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Original Article

Seroprevalence and risk factors of Brucellosis in small ruminants slaughtered at Debre Ziet and Modjo export abattoirs, Ethiopia

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Abstract

Introduction: Brucellosis is a global zoonotic disease and major public and animal health problem in many parts of the world, particularly in places where livestock is a major source of food and income. This cross-sectional study was conducted between November 2012 and May 2013 to determine the seroprevalence and assess potential risk factors of brucellosis in small ruminants in five export abattoirs at Debre Ziet and Modjo, Oromia Regional State, Ethiopia.

Methodology: Serology and questionnaire were the methods used. In this investigation, 853 sera samples of 485 caprines and 368 ovines brought for slaughter were selected randomly. The Rose Bengal plate test and complement fixation test were conducted using sera samples at National Animal Health Diagnostic and Investigation Center (NAHDIC) serology laboratory. Data collection sheets were used to gather information on possible risk factors believed to influence the occurrence of *Brucella* infection in small ruminants such as age, species, breed, body condition score, and origin of small ruminants.

Results: Brucellosis was found in 17 (1.99%) and 15 (1.76%) small ruminants using the Rose Bengal plate test and complement fixation test, respectively. The univariate and multivariate logistic regression analysis showed that age and body condition score of the animals were risk factors to *Brucella* infection ($p = 0.008$ and $p = 0.001$, respectively) in small ruminants.

Conclusions: Based on this survey, brucellosis is a potential problem in small ruminants in Ethiopia that should be further explored.

Key words: Brucellosis; CFT Ethiopia; RBPT; seroprevalence; small ruminants.

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Introduction

There are a number of diseases seriously affecting human and animal health as well as animal production. Worldwide, brucellosis is a disease that brings reproductive failure to livestock and serious health problems to humans [1]. Even though brucellosis has been eradicated from developed countries, it remains endemic in many parts of the world, including Latin America, the Middle East, western Asia, some Mediterranean regions, and Africa. Brucellosis is an infectious disease caused by bacteria of the genus *Brucella*, characterized by abortion and infertility in several mammal species, and considered to be one of the most important zoonoses worldwide [2]. Several closely related species of the genus *Brucella* have been recognized, namely*B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. pinnipedialis*, *B. ceti*, *B. microti*, and *B. inopinata* [3-5].

Brucellosis in small ruminants is mainly caused by *B. melitensis*; however, *B. ovis* is also an important cause of orchitis and epididymitis in rams and occasionally infects ewes [6,7]. In addition, a few cases of *B. abortus* and *B. suis* have been reported [8]. This shows that there is a cross-transmission of some *Brucella* species among different livestock species [9]. Generally, brucellosis in caprines and ovines is clinically characterized by the following symptoms: abortion, stillbirth, placental retention, weak offspring, acute orchitis, and epididymitis in males, which may develop into infertility [10].

Despite having a large number of small ruminants compared to other African countries, Ethiopia does not exploit small ruminant production to its full potential. This is due to several factors, among which is the high disease burden that limits the economic return and productivity from small ruminants; brucellosis is one of these diseases [11]. Small ruminant brucellosis is endemic in Ethiopia. Various prevalence rates have been reported: a study conducted between November 2004 and April 2005 in sheep and goats in Afar and Somali pastoral areas reported a rate of 9.7% [12]; a study conducted between December 2005 and June 2006 in a pastoral region of Afar reported rates of 5.8% in goats and 3.2% in sheep [13]; a study

conducted between October 2008 and March 2009 in sheep in south Wollo zone reported a rate of 2.5% [14]; a study conducted between October 2008 and March 2009 in South Omo Zone reported a rate of 4.2% in goats [15]; a study conducted between November 2010 and April 2011 in Dire-Dawa region reported rates of 9.86% in goats and 8.77% in sheep [8]; and a study conducted between November 2009 and April 2010 in Yabello district reported rates of 1.17% in sheep and 1.88% in goats [16].

Though various prevalence studies of brucellosis were carried out in different agro-ecological zones, very few investigative works have been done in abattoirs, where large numbers of animals and breeds of small ruminants are slaughtered daily. Therefore, the objectives of this study were to estimate the seroprevalence of brucellosis and to assess the potential risk factors of *Brucella* infection in small ruminants slaughtered in selected export abattoirs at Debre Zeit and Modjo, Oromia Regional State, Ethiopia.

Methodology

Study design

A cross-sectional study was carried out from November 2012 to May 2013 to determine the seroprevalence of brucellosis and assess the associated potential risk factors in small ruminants in selected export abattoirs, namely Hashim, Elfora, Luna, Organic, and Modjo. To ensure the confidentiality of the participating abattoirs, code numbers were used.

Sample size determination

The sample size was calculated using the method recommended by Thrusfield [16] for simple random sampling. The sample size for small ruminants was determined based on the recent seroprevalence studies of brucellosis in Ethiopia. According to Negash *et al*. [8], prevalence rates of 9.7% and 4.8% in caprines and ovines, respectively, were determined. Accordingly, the minimum sample for ovines and caprines were 134 and 70, respectively, with 95% confidence interval and 5% margin of error. However, to increase the power of the study, the number of ovines and caprines were increased to 368 and 485, respectively.

$$N = \frac{(1.96)^2 \times Pexp\,(1 - Pexp)}{d^2}$$

where:
Pexp: expected prevalence = 9.7% in caprines and 4.8% in ovines
d^2: absolute precision (5%)
CI: confidence interval (95%)

Study animals

The target population was all caprines and ovines presented to be slaughtered in the five export abattoirs during the study period, approximately 6,000 small ruminants, 80% of which were caprines. Overall, 853 small ruminants (368 ovines and 485 caprines) were randomly selected as the study subjects. Only male animals were slaughtered and exported, and eight breeds of caprines and ovines that originated from 10 regions of the country were involved in this study.

Ethical considerations

Ethical clearance was obtained from Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University institutional review board (IRB), and permission was solicited from export abattoirs.

Sampling techniques

The total number of small ruminants to be sampled was allocated to five export abattoirs proportionally, based on the number of animals slaughtered during the study period. Samples were then drawn from the target population randomly during each visit.

Questionnaires

Data collection sheets were used to gather information on the sampled animals' characteristics and origins, including age, species, breed, body condition score, and source. The questionnaire was administered by the principal investigator.

Blood sample collection

Approximately 7–10 mL of blood was collected from the jugular vein of each sampled animal using a plain vacutainer tube (BD vacutate, Oxford, UK) and needles using aseptic techniques. Each vacutainer tube was marked with a permanent marker, recording the case number of the respective study subject and the name of the abattoir from which it originated. The blood samples were put at an inclined position in order to allow clotting for one to two hours at room temperature to get clear serum and to minimize hemolysis of blood. They were then stored overnight horizontally at 4°C, and the serum was separated from the clot by centrifugation at 2,500 rpm for 10 minutes at room temperature. Finally, the serum was transferred to a sterile labeled vial and stored in deep freezer (-20°C) until further testing.

Body condition scoringand age determination

Body condition scoring was used to estimate the small ruminants' muscle and fat depositions. Body

condition scores ranged from 1 (very thin) to 5 (very fat), and were based on feeling around the vertebrae in the loin region [17]. This method can be used by farmers, pastoralists, researchers, and producers to make management decisions regarding the health of their animals and the quality and quantity of feed needed to optimize performance. If animals are in poor body condition, the animals may be underfed or have a disease. If the animal has a good body condition score, the animal is likely healthy and well fed. The ages of the small ruminants were determined based on dentition [18].

Serological tests

Two types of serological tests were employed as a screening and confirmatory test for the detection of *Brucella* antibody: the Rose Bengal plate test (RBPT) and the complement fixation test (CFT), respectively. Both tests were done at the National Animal Health Diagnostic and Investigation Center (NAHDIC).

RBPT was used as a screening test on the serum samples collected for the presence of *Brucella* agglutinins. Equal volumes of test serum and *B. abortus* antigen strain 99(Animal Health and Veterinary Laboratories Agency (AHVLA) Weybridge, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom) (30 µL) were placed in a plate and mixed thoroughly with a toothpick, then rocked for four minutes using both hands. Then the results were interpreted according to the presence and degree of agglutination. Samples with no agglutination (0) were recorded as negative, while those with +, ++, and +++ were recorded as positive. The test was conducted as described previously by other authors [19-21].

Sera tested positive by RBPT were further confirmed using CFT. The complement systems consist of a series of protein that, if triggered by an antigen antibody complex, react in a sequential manner to cause cell lysis. The test has two steps. The first step is antigen; test serum and complement are mixed and incubated. The second step is an indicator system that consists of sheep red blood cells (SRBC) and an amboceptor that sensitizes red blood cells to the action of the complements. If the test serum contains antibodies to *Brucella*, an antigen-antibody complex is formed; the complement is used up, and no lysis of SRBC occurs. If the test serum does not contain *Brucella* antibodies (negative reaction), the complement will not be fixed and lysis of SRBC would occur [19].

Data collection and analysis techniques

Results from serological tests and data collection sheets were coded and stored in Epi Data version 3.1, and then transferred to STATA version 11.0 for data analysis. Positive samples confirmed using CFT were considered seropositive and taken for further data analyses. Categorical data were expressed in percentages, and prevalence was calculated by dividing the number of positive sera samples by the total number of samples examined. Univariate and multivariate logistic regression, odds ratio, 95% confidence interval, and the Chi-square and Fisher's exact tests were computed to determine the degree of association of animal characteristics with *Brucella* seropositivity in small ruminants. For all analysis, a p value < 0.05 was taken as significant.

Results

The results of brucellosis serology are shown in Table 1. Of the 853 sera samples from caprines and ovines screened with RBPT, 17 (1.99%) were found to be positive. Sera samples screened positive were subjected to complement fixation tests, and 1.76% (95% CI: 0.99–2.88) were found to be positive for *Brucella* infection. On a species level, the prevalence of brucellosis in caprines was no different than in ovines. In terms of the age group and body condition scores of the animals, a higher number of *Brucella* infections was observed in older and very thin animals compared to that observed in animals that were younger and in medium condition.

There were no differences in brucellosis seropositivity found between the different abattoirs. Relatively higher rates of *Brucella* infection were observed in Afar goat breeds and animals that originated from the Afar region compared to animals of other breeds and origins; however, this difference was not statistically significant (Table 2).

The results of the univariate logistic regression analysis are summarized in Tables 3 and 4. The ages and body condition scores of the small ruminants showed statistically significant differences as potential risk factors for *Brucella* infection. Animals older than 24 months of age were 10 times more likely to be infected with brucellosis than were animals younger than 12 months of age ($p = 0.001$; OR = 9.909; CI: 2.411–40.724). Similarly, very thin animals were at high risk of *Brucella* infection compared to animals of medium body condition ($p = 0.000$; OR = 10.518; CI: 3.023–36.591).

Table 1. Seroprevalence of brucellosis in small ruminants slaughtered at Debre Zeit and Modjo abattoirs by species, ages, and body condition scores (n = 853)

Variable	N	RBPT positive No. (%)	CFT positive No. (%)	95% CI for CFT	Fisher's exact test p value
Species					
Caprine	485	9 (1.86)	9 (1.86)	0.66–3.06	
Ovine	368	8 (2.17)	6 (1.63)	0.34–2.92	0.804*
Age (in months)					
≤ 12	549	5 (0.91)	4 (0.73)	0.02–1.44	
13–24	245	7 (2.86)	7 (2.86)	0.77–4.94	0.002
> 24	59	5 (8.47)	3 (5.08)	> 0–10.69	
BCS					
Very thin	106	8 (7.55)	7 (6.60)	1.88–11.33	
Thin	148	5 (3.38)	4 (2.70)	0.09–5.32	0.000
Medium	599	4 (0.67)	4 (0.67)	0.16–1.32	
Total	**853**	**17 (1.99)**	**15 (1.76)**	**0.88–2.64**	

*Chi-square; RBPT: Rose Bengal plate test; CFT: complement fixation test.

Table 2. Distribution of *Brucella* seropositivity by breeds and origins of small ruminants slaughtered at Debre Zeit and Modjo (n = 853)

Categories	N	RBPT positive No. (%)	CFT positive No. (%)	95% CI for CFT	Fisher's exact test p value
Abattoirs					
Abattoir 1	95	3 (3.16)	2 (2.11)	-0.01–0.06	
Abattoir 2	192	4 (2.08)	4 (2.08)	0.01–0.07	
Abattoir 3	136	2 (1.47)	2 (1.47)	-0.09–0.05	0.826
Abattoir 4	219	5 (2.28)	5 (2.28)	0.02–0.08	
Abattoir 5	211	3 (1.42)	2 (0.95)	-0.00–0.04	
Breeds					
	Caprines				
Long Ear Somali	222	5 (2.25)	5 (2.25)	0.01–0.03	
Woito-Guji	69	-	-		
Arsi-Bale	72	2 (2.78)	2 (2.78)	-0.02–0.06	
Afar	55	2 (3.64)	2 (3.64)	-0.03–0.07	0.779
Central Highland	35	-	-		
Harerge Highland	32	-	-		
	Ovines				
Black Head Somali	257	7 (2.72)	5 (1.95)	0.02–0.08	
Arsi-Bale	111	1 (0.90)	1 (0.90)	-0.01–0.03	
Origin					
Borana	462	12 (2.60)	10 (2.37)	0.07–0.13	
Arba Minch	35	-	-		
Bale	45	1 (2.22)	1 (2.22)	-0.03–0.05	
Afar	55	2 (3.64)	2 (3.64)	-0.03–0.07	0.968
Ambo	32	-	-		
Dauro	23	-	-		
Dessie	5	-	-		
Ginka	58	1 (1.72)	1 (1.72)	-0.02–0.04	
Measo	32	-	-		
Arsi	99	1 (1.01)	1 (1.01)	-0.02–0.04	
Konso	7	-			

RBPT: Rose Bengal plate test; CFT: complement fixation test.

Table 3. Univariate logistic regression analysis of the association between risk factors and *Brucella* seropositivity in small ruminants slaughtered at Debre Zeit and Modjo abattoirs

Risk factors	Sera examined	CFT positive No. (%)	Odds ratio (OR)	95% confidence interval for OR	P value
Species					
Ovine	368	6 (1.63)	1		
Caprine	485	9 (1.86)	1.141	0.402–3.234	0.804
Age (in months)*					
≤ 12	549	4 (0.73)	1		
13–24	245	7 (2.86)	4.007	1.162–13.818	0.028
> 24	59	4 (6.78)	9.909	2.411–40.724	0.001
Body condition score*					
Medium	599	4 (0.67)	1		
Thin	148	4 (2.70)	4.132	1.021–16.719	0.047
Very thin	106	7 (6.60)	10.518	3.023–36.591	0.000
Total	**853**	**15 (1.76)**			

*included in multivariate regression.

Table 4. Analysis of the association between risk factors and prevalence of brucellosis in small ruminant slaughtered at Debre Zeit and Modjo abattoirs using univariate logistic regression

Risk factors	N	CFT positive No. (%)	Odds ratio (OR)	95% confidence interval for OR	P value
Breeds					
	Caprines				
Long Ear Somali	222	5 (2.25)	1		
Woito-Guji	69	-	-	-	-
Arsi-Bale	70	2 (2.78)	1.240	0.235–6.533	0.800
Afar	53	2 (3.64)	1.638	0.309–8.675	0.562
Central Highland	35	-	-	-	-
Harerge Highland	32	-	-	-	-
	Ovines				
Black Head Somali	257	5 (1.95)	0.861	0.246–3.014	0.815
Arsi-Bale	111	1 (0.90)	0.395	0.046–3.419	0.399
Orgin					
Borana	462	10 (2.37)	1		
Arba Minch	35	-	-		
Bale	45	1 (2.22)	1.027	0.128–8.230	0.980
Afar	55	2 (3.64)	1.706	0.099–6.323	0.494
Ambo	32	-	-		
Dauro	23	-	-		
Dessie	5	-	0.793	0.099–6.322	0.826
Ginka	58	1 (1.72)	-		
Measo	32	-	-		
Arsi	99	1 (1.01)	0.461	0.058–3.655	0.452
Konso	7		-		
Total	**853**	**15 (1.76)**			

Table 5. Multivariate logistic regression analysis between potential risk factors associated with small ruminant brucellosis slaughtered at Debre Zeit and Modjo abattoirs

Risk factors	N	CFT positive No. (%)	Odds ratio (OR)	95% confidence interval for OR	P value
Age (in months)					
< 12	549	4 (0.73)	1		
12–24	245	7 (2.86)	3.750	1.076–13.066	0.038
> 24	59	4 (6.78)	7.077	1.666–30.072	0.008
Body condition score					
Medium	599	4 (0.67)	1		
Thin	148	4 (2.70)	3.786	0.927–15.458	0.064
Very thin	106	7 (6.60)	8.747	2.462–31.073	0.001
Total	**853**	**15 (1.76)**			

However, a statistically significant difference was not observed among risk factors of species, abattoirs, breeds, and origins of small ruminants with *Brucella* seropositivtiy ($p > 0.05$).

The results of multivariate logistic regression analysis are shown in Table 5. Age and body condition score of small ruminants showed statistically significant differences as risk factors for *Brucella* infection. Small ruminants older than 24 months of age and of very thin body condition exhibited higher odds of *Brucella* infection than did young and medium body condition animals, respectively ($p = 0.008$; OR = 7.201; CI: 1.684–30.785 and $p = 0.001$; OR = 9.059; CI: 2.504–32.766).

Discussion

In developing countries such as Ethiopia where there is a huge population of livestock and a very high portion of the human population live in rural areas, investigating the status of brucellosis both in livestock and humans is of paramount importance to safeguard public and animal health. As in other developing countries, brucellosis has not been brought under control in Ethiopian livestock, which might be due to mismanagement of animal quarantine, trans-boundary animal movement, lack of eradication and vaccination programs, and lack of awareness of the disease among pastoralists, farmers, and the general public [22]. The current study resulted in serological evidence of brucellosis in slaughtered small ruminants in Debre Zeit and Modjo export abattoirs.

The overall seroprevalence of brucellosis in small ruminants was found to be 1.76%; on a species level, the prevalence of caprine and ovine brucellosis was 1.86% and 1.63%, respectively. Similar findings were reported by Teshale *et al*. [12], with prevalence rates of 1.2% and 1.9%, and by Bekele *et al*. [23], with prevalence rates of 1.2% and 1.6% in ovines and caprines in Somali pastoral regions, respectively. This might be due to similar sources of small ruminants, since most small ruminants slaughtered in the export abattoirs came from pastoral and agro-pastoral areas of the country. Boukary *et al*. [24] reported an overall true brucellosis prevalence of 3.6% in sheep in Nigeria; Negash *et al*. [8] reported a prevalence of 9.39% in caprines and 8.77% in ovines in Dire-Dawa regions; similarly, Ashenafi *et al.* [13] observed prevalence rates of 3.2% and 5.8% in ovines and caprines in the pastoral areas of the Afar region, respectively. All export abattoirs in Ethiopia slaughtered and exported only male animals who were relatively young and healthy. Therefore, the lower prevalence rates found in this study could be because of the male sub-population investigated. Males are less susceptible to brucellosis due to the absence of carbon 4-sugar erythritol, which stimulates the growth and multiplication of *Brucella* organisms [25,26]. In addition, the serological response of male animals is limited; therefore, *Brucella*-infected animals are usually observed to be non-reactors or show low antibody titers [27]. Nonetheless, the relatively young and healthy animals might undermine the prevalence of *Brucella* seropositivity.

In this investigation, seroprevalence of brucellosis was not significantly higher in caprines (1.86%) than in ovines (1.63%). Similar findings were reported by Ashenafi *et al*. [13] and Bekele *et al.* [23]. However, preference for specific hosts was recognized in *Brucella* species; caprines were the primary hosts for *B. melitensis* [28]. Moreover, caprines excrete *Brucella* organisms for a longer period and in greater amounts than do ovines, which could create a favorable condition for further spread of infection within caprine flocks [28]. The similarity of prevalence in both species found in this study are probably due to the exposure to similar sources of

infection and management, as small ruminants flocks are often mixed.

Similar brucellosis seroprevalence was observed in small ruminant breeds that came from pastoral and agro-pastoral communities, and no statistically significant difference was found between breeds. This might be due to the large number of different breeds of small ruminants owned by these communities, which graze together in communal grazing lands or use the same watering points.

Using univariate and then multivariate logistic regression analysis, only ages and body condition scores of the animals showed statistically significant differences in risk factors for *Brucella* infection. Ovines and caprines older than 24 months of age were seven times more likely to get *Brucella* infection than were animals younger than 12 months of age. This observation was consent with the findings of Ashenafi *et al*. [13], Ashagrie *et al*. [15], and Bekele *et al*. [23]. The possible explanation is that older animals could have greater chances of exposure to infected herds or animals [29]. Furthermore, younger animals might be more resistant to *Brucella* infection [28,30,31].

Statistically significant differences were also observed between the prevalence of brucellosis and the body condition scores of the animals. Very thin animals were eight times more prone to infection with *Brucella* than were animals of medium body conditions. This might be due to either the possible association of higher susceptibility to other infectious diseases when the animals are already infected with brucellosis, or to the weakening effect of *Brucella*, both leading to loss of body weight [32].

Export abattoirs in Ethiopia are obliged to slaughter and export only male, young, and relatively healthy animals. Because female animals were excluded, the results of this seroprevalence study are not representative of the general small ruminant population of the region; it should be noted that brucellosis is an important risk in pregnancy.

Conclusions

This study showed that the prevalence of brucellosis in male small ruminants was very low compared with other studies in the country. However, the presence of seropositive animals not only shows the presence of the disease in export abattoirs, it also indicates the presence of foci of infection in several regions of origin of the exported animals. Therefore, a comprehensive surveillance system and efficient control methods (vaccination) would be beneficial to the livestock and export sector to limit further distribution of the disease. The priority in Ethiopia should be control, prevention, and disease surveillance, as in other endemic countries. A high degree of public participation is an important factor for successfully controlling the disease. Obtaining disease information and educating the community will help to increase awareness of the disease, increase community participation, and promote acceptance of control measures in livestock. Control and preventive measures may not be understood or accepted by traditional livestock communities because they may interfere with their lifestyle, food habits, and farming practices. Therefore, proper understanding of the causes and risks of brucellosis and of the benefits of preventive measures is essential for the acceptance of such measures [33]. Brucellosis is a public health concern, and protection of workers at abattoirs and awareness of farmers and consumers is also desirable to prevent transmission to humans.

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