

Seroprevalence and Associated Factors of Ovine and Caprine Brucellosis in Dubti District of Afar Region, Ethiopia

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Abstract

Background: Brucellosis is a bacterial zoonotic disease that causes reproductive losses in animals and poses public health hazards as well as economic impact in sub-Saharan Africa including Ethiopia. The present study was undertaken with the objective to estimate the seroprevalence and identify associated risk factors of small ruminant brucellosis.

Methods: A cross-sectional study was carried out on the seroepidemiology of ovine and caprine brucellosis from February to April 2022 in six selected kebeles of Dubti district, Afar region, Ethiopia. A total of 300 serum samples were collected from apparently healthy sheep (n=69) and goats (n=231) and tested using Rose Bengal Plate Test (RBPT) as a Screening test for *Brucella* antigens, while indirect enzyme linked immune-sorbent assay (I-ELISA) was used as a confirmatory serological test of reactors by Rose Bengal Plate Test at Animal Health Institution (AHI). Descriptive statistics, Pearson's chi-square (X^2) and Univariable logistic regression analyses were used in this study.

Results: The overall seroprevalence of *Brucella* infection in small ruminants in the present study was found to be 6.7% (n=20/300) and the species level seroprevalence rate was found to be 7.2% (n=5/69) in sheep and 6.5% (n=15/231) in goats in the study areas. Associated risk factor analysis of chi-square and Univariable logistic regression analysis showed that seropositivity of *Brucella* infection with species, sex, species, age, parity, district and abortion history were found statistically insignificant ($P>0.05$).

Conclusion: The present study finding revealed that *Brucella* infection is circulating in small ruminants in the study area in particular and afar region in general. Further studies should be carried out on the entire region to determine *Brucella* seroprevalence, molecular based isolation and biotype identification to develop appropriate control strategies of the disease.

Keywords: Afar; Brucellosis; ELISA; RBPT; Sero-prevalnce; Small ruminant.

Introduction

In Ethiopia, the majority of the community relies largely on the livestock sector as a source of meat, milk, draft power, and revenue, particularly in pastoral and agro-pastoral regions [1]. Small ruminants are desirable livestock species since they grow more quickly, require less maintenance, have shorter production cycles, and are better adaptable to harsh climates than large ruminants [2]. Despite having a huge population of small

ruminants, Ethiopia is unable to make the best use of this resource due to a number of factors, including widespread infectious diseases, a lack of a suitable disease management strategy, inadequate feeding, poor management, a lack of genetic empowerment, and a lack of government attention [1]. Among those infectious diseases of small ruminants, brucellosis is a major problem and widely distributed in all regions of the coun-

try [3] and hampers the productivity of small ruminants. Brucellosis is considered the world's most common bacterial zoonosis and highly contagious and economically important with public health importance. The World Health Organization (WHO) ranks brucellosis as the second-most significant zoonotic disease in the world, with major economic and public health consequences in sub-Saharan Africa, including Ethiopia [4].

In many regions of the world, particularly in those where livestock is a significant source of food and money, brucellosis is a ubiquitous zoonotic disease that poses a serious threat to human and animal health. Brucellosis is a substantial public health threat in pastoral and agro-pastoral communities where intimate contact with animals, the use of raw milk, and a lack of zoonotic disease awareness make it easier for people to contract the disease from livestock. Almost all pastoral communities consume raw milk, which poses a risk to the pastoralists as it is the primary source of brucellosis infection [5]. According to their biological makeup, *Brucella* species are facultative intracellular, Gram-negative, flagellated, immobile, oxidase-, catalase-, and urease-positive, non-spore forming, non-capsulated, and partially acid-fast Coccobacilli. They also lack endospores and native plasmids [6].

In livestock, *Brucella abortus* (*B. abortus*), *Brucella melitensis* (*B. melitensis*), *Brucella suis* (*B. suis*), *Brucella canis* (*B. canis*), and *Brucella ovis* (*B. ovis*) are the primary causes of Brucellosis. The two species that usually causes brucellosis in sheep and goats are *B. melitensis* (biovars 1, 2, or 3) and *B. ovis* [7]. *Brucella melitensis* and *B. ovis* infections have significant veterinary and public health importance and result in significant economic losses associated to abortion, neonatal death, decreased fertility, and decreased milk supply. The major ways that brucellosis being transmitted are through inhalation, contact with an infected fetus, or ingesting organisms along with contaminated food and drink. The urine, milk, vaginal discharge, semen, and delivery secretions of sick animals all contain large quantities of organisms [8]. Various studies in Ethiopia have shown that small ruminant Brucellosis is widespread, particularly in the nation's pastoral areas. The practice of consuming raw milk, handling tainted animal waste, combining the care of different animal species, and herding a large number of animals are all thought to be contributing factors to the high prevalence of the disease in the Afar region [9]. Moreover, recurring natural disasters in the area, such as concurrent drought and flooding, the introduction of new animals as replacement stock from other regions, and food security initiatives for vulnerable communities might all be sources of pathogen introduction [10]. In the current study area, there is a paucity of up-to-date information concerning the disease in small ruminants. Therefore, the objectives of this study were to update the seroprevalence of brucellosis in small ruminants and to identify associated risk factors in Dubti district of Afar region.

Materials and methods

Description of the study area

The Afar region is located in the northeast part of Ethiopia. Administratively, the region is divided into five zones which are further subdivided into 35 districts and more than 358 Peasant Associations (PAs). The current study was conducted in zone one, Dubti district and in five selected kebeles of the district. Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region.

Study population

The current study was conducted in small ruminants kept under extensive management system in five randomly selected kebeles of Dubti district of Afar region. All male or female of sheep and goats in the study area with the age of 6 months and above were considered as the study animals. Vaccination was not considered as there is no vaccination practice against *Brucella* infection in all livestock species in Ethiopia yet. The study populations were classified into young (6 months up to two years) and adult (greater than two years).

Study design

A cross-sectional study design was conducted from March, 2022 to April, 2022, to estimate the seroprevalence of brucellosis in small ruminants in selected Kebeles of Dubti district and assess potential risk factors associated with the disease. A questionnaire targeted on different variables such as age, sex, parity, abortion history, herd size was introduced to herd owners to assess the associated risk factors in the area

Sample size determination

Sample size was determined using a method recommended by [11]. Based on the expected Brucellosis prevalence of 2.4% from previous Zonal level study reported by [7]. The sample size was calculated as.

$$n = \frac{Z^2 \times P_{exp} (1 - P_{exp})}{d^2}$$

Where: n = required sampling units.

Z = Multiplier from normal distribution at 95% Confidence interval (1.96).

P_{exp} = Estimated (expected) prevalence 50% (0.5).

(1-P) = Probability of having no disease 50% (0.5).

D = Desired absolute precision 5% (0.05).

Based on the above formula, the total sample size to be collected was 36 sera. However, this is very small sample size. To increase the precision and make more representative of the small ruminant population in the district, the sample size was maximized to 300. Therefore, the total sample that was sampled during this study was 300 blood sera. Sampling was proportionally distributed based on the total small ruminants' population in the study district's kebeles. The number of sheep and goats samples was not proportional but as it randomly selected and sampled in the field.

Sample collection and sampling methodology

Approximately 8-9 ml of blood was collected from the jugular vein of each sheep and goats using plain vacutainer tubes and needles following the necessary ethics described under the ethical declaration section. The tubes were labeled individually and were kept in icebox. The samples were allowed to stand overnight to allow serum separation. The sera were then collected into sterile cryogenic tubes. Then, the separated serum was labeled and kept under refrigeration (-20°C) until transportation to Animal Health Institution (AHI), Sebeta, Ethiopia for serological analysis. The shipment of the samples was done using an ice box with ice pack. Relevant data of the study was recorded along with blood serum collection. The individual animal details such as the identity of the animal, species, sex, age, history of abortion and parity were registered. Zone and study district was purposively selected to get transport access as sam-

ple for the current study was collected together with a team who came from Animal Health Institute (AHI) to Dubti district for its own purpose. However, the five kebeles were randomly selected from the list of different kebeles in the district. In addition, individual animals were also randomly sampled from the herd.

Serological laboratory analysis

Rose Bengal Plate Test (RBPT): Modified Rose Bengal Plate Test (PBPT) was used as a screening test for all sera samples collected for the presence of *Brucella* agglutinins. All collected sera samples were tested for the presence of antibodies against the natural infection by *Brucella* following the protocol of the. The test was carried out in bacterial serology unit at Animal Health Institution (AHI), Sebeta. Briefly, 30 µl of stained rose Bengal antigen was dispensed on to card plate and then 75 µl of sera samples were dropped alongside the stained Rose Bengal Brucella antigen. Using the tip of the micropipette tips, the sera were mixed and examined for agglutination. Positive and negative controls were employed for interpretation of the results. Agglutinations were recorded as 0, +, ++ and +++ according to the degree of agglutination. Those samples with no agglutination were recorded as negative while those with +, ++ and +++ were recorded as positive.

Indirect Enzyme Linked Immunosorbant Assay (I-ELISA): All positive samples with RBPT were further subjected to indirect Enzyme Linked Immune-Sorbent Assay (I-ELISA) test as a confirmatory test at the National AHI, This kit detects anti *brucella* lipopolysaccharide antibodies in bovine sera. Indirect Enzyme Linked. ImmuneSorbent Assay (IELISA) test was done according the manufacture's manual. Before use and were homogenized by inversion all reagents and sera samples allowed to come to room temperature then 190 µl of the dilution Buffer 2 was added to all the 96 micro plate wells and 10 µl of the Negative control was added to well A1 and B1, 10 µl of the Positive control was added to wells C1 and D1 and 10 µl of sera samples to be tested were added in to the remaining wells. Then after the wells was covered with adhesive film, the plate was incubated at 37°C for 45 minutes, then after the content was discarded and each well was washed three times with 300 µl of the wash solution. 100 µl of the Conjugate 1x was added to each wells and then incubated for 30 minutes, Then after the content of the wells was discarded and each well was washed three times with 300 µl of the wash solution again. 100 µl of buffered peroxides substrate was added into each well and mixed by plate agitator to ensure correct homogenization. After incubation at room temperature for 15 minute shielded from light, 100 µl of stop solution was added and mixed with plate agitator. The sample was then put in to the ELISA reader finally the result was obtained by printing from the computer connected to read under the microreader at 450 nm ELISA reader.

Ethical consideration and consent to participate

Ethical clearance for this study was obtained from Animal Health Institution by Animal Research Ethical and Review committee with certificate Ref. No: VM/ERC/10/03/12/2022. Before sample collection, the owners of the animals were informed with the objectives of the study and verbal consent had been obtained to take blood samples from sheep and goats, and this issue was included in the ethical clearance obtained. During sample collection sheep and goats were treated with best practice of veterinary care.

Data management and analysis: The data gathered through the laboratory analysis and questionnaire survey were stored in a Microsoft Excel spreadsheet and analyzed using STATA version 14.0 for Windows (Stata Corp. College Station, USA). During the statistical analysis, for all the risk factors, the first level of each independent variable was used as a reference category. Seroprevalence was computed by dividing the total number of sheep and goat tested positive by confirmatory test I-ELISA by the total number of sheep and goat sera tested. Chi-square (X^2) and Univariable logistic regression analysis was used to assess the association between seropositivity and explanatory variables. A Confidence Interval (CI) of 95% and 5% cut-off value was set for significance. For all analyses, $P < 0.05$ was taken as statistically significant.

Results

In the current study, out of 300 sera collected from the study population and tested using Rose Bengal Plate Test (PBPT) 34(11.3%) and all the positive serum samples by PBPT were tested for confirmatory by I-ELISA. The confirmatory test revealed that 6.7% ($n=20/300$; 95% CI of 4.15-7.95) were found to be positive for the presence of antibodies against Brucella antigen infection. The demographic characteristics of study population were presented in Table 1. Majority of study population, 87.3% ($n=262$) were females while about 12.67% ($n=38$) of them were males.

Table 1: The demographic characteristics of the tested animals.

Variables	Category	Sample size	Percentage
Sex	Male	38	12.67%
	Female	262	87.3 %
Species	Ovine	69	23 %
	Caprine	231	77 %
Age	Young	79	26.3 %
	Adult	221	73.66 %
Parity	Yes	115	38.33 %
	No	185	61.66 %
Abortion history	Yes	13	4.33 %
	No	287	95.66%
District	1. Harakis and gansuri	60	2(3.3)%
	2. Ayerolef and Gebelaytu	60	3(5) %
	3. Aredo & Larigoh	60	6(10) %
	4. Debal	44	1(2.3) %
	5. 01	76	8(10.5) %

Seroprevalence of brucella infection

Out of 300 sheep and goats sera tested by Rose Bengal Plate Test (RBPT) 11.3% ($n=34$) were positive and all the positive sera samples were tested for confirmatory by I-ELISA 20 (6.7%) samples were positive against Brucella antibodies as depicted in (Figure 1). In this study, the seroprevalence of Brucella infection among the species level was 7.2% in sheep and 6.5% in goats in the study district of Afar region. From the total sera tested, all samples were females with a prevalence of 6.7% ($n=20/300$).

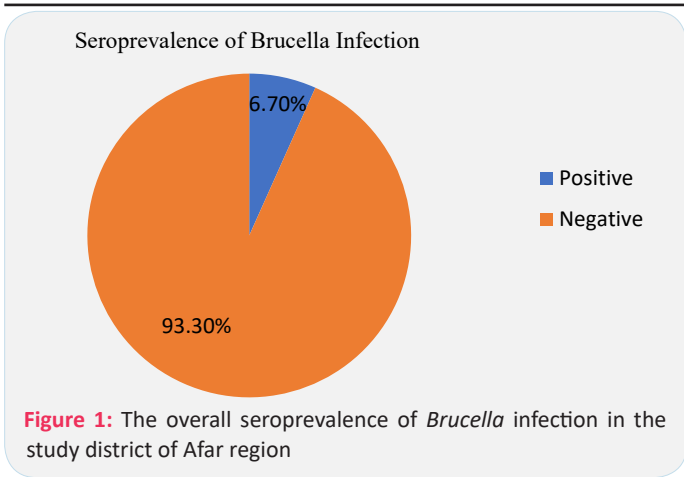


Figure 1: The overall seroprevalence of *Brucella* infection in the study district of Afar region

Associated Factors of Brucella infection

Associated risk factors such as species, sex, age, parity, and district and abortion history were assessed using structured questionnaire for every sampled herd for the occurrence of Brucella infection seroprevalence as depicted in (Table 2). In the current study, the result of Brucella infection seroprevalence across the geographical areas was found to be varied. In different age groups, the seroprevalence in the study population, young age group was 8.9%, and adult aged group was 5.9%. The seroprevalence of Brucella infection between sex groups showed that all samples were female (6.7%). In the present study, the chi-square (χ^2) analysis result indicated that among the associated risk factors with brucellosis seroprevalence occurrence; none of the variables were found to be statistically significant as depicted (Table 2).

Table 2: Chi-square analysis results of associated factor of brucellosis seroprevalence.

Variables	Category	Sample size	Seropositive%	Chi-square (χ^2)	P value
Sex	Male	38	0(0)	3.1080	0.078
	Female	262	20(7.6)		
Species	Ovine	69	5(7.2)	0.484	0.826
	Caprine	231	15(6.5)		
Age	Young	79	7(8.9)	0.8297	0.362
	Adult	221	13(5.9)		
Parity	Yes	115	12(6.5)	0.0252	0.874
	No	185	8(6.9)		
Abortion history	Yes	287	18(6.3)	1.6598	0.198
	No	13	2(1.5)		

Table 3: Univariable analysis results of associated factors for brucellosis seroprevalence.

Variables	Category	Seropositive	Seroprevalence	P value	95% CI
Sex	Male	0	0%	0.089	0.000-1.765
	Female	20	100%	Ref	Ref
Species	Ovine	5	25%	1.000	0.416- 3.909
	Caprine	15	75%	Ref	Ref
Age	Young	7	35%	0.430	0.228-3.057
	Adult	13	65%	Ref	Ref
Parity	Yes	8	40%	1.000	0.185-2.291
	No	12	60%	Ref	Ref
Abortion history	Yes	18	90%	Ref	Ref
	No	2	10%	0.212	0.409-10.347

Univariate logistic regression analysis result revealed that the associated risk factors that had no significant association with ELISA seropositivity were species (P=1.000), sex (P=0.089), age group (P=0.430), parity (P=1.000) and age (P=0.212) as depicted (Table 3).

Discussion

Human-animal intimacy and close contact are highly common in Ethiopia, particularly in pastoral areas where the livestock are the community’s primary source of income. In many nations around the world, brucellosis is a serious zoonotic disease that causes abortion in naturally infected small ruminants. This disease has an indirect and direct impact on humans and causes significant economic losses [12]. To safeguard the health

of both people and animals, it is important to look at the occurrence of brucellosis in both humans and livestock. According to the results of the current study, the seroprevalence of brucellosis in small ruminants overall in the study district of the Afar region was 6.7% (n=20/300), and the prevalence of the disease at the species level in caprine and ovine animals was 6.5% and 7.2%, respectively. The current seroprevalence result (6.7%) was in accordance with [12], who reported 5.6% from the Afar and Somali regional states, [13], who reported 2.6% seroprevalence in selected settlements of Dire Dawa Administrative Council Area, Eastern Ethiopia, and [14] that estimate 5.4% of small ruminant brucellosis in southern Ethiopia, [15] detected 5.42% from Oromia and Somali regional states; [16] discovered 5.87% in the Amhara regional state; [17] in the Borena zone,

Oromia region with prevalences of 1.17 and 1.88% in sheep and goats, respectively; [7] with a prevalence of 3.3% in small ruminant brucellosis and [19], who reported 6.7% in selected export abattoirs, Ethiopia. The finding of the current study was also comparable with previous studies report from other countries; 5.2% in Nigeria as reported by [20] and others. However, the seroprevalence result of the present study was relatively higher than previous studies by [7], who recorded a prevalence of 2.4% in Zone 1 of the Afar region; [21] detected 0.4% in Bahir Dar; [22], who demonstrated 0.9% in Somali and Oromia; and [22] observed 0.53% in Bale and Boran; [23], who reported 1.7% in goats and 1.6% in sheep in the Somali region; [24] estimated 0.6%; and [25] who reported 1.56% in Jijiga District, Somali Regional State, Eastern Ethiopia.

In contrast to the results of the current study, [26] found a higher seroprevalence of small ruminant brucellosis of 13.6% in Tallalak area of Afar region; and [27] recorded 13.7% in Chifra and Ewa districts of Afar region, Ethiopia; [28] found 12.4%, [29] approximated 16%, and [30] reported 9.11% in a few areas under the Dire Dawa administration. The difference in the seroprevalence of brucellosis among small ruminants between the previous result and the current result could be the result of agro-ecological differences, variation in the composition of the sampled animals, discrepancies in the sensitivity or specificity of the serological test methods used, the type of management employed, the sample size, the breeds of small ruminants owned by these communities, the size of the flocks in the study population, or the reintroduction of from other brucellosis prevalent areas, a difference in sampling method, and other factors. Even though goats were reported to have a higher prevalence of brucellosis than sheep, the difference was not statistically ($P>0.05$) significant. The seroprevalence of brucellosis was, however, substantially ($P<0.05$) greater in goats than in sheep, according to [28,23]. The greater susceptibility of goats to brucella infection than sheep and the fact that sheep do not excrete brucella organisms for longer periods of time than goats, which in turn can reduce the potential for the disease to spread among sheep flocks, may be the causes of the higher prevalence of brucellosis in goats than in sheep.

The current study also indicated that only female sheep and goats had positive serum using I-ELISA. The possible reason that could be fewer males ($n=38$) tested than females ($n=262$) may account for the lack of male seroreactor animals in this study. Moreover, it has been noted that male animals are typically more resistant to *Brucella* infection than female animals [31]. According to [32], the absence of erythritol in male animals makes them less prone to infection. Additionally, it has been shown that male animals' limited serological responses to *Brucella* infection result in testes that are typically non-reactors or have low antibody titers [33]. The seroprevalence of small ruminant brucellosis indicates that adults have a substantially greater seroprevalence than young animals although being statistically insignificant. This could be because following sexual maturity, particularly after pregnancy, susceptibility increases. The presence of erythritol hormones and other substances in the uterus, placenta, and fetal fluids favors the growth of *B. melitensis*, the major organism causing infection in sheep and goats [34]. The results of this study were in agreement with previous studies by [35], who reported a prevalence of 5.3% in adult animals and 1.5% in younger sheep and goats. It has been documented that both sexes of sexually immature animals are more likely to become infected with brucella than their more sexually mature counterparts. Erythritol and sex hormones,

which encourage the growth and multiplication of brucella species organisms, which concentration tends to rise with age and sex maturity, would be the main offenders behind this. Analysis of the current study's data showed no statistically significant association ($P>0.05$) between parities and the disease's seroprevalence. However, according to [36,29] seropositivity in female sheep and goats with a history of no parity may be caused by the female animals being repeatedly exposed to parturition and other physiological stressors, which increases the likelihood that the animals will become infected with *Brucella*.

In the current study, brucella seropositivity was observed more frequently among sheep and goats with a history of abortion (6.3%) than animals that have no history of abortion (1.5%), however, the difference noted was not statistically significant ($P>0.05$) in the seroprevalence of *Brucella* antibodies between aborted and non-aborted sheep and goats. This suggests that brucellosis may not be associated with abortion in sheep and goat in study areas. This could be true as abortion in sheep and goats has multiple infectious causes other than brucellosis. It is widely known that *Brucella* infection seropositivity is not always associated to abortion. To confirm that the pathogen is responsible for abortion, it is crucial to determine the real reasons in the tissues of the aborted fetus or placenta, as well as in the aborting sheep and goats [37]. This result is consistent with the findings of [38-40], who indicated that there was no association between the risk of *Brucella* seropositivity and abortion. This finding, however, contradicts with those of a few other Ethiopian studies [41,42] that asserted brucellosis was associated with abortion in sheep and goats. This variation might be driven on by differences in the regional agro-ecology, breed, management, and husbandry practices. Additionally, there may be variations among the study areas in terms of factors that can facilitate the spread of various causes of abortion [43].

Conclusion

The result of the present study showed that the seroprevalence of small ruminant brucellosis in the study area was relatively low as compared to other finding in Afar region but still relatively higher prevalence compared to other study findings recorded in other regions of Ethiopia. Hence, it is important to carry out further study to identify the circulating *Brucella* species using molecular tools and other potential causes of abortion in sheep and goats. Besides, this study warrants the need for further investigation on *Brucella* infection and its public health impact in addition to reproduction and production effect in the study area. Further studies which cover more districts with increased sample size need to be carried out to know the general small ruminants Brucellosis in the region in particular and in the country in general.

Declaration

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Author contribution indication: TD: Contributed to conception of the research idea, designing and data collection, data analysis, interpretation of data, writing and editing of the manuscript.

TK: Contributed to conception of the research idea, data col-

lection, Methodology, writing and review of the manuscript.

GK: Contributed to conception of the research idea, data collection, methodology, writing review of the manuscript, diagnostic testing and laboratory analysis

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Availability of data and materials: The datasets generated and/or analyzed during the current study are not publicly available due to the confidentiality agreements made all authors, but could be available from the corresponding author on reasonable request.

Ethics approval and consent to participate: Written ethical approval and informed consent for this study was obtained from AHI, Research Ethics and Review committee (reference no: VM/ERC/10/03/12/2022). Written informed consent was also obtained from the herd owners to take samples from their small ruminants and for further research use purpose. The reason for this written informed consent is that, participants were required for interview and the individual participant was not subjected to any harm as much as their privacy is kept confidential. Confidentiality of collected data and the scientific honesty during write up was considered. These written informed consents were documented.

Consent for publication: Not applicable

No coauthors and the author declare that no competing interests.

Conflict of interest: The authors declare they have no competing interests.

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