing with clotted blood using a micropipette and stored at -20 ℃ until Processed. Antibodies against Brucella infection in the herd was screened using Modified Rose Bengal Plate Test according to the OIE protocol [16]. In brief, 75 Sera samples and 25 Brucella antigen strain 99 obtained from (ID.vet, RSA-RB-016, 0112 GB, 310, rue Louis Pasteur-Grable's- FRANCE) were mixed onto 12 wells glass slid white plate using an applicator stick and rocked for about 4 minutes. Visible agglutination were considered as +, ++, +++ for positive samples and no agglutinations at 4 minutes were interpreted as negative [16]. The complement fixation test (CFT) is regarded as being the confirmatory test for the serological detection of Brucella infection in animals (IOE, 2016). Is a blood test that can determine the presence of

antigen specific antibodies by incubating patient serum with a defined amount of antigen and complement Animals are considered positive if tested positive on both RBPT and CFT.

2.6. Data Management and Analysis

The data gathered from the questionnaire and laboratory test was entered into a Microsoft Excel spreadsheet. The necessary statistical analysis was performed using STATA/IC 13 after coding and editing (2013, College Station). Descriptive statistics were used to ascertain both the dependent and independent variables' frequency and proportion. To determine whether there was a significant difference between the expected outcome (brucellosis) and the independent factors (species, sex, age, parity, abortion, and origin of animals), the χ^2 test was also used. Using univariate logistic regression analysis, the different possible risk factors were evaluated for their association with the seroprevalence of brucellosis in small ruminants. To measure strength of the associations and to calculate the odds ratio logistic regression analysis was applied.

3. Result

3.1. Sero-prevalence of Small Ruminant Brucellosis

The overall sero-prevalence of brucellosis in the study area was 3% (19/600) using complement fixation test (CFT). The prevalence of brucellosis as detected using the RBPT and CFT for each species was presented in Table 1. The sero- prevalence of *Brucella* infection in sheep (4.8%) was greater than in goats (0.8%) and has shown a statistically significant difference ($p < 0.05$). The higher prevalence was recorded in Ovine (4.8%, 17/355) as compared to caprine (0.8%, 2/245) Table 1.

Table 1. *Sero-prevalence of small ruminant brucellosis by species of animal.*

S/N	Species	No of sampled Animals	Sero-positivity rate	
			RBT	CFT
1	Ovine	355	40 (11.2%)	17 (4.8%)
2	Caprine	245	2 (0.8%)	2 (0.8%)
3	Total	600	42 (7%)	19 (3%)

3.2. Association of Risk Factors with Sero-prevalence and Logistic Regression Analysis

The chi-square (χ^2) was applied to determine the existence of association between sero-positivity and potential risk factors. To measure the strength of associations and to calculate the odds ratio logistic regression analysis was applied. Confidence interval (CI) of 95% and 5% cut-off value was established for significance. P value of < 0.05 was taken as statistically significant. Species had a statistically significant association with *Brucella* sero-positivity ($p < 0.05$). Ovine had 6.11 times more likely to contract brucellosis as compared to Caprine with (P = 0.006) in this study. Animals with Parity have 0.952 times likely to infected than those without Parity and animals with abortion history has 5.32 times more likely to be seropositive for brucellosis than those without such history (OR =5.32, 95% CI = 0.608-46.553). Distribution of the disease by district was higher in N/Mecha than Wemberma but all these were not statistically significant (Table 2).

Table 2. *Potential risk factors association with brucellosis in small ruminants.*

S/N	Variable	Categorie	No of sampled animals	Positivity rate	CRUDE OR	P value	χ^2	[95% CI]
1	Species	Ovine	355	17 (4.8%)	6.11	0.006	7.4597	1.48-26.69
		Caprine	245	2 (0.8%)				
2	Sex	Male	69	2 (3.6%)	0.903	0.9	0.0183	0.204-3.99

S/N	Variable	Categorie	No of sampled animals	Positivity rate	CRUDE OR	P value	χ^2	[95% CI]
		Female	531	17 (3.6%)				
3	Parity	Yes	504	15 (3%)	0.952	0.93	0.0059	0.271-3.335
		No	27	1 (3.7%)				
4	Abortion history	Yes	7	1 (14.3%)	5.32	0.091	2.8556	0.608-46.553
		No	524	16 (3%)				
5	District	Wemberma	300	7 (2.3%)	0.573	0.244	1.3588	0.222-1.477
		N/Mecha	300	12 (4%)				

4. Discussion

Out of 600 (355 sheep and 245 gats) sera samples tested for small ruminant brucellosis in the North Mecha and Wenbrima districts, 42 (7%) tested positive on the RBPT, and 19 (3%) tested positive for CFT for brucellosis. The overall sero-prevalence of brucellosis in the study area was 3% (95% CI: 1.48-26.69) in line with previous reports of 3.5% [11], 3.3% [10], and 3.3% [3] in different parts of the country. Conversely, low prevalence of 0.9% [6], 0.7% [12], 1.76% [14], 1.79% [9], 1.5% [1] and 0.24% [5] was recorded. In different parts of Ethiopia higher prevalence of the disease was also recorded. Both sexes of animals have equal chances of acquiring infection (3.6%). but statistically, had no significant effect. This finding contradicts previous research, which may be due to different factors. The species of the animals had a significant effect on sero-positivity ($P < 0.05$). In this study, the odds of being sero-positive for Brucella were found to be 6.11 times higher in sheep (OR = 6.11, 95% CI = (1.48, 26.69)) compared to goats. This finding contradicts previous research works, which may be due to different factors [20]. On Pearson's Chi square test X^2) analysis as indicated in (Table 2), there was no statistically significant association ($P>0.05$) between prevalence of brucellosis and the risk factors such as sex, parity, abortion history and district.

5. Conclusion and Recommendations

This study revealed a high seroprevalence of brucellosis in sheep as compared to goats in North Mecha than Wemberma districts. Extensive farming system in the study area were found to be at high risk of acquiring *Brucella* infection because of customs surrounding the consumption of raw milk, traditional animal husbandry, the practice of handling animals with reproductive difficulties with their bare hands, and a lack of knowledge regarding the zoonotic nature of the disease. And also mixing herds with free grazing is commonly practiced in the extensive production system which facilitates disease dissemination and subsequent cross transmissions of brucellosis among species of animals. Therefore, it is advised in the research areas to implement suitable control measures in the smallholder production system and to improve management systems. It is necessary to conduct more epidemiological studies in addition to identifying and isolating the *Brucella* biotype that is causing the infection.

Abbreviations

AHI	Animal Health Institute
CFT	Complement Fixation Test
LFSDP	Livestock & Fisheries Sector Development Project

Acknowledgments

The authors would like to express recognition to Animal Health Institute (AHI) and Livestock & Fisheries Sector Development Project (LFSDP) for their support. Likewise, the authors would like to express honest gratitude to Wemberima and North Mecha district livestock owners and district animal health staff for their cooperation during sample collection.

Availability of Data and Materials

Due to the confidentiality agreements made, the datasets generated and/or analyzed during the study are not publicly available but could be accessible from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

The current study involved only animal samples, it did not involve human participants. Informed consent was obtained from the herd owners to take samples from their animals which were documented. Confidentiality of data obtained and the scientific morality was considered.

Funding

This research work was financially supported by Animal Health Institute (AHI) and Livestock & Fisheries Sector Development Project (LFSDP).

Conflicts of Interest

The authors declare no conflicts of interest.

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