Seroepidemiology of Ovine Brucellosis in East and West Shewa Zones of Oromia Regional State, Central Ethiopia

Article *in* Journal of Veterinary Science and Technology · January 2015

DOI: 10.4172/2157-7579.1000265

1 author:

Endrias Gebremedhin
Ambo University
60 PUBLICATIONS **710** CITATIONS

SEE PROFILE

Veterinary Science & Technology

Gebremedhin, J Veterinar Sci Technol 2015, 6:6
http://dx.doi.org/10.4172/2157-7579.1000265

Research Article Open Access

Seroepidemiology of Ovine Brucellosis in East and West Shewa Zones of Oromia Regional State, Central Ethiopia

Endrias Zewdu Gebremedhin*

Ambo University, College of Agriculture and Veterinary Sciences, Department of Veterinary Laboratory Technology, Ambo-19, Ethiopia

***Corresponding author:** Endrias Zewdu Gebremedhin, Ambo University, College of Agriculture and Veterinary Sciences, Department of Veterinary Laboratory Technology, Ambo-19, Ethiopia, Tel: +251 11 236 2006; E-mail: endrias.zewdu@gmail.com

Rec date: Aug 17, 2015; **Acc date:** Oct 14, 2015; **Pub date:** Oct 16, 2015

Abstract

Background

A seroepidemiological study of ovine brucellosis was carried out in Ada'a-Liben, Ambo and Fentale districts of Central Ethiopia from November 2010 to May 2012. A cross-sectional two stage cluster sampling method was used in order to collect 1119 sera samples from 227 flocks. Additionally, a questionnaire survey was conducted to collect information about risk factors. Modified Rose Bengal Plate Test (mRBPT) and Complement Fixation Test (CFT) were used as screening and confirmatory tests, respectively. A logistic regression was used to compute the odd ratios associated with potential risk factors.

Results

Overall, the results revealed that 16.74% (95% Confidence interval [CI]: 11.85, 21.63) and 3.57% [95% CI: 2.49, 4.66] of the tested flocks and animals, respectively, had antibodies against *Brucella* sp. by CFT. The highest animal level seroprevalence was recorded from Fentale district (4.97%) followed by Ada'a-Liben (3.0%) and Ambo (2.09%) districts. Univariable logistic regression analysis of potential risk factors revealed that district, breed, still birth and neonatal losses were significantly associated with brucella seroprevalence at both individual animal and flock level ($P<0.05$). Multivariable logistic regression model revealed history of still birth as an independent predictors of seropositivity at individual animal level (adjusted Odds ratio [aOR]=2.55, 95% CI: 1.19, 5.45; $P=0.016$). Of the variable offered to the multivariable model (district, history of still birth and neonatal losses), none of them were found to be independent predictors of flock level seropositivity ($P>0.05$).

Conclusions

Ovine brucellosis is endemic at moderately high prevalence in the study areas. History of still birth was significantly associated with ovine brucellosis. Further epidemiological studies that include isolation, biotyping and molecular identification of *Brucella* sp. and education of people are suggested for better control.

Keywords: Central Ethiopia; CFT; Ovine brucellosis; MRBPT; Risk factor; Seroepidemiology of ovine brucellosis

Introduction

Sheep are important for mutton, wool and milk production throughout the world [1]. In Ethiopia there are an estimated 26.12 million sheep [2]. Brucellosis in goats and sheep is normally caused by a Gram-negative coccobacillary rod, *Brucella melitensis* (biovars 1, 2 or 3) although *Brucella abortus* may also cause clinical brucellosis. The disease is characterized by abortion in late pregnancy and subsequent high rate of infertility [3,4]. Brucellosis, especially caused by *Brucella melitensis*, remains one of the most common diseases with major veterinary and public health significance worldwide with more than 500,000 human cases reported annually [4-6]. The primary routes of transmission of ovine brucellosis are the placenta, fetal fluids and vaginal discharges excreted by infected ewes during abortion or full-term parturition. Shedding of brucella is also common in udder secretions and semen [4,5]. Sheep and goats and their products are one of the sources of infection for humans. Infection of humans' takes place through contact with infected animals or consumption of their products, mostly milk and milk products, especially cheese made from unpasteurized milk of sheep and goats and rennet from infected lambs and kids [6].

Although brucellosis is controlled from a number of industrialized nations by routine surveillance, vaccination and stumping out, the disease continues to be a major public and animal health problem in many regions of the world [4,7,8]. Brucellosis is a major animal health as well as public health problem in Ethiopia; particularly among pastoral communities due to low awareness of the disease, culture of raw milk consumption and close contact with animals [9]. Serological evidence of brucella infection in cattle, sheep, goat, camels and humans has been reported from different regions of the country. Table 1 summarized prevalence results of ovine brucellosis of past studies in different regions of Ethiopia. These findings lack geographic representativeness across the different agro-ecological areas. Moreover,

considering the huge sheep population and economic and public health impacts of the disease, epidemiological knowledge is still inadequate towards overall understanding and subsequent control programs of brucellosis in Ethiopia. The objectives of the present study were to estimate the seroprevalence of ovine brucellosis and its associated risk factors in Ambo, Ada'a-Liben and Fentale districts of Central Ethiopia.

Area	Test used	Number Tested	Prevalence (%)	References
Afar, eastern Ethiopia	CFT	563	3.2	[10]
Afar and Somali pastoral area of eastern Ethiopia	I-ELISA	928	5.6	[11]
South Wollo, North Eastern Ethiopia	CFT	800	1.5	[12]
In and around Debre Birhan region, Ethiopia	CFT	384	1.3	[13]
In and around Bahir Dar, Northwest Ethiopia	CFT	270	0.0	[14]
Jijiga district, Somali Regional State, Eastern Ethiopia	CFT	421	1.2	[15]
Central highlands of Ethiopia	RBPT	1507	1.5	[16]
Selected sites of Dire Dawa region, Eastern Ethiopia	CFT	171	8.77	[17]
Adama (Boku sheep export Farm), (origin Adama), Central Ethiopia	CFT	662	0.91	[18]
Adama (Boku sheep export Farm), (origin Arsi) Central Ethiopia	CFT	630	0.63	[18]
Adama (Boku sheep export Farm), (origin Bale), Southeastern Ethiopia	CFT	738	0.41	[18]
Southern Zone of Tigray Region, Northern Ethiopia	CFT	490	1.4	[19].

Table 1: Seroprevalence of ovine brucellosis in Ethiopia. CFT=Complement Fixation Test; RBPT=Rose Bengal Plate Test; I-ELISA=Indirect-Enzyme Linked Immunosorbent Assay.

Materials and Methods

Description of the study districts and population

The study was conducted in three districts of Oromia Regional State, Central Ethiopia, where there is no history of brucellosis vaccination. Ambo, Ada'a-Liben and Fentale districts were purposively chosen as study districts to represent the highland, midland and lowland agro-ecologies of Oromia Regional State, respectively. The districts are separated from each other by 150 to 289 kms. The altitude in meters above sea level (masl), population and climatic data of the study districts were depicted on Table 2.

Sampling district	Location	Altitude (masl)	Rainfall (mm)	Annual Temp (oC)	Sheep Population	No. sampled
Ambo	37°32'- 38°3'E 8°47' – 9°20'N	1400-3045	800-1000	15 - 29	52714	382
Ada'a-Liben	38°38'E 08°44'N	1500 - >2000	839 (mean)	7.9 - 28	55305	233
Fentale	36023'– 39054'E 8o54'N	955 - 2007	553 (mean)	29 - 38	69482	504
Total					177501	1119

Table 2: Basic data of Ambo, Ada'a-Liben and Fentale districts. Source: IPMS [20], CSA [2]; Anonymous [21]; masl=meters above sea level.

Fentale district has an arid to semi-arid climate and the production system is predominantly pastoral and agro-pastoral. Sedentary farming dominated by extensive type of management system is a feature of the highlands and midlands of Ambo and Ada'a-Liben districts. However, semi-intensive farming is practiced in some urban and peri-urban areas.

Afar, Arsi-Bale and Horro breeds of sheep predominate in Central Ethiopia. Sheep are kept for mutton production in most parts of the country; however, pastoralists in Fentale district also use sheep for milk production. In this study, sheep of both sexes above six months old were included.

Study design and sample size

A cross-sectional study with a two-stage cluster sampling design was carried out from November 2010 to May 2012 in order to estimate the flock and individual animal level seroprevalence of ovine brucellosis. Peasant associations (PA's) within each district were purposively selected based on farmers' willingness, logistics and accessibility. An expected prevalence of 8.77% [9] and 3% absolute precision were used to get the calculated sample size (n=342) followed by a nearly three times inflation. This is because of the absence of variance data between clusters and our interest of having a more precise estimate [10,11]. The required sample size (1119) was allocated to each district proportionally based on their sheep population. The number of sheep flocks (227) to visit was determined by dividing the total sample size (Table 2) with the number of sheep to be sampled within each flock (five). For sample size calculation the average number of sheep per household suitable for sampling (≥ 6 months) was assumed to be five. These flocks were selected using list of willing household heads as sampling frame which was recorded during the initial meeting held to identify households willing to participate in the study. In a flock with ≤ five sheep, all were sampled. However, from a flock comprised of more than five sheep a random sample of five animals were selected.

Blood collection and serum separation

Approximately 5 ml of whole blood samples were collected by venipuncture from the jugular vein using disposable plain vacutainer tubes and needles (BD Vacutainer Systems, Plymouth, UK). The blood samples were allowed to clot and then centrifuged at 2250 x g for 5 min. The serum was collected into 1.5 ml Eppendorf tubes (Eppendorf-AG, Hamburg, Germany) and transported to the College of Veterinary Medicine and Agriculture, Debre-Zeit, using an ice box and stored at -20°C until serologically tested.

Questionnaire survey

A close-ended questionnaire was developed and filled in for each flock by interviewing flock owners or herders during sampling in order to assess potential risk factors for ovine brucellosis. Those included sex (male, female), age, altitude (highland ≥ 2300, midland 1500-2300, lowland ≤ 1500 masl), flock size (large ≥ 50, small<50 animals), production system (sedentary, agro-pastoral, pastoral), breed (Horro, Arsi-Bale, Afar), type of management (extensive: free ranging without supplementary feed; semi-intensive: supplementary feed provided), source of water (tap, river, stagnant [pond, lake, well], mixed), residential place (urban, peri-urban, rural), presence of dogs (yes, no), presence of goats (yes, no), abortion (yes, no), still birth (yes, no), neonatal loss (yes, no) and sanitation (poor, fair). Animal age determination was made based on dentition [12] and herders' information.

Modified rose bengal plate test (mRBPT)

All the collected serum samples were tested for the presence of antibodies against ovine brucellosis following the protocol of the OIE [4,13]. In order to improve the sensitivity of the RBPT and minimizes the discrepancies between RBPT and Complement Fixation Test (CFT) results, we used three volumes of serum and one volume of antigen (e.g. 75 µl and 25 µl, respectively) in place of an equal volume of each as recommended by OIE [4]. Thus, mRBPT was employed for screening purpose. After mixing of test and control sera with the antigen the plates were gently shaken by hand for about 4 minutes. The results were interpreted according to Nielson and Punkan [14], "0" as negative (No agglutination), "+" (Barely perceptible agglutination), "+ +" (Fine agglutination and some clearing) and "+++" (Course clumping, definite with clearing)

Complement fixation test (CFT)

Modified rose Bengal plate test positive sera were stored at -20°C until tested by CFT for confirmation. The protocol described by Mac Millan [21] which uses standard *B. abortus* antigen (Veterinary Laboratories Agency, Addlestone, United Kingdom), Amboceptor (Biomerieux, France), 2% sheep red blood cell (RBC), and positive and negative control antisera was used. The complement was obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany. Sera with strong reaction at dilution of 1:5 with a strong reaction of approximately 100% fixation of the complement (4+), more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least 50% fixation of complement (2+) at a dilution of 1:10 and 1:20 were classified as positive [13]. The test was done at the National Veterinary Institute (NVI) at Debre-Zeit, Ethiopia.

Data management and analysis

The data gathered through the questionnaire survey and laboratory testing was stored in Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for windows (Stata Corp. College Station, USA). The categories of the variables were: Altitude (lowland, mid land, high land), sex (male vs. female), age (young vs. adult), flock size (large vs. small taking 50 as a cut-off), management type (extensive vs. semi-extensive), residential place (urban vs. rural), source of drinking water (stagnant, river, tap, mixed), presence of goats in household (yes vs.no), presence of dogs (yes vs. no), history of abortion (yes vs. no), still birth (yes vs. no) and neonatal losses (yes vs. no). Hence, all variables were handled as categorical variables. During the statistical analysis, for all the risk factors, the first level of each independent variable was used as a reference category. Variables with more than two categories were transformed into indicator (dummy) variables. A sheep was considered brucella seropositive provided that both mRBPT and CFT gave positive result. Flocks containing at least one seropositive animal were considered positive. Seroprevalence was calculated by dividing the total number of sheep tested positive by CFT by the total number of sheep tested. Similarly, flock-level seroprevalence was calculated as the number of flocks with at least one positive animal by CFT divided by the total number of flocks tested. Chi-square test was used to assess association between seropositivity and explanatory variables. A logistic regression was used to compute the odd ratios associated with potential risk factors. During the analysis the clustering nature of the outcome within flock was considered by including flock as a clustering variable. This enabled us to use clustered sandwich estimator i.e., robust standard error rather than the standard error of the parameters estimated using maximum likelihood method. Non-collinear variables with $P<0.20$ in the univariable analysis were included to the multivariable model. The 95% confidence level was used and results were considered significant at $P \leq 0.05$.

Results

Overall prevalence

Ovine brucellosis was detected by CFT in 16.74% (38/227; 95% CI; 11.85, 21.63) of the sheep flocks investigated. However, in spite of the relatively higher number of flocks affected by brucellosis only 3.57% (40/1119; 95% CI: 2.49, 4.66) of sheep screened gave positive result for CFT. Although statistically not significant (P>0.05), higher animal level (4.96%) and flock level (22.55%) seroprevalence was detected in Fentale district as compared to Ambo district (Table 3).

Districts	Animal level seroprevalence					Flock level seroprevalence		
	Tested	*mRBPT		CFT		CFT		
		Pos. (%)	95% CI	Pos. (%)	95% CI	Tested	%	95% CI
Ambo	382	10 (2.62)	1.01, 4.22	8 (2.09)	0.65, 3.53	78	10.26	3.44, 17.07
Ada'a-Liben	233	7 (3.00)	0.81, 5.20	7 (3.00)	0.80, 5.20	48	14.89	4.55, 25.24
Fentale	504	32 (6.35)	4.22, 8.48	25(4.97)	3.06, 6.86	101	22.55	14.36, 30.74
Total	1119	49 (4.38)	3.18, 5.58	40(3.57)	2.49, 4.66	227	16.74	11.85, 21.63

Table 3: Animal and flock level seroprevalence of ovine brucellosis in Ambo, Fentale and Ada'a-Liben districts, Central Ethiopia. *statistically significant, Pearson χ^2=8.5538, P=0.014, Pos.=positive, CI=Confidence Interval, mRBPT=Modified Rose Bengal Plate Test.

Brucella infection at "*kebele*" (smaller administrative unit of a district) level showed that 3 of 10 (30%) kebeles from Ambo, 3 of 4 (75%) kebeles from Ada'a-Liben and 7 of 7 (100%) kebeles from Fentale districts contain at least one seropositive animal.

Risk factor analysis

Results of univariable and multivariable logistic regression analyses of animal level seroprevalence of Brucella infection were summarized below in Table 4. District, breed, still birth and neonatal loss were significantly associated with brucella seropositivity at animal level (P<0.05) by univariable analysis. Multicollinearity (r ≥ 0.5) was observed between some of the potential risk factors investigated; namely presence of goats vs district (0.7), altitude vs district (0.94), altitude vs presence of goats (0.71), breed vs district (0.99), breed vs altitude (0.94), breed vs presence of goats (0.72), production system vs district (0.82), production system vs presence of goats (0.72), production system vs altitude (0.80) and production system vs breed (0.82). Of the collinear variables those expected to have biological relation with brucellosis were selected for multivariable analysis (district). Accordingly, district, history of still birth and neonatal losses were included in the multivariable model. In the final model history of still birth was retained as independent predictor P<0.05 (Table 4).

Variables		*n	CFT pos. (%)	Univariable		Multivariable	
				cOR (95% CI)	P	aOR (95% CI)	P
District	Ambo	383	8 (2.09)	1.00	-	-	
	Ada'a-Liben	233	7 (3.00)	1.45 (0.55, 3.85)	0.454	1.29 (0.48, 3.48)	0.620
	Fentale	503	25 (4.97)	2.45 (1.13, 5.34)	0.024	1.63 (0.63, 4.27)	0.315
Altitude	Highland	273	6 (2.20)	1.00			
	Midland	343	9 (2.62)	1.20 (0.44, 3.25)	0.721		
	Lowland	503	25 (4.97)	2.33 (0.98, 5.55)	0.057		
Breed	Horro	379	8 (2.11)	1.00			
	Arsi-Bale	240	9 (3.75)	1.81 (0.69, 4.76)	0.231		
	Afar	500	23 (4.60)	2.24 (1.03, 4.87)	0.043		
Sex	Female	911	31 (3.40)	1.00			
	Male	208	9 (4.33)	1.28 (0.57, 2.90)	0.548		
Age	Adult(≥1 yr)	886	30 (3.39)	1.00			
	Young(<1yr)	233	10 (4.29)	1.28 (0.64, 2.56)	0.485		

Presence of goats	No	582	17 (2.92)	1.00			
	Yes	537	23 (4.28)	1.49 (0.79, 2.79)	0.216		
Flock Size	Small	625	22 (3.52)	1.00			
	Large	494	18 (3.64)	1.04 (0.56, 1.93)	0.910		
Manage-ment	Extensive	896	30 (3.35)	1.00			
	Semi-int.	223	10 (4.48)	1.36 (0.65, 2.83)	0.419		
Residential place	Urban& P	274	8 (2.92)	-			
	Rural	845	32 (3.79)	1.31 (0.57, 2.99)	0.524		
Water source	Mixed	80	2 (2.50)	1.00			
	Stagnant	167	5 (2.99)	1.20 (0.25, 5.89)	0.817		
	River	816	31 (3.80)	1.54 (0.39, 6.09)	0.538		
	Tap	56	2 (3.57)	1.44(0.13, 15.65)	0.762		
Production system	Sedentary	620	17 (2.75)	1.00			
	Agropast.	305	15 (4.92)	1.83 (0.91, 3.69)	0.089		
	Pastoral	194	8 (4.12)	1.53 (0.68, 3.42)	0.306		
Presence of dogs	No	534	17 (3.18)	1.00			
	Yes	585	23 (3.93)	1.24 (0.67, 2.31)	0.488		
Abortion	No	750	27 (3.60)	1.00			
	Yes	161	4 (2.48)	1.47 (0.53, 4.06)	0.462		
Still birth	No	694	16 (2.31)	1.00			
	Yes	217	15 (6.91)	3.15 (1.60, 6.18)	0.001	2.55 (1.19, 5.45)	0.016
Neonatal loss	No	572	15(2.62)	1.00			
	Yes	339	16 (4.72)	1.84 (0.93, 3.64)	0.080	1.05 (0.45, 2.47)	0.915
Farm sanitation	Fair	106	4 (3.77)	1.00			
	Poor	1013	36 (3.55)	1.06 (0.31, 3.65)	0.921		

Table 4: Results from logistic regression analysis on the predictors of animal level ovine brucellosis in Ambo, Ada'a-Liben and Fentale districts, Central Ethiopia. *n=number tested, a Total number of sheep tested, cOR=crude Odds Ratio, aOR=adjusted Odds Ratio, yr=year, CI=Confidence Interval, Semi-int.=semi-intensive, Urban &P=Urban and peri-urban, agropast=agropastoral.

At flock level, univariable logistic regression analysis showed that sheep flocks from Fentale district were 2.55 times more likely to have at least one seropositive animal as compared to flocks of Ambo district (P=0.035). Flocks with history of still birth and neonatal losses were 2.89 and 2.25 times, respectively, more likely to have seropositive sheep as compared to those flocks with no history of still birth (P=0.005) and neonatal losses (P=0.025). Significant association was not observed between brucella seropositivity and: altitude, presence of goats, flock size, management, residential place, source of water, production system, presence of dogs, history of abortion and farm sanitation (P>0.05) (Table 5). The following variables were collinear: altitude vs district (0.94), presence of goats' vs district (0.7), history of neonatal losses with: altitude (0.55), production system (0.55) and presence of goats (0.52), production system with: district (0.82), altitude (0.8) and presence of goats (0.72). Only district, history of neonatal losses and still birth were fit for multivariable logistic regression model, the rest failed due to either collinearity or high univariable P-value (P>0.25). None of these variables were found to be independent predictors of flock level seropositivity (P>0.05) (Table 5).

Discussion

The study was conducted with the intention of estimating seroprevalence and potential risk factors for acquiring ovine brucellosis. The overall seroprevalence (3.57%, 95% CI: 2.49, 4.66) recorded in the present study in the absence of brucella vaccination program in Ethiopia indicates that the disease is endemic at moderately high level. The result of the present study (3.57%) is in accordance with the findings of Ashenafi et al. [10]. In contrast, lower seroprevalence [15-21] have been reported previously. Higher

seroprevalences have also been reported previously from pastoral areas of Eastern Ethiopia (Afar, Somali and Dire Dawa regions) [9,22]. Recently higher prevalence (22.8%) of caprine brucellosis was reported in pastoralist communities adjacent to Awash National Park, Ethiopia [23].

Variables		**n	CFT pos. (%)	Univariable		Multivariable	
				cOR(95% CI)	P	aOR (95% CI)	P
District	Ambo	78	8 (10.26)	1.00		-	
	Adea	47	7 (14.89)	1.53 (0.52, 4.55)	0.443	1.47 (0.49, 4.44)	0.490
	Fentale	102	23 (22.55)	2.55 (1.07, 6.07)	0.035	1.39 (0.43, 4.47)	0.577
Altitude	Highland	55	6 (10.91)	1.00			
	Midland	70	9 (12.86)	1.20 (0.40, 3.63)	0.740		
	Lowland	102	23 (22.55)	2.38 (0.90, 6.25)	0.080		
Presence of goats	No	118	16 (13.56)	1.00			
	Yes	109	22 (20.18)	1.61 (0.80, 3.27)	0.185		
Flock Size	Small	128	21 (16.41)	1.00			
	Large	99	17 (17.17)	1.06 (0.52, 2.13)	0.879		
Management	Extensive	181	29 (16.02)	1.00			
	Semi-int.	46	9 (19.57)	1.27 (0.56, 2.93)	0.567		
Residential place	Urban& P	56	7 (12.50)	1.00			
	Rural	171	31 (18.13)	1.55 (0.64, 3.75)	0.331		
Water source	Tap	11	1 (9.09)	1.00			
	Mixed Stagnant	16 33	2 (12.50) 5 (15.15)	1.43 (0.11, 18.10) 1.79 (0.18, 17.29)	0.783 0.617		
	River	167	30 (17.96)	2.19 (0.27, 17.84)	0.464		
Production system	Sedentary	126	16 (12.70)	1.00			
	Agropast.	62	14 (22.58)	2.01 (0.91, 4.44)	0.086		
	Pastoral	39	8 (20.51)	1.77 (0.69, 4.54)	0.232		
Presence of dogs	No	107	17 (15.89)	1.00			
	Yes	120	21 (17.50)	1.12 (0.56, 2.27)	0.746		
Abortion	No	113	18 (15.93)	1.00			
	Yes	114	20 (17.54)	1.12 (0.56, 2.26)	0.745		
Still birth	No	173	22 (12.72)	1.00			
	Yes	54	16 (29.63)	2.89 (1.38, 6.04)	0.005	2.12 (0.88, 5.14)	0.095
Neonatal loss	No	139	17 (12.23)	1.00			
	Yes	88	21 (23.86)	2.25 (1.11, 4.56)	0.025	1.50 (0.56, 4.03)	0.425
Farm sanitation	Fair	23	3 (13.04)	1.00			
	Poor	204	35 (17.16)	1.38 (0.39, 4.91)	0.619		

Table 5: Results from logistic regression analysis on the predictors of flock level ovine brucellosis in Ambo, Adea and Fentale districts, Central Ethiopia. **n=number of flocks tested.

The variation in prevalence of ovine brucellosis between the present study and that of the aforementioned studies might be related to variation in management practices and hygiene [9], population density and mixing of herds of different ruminant species [19,24], agro-ecology and sensitivity of serological tests employed [22,25].

Univariable logistic regression showed that the odds of acquiring brucellosis in Fentale district is 2.5 times higher as compared to sheep in Ambo district (P=0.024), however, significant difference between districts was not evident in the final model. In agreement with this finding, Ashenafi et al. [26] also found no significant difference in prevalence of small ruminant brucellosis between study districts in Afar region, eastern Ethiopia. Of the investigated "kebeles" 100% (7/7) in Fentale, 75% (3/4) in Ada'a-Liben and 30% (3/10) in Ambo districts contain at least one brucella seropositive sheep. This indicates that brucella infection has marked difference in spacial distribution among "kebeles" of the study districts since the infection is widely distributed in "kebeles" of Fentale and Ada'a-Liben districts as compared to Ambo district. The relatively higher seroprevalence in Fentale district (4.97%) (where raw milk consumption is major food for pastoral communities) as compared to Ambo (2.09%) and Ada'a-Liben (3.0%) districts could be partly ascribed to the relatively larger flock size as well as animal density in Fentale district which contributes for close contact between infected and non-infected animals at watering points, communal grazing areas and in house /enclosures at night. Furthermore, the free movement of sheep from one area to another area [5,8], limited veterinary support services and husbandry practices [8], absence of systematic culling program (leading to retention of perhaps seropositive sheep thereby favoring spread) and general poor sanitary practices might have additionally contributed for the relatively higher seroprevalence in Fentale district.

Although brucellosis is primarily a disease of sexually mature animals [5], in the current study, significant difference in seropositivity was not found between sexually mature (adult) and immature sheep (young) (X^2 = 0.4392 (1), P = 0.508) which is in agreement with the reports of Negash et al. [9]. However, unlike the present finding, significantly high seroprevalence was reported in sexually mature (adult) than young sheep [9,19,27-29].

In accord with the reports of Ashenafi et al. [27], Teshale et al. [22], Yesuf et al. [28] and Bekele et al. [19], there was no significant difference in the seroprevalence of brucellosis between female (3.4%) and male (4.33%) sheep.

In the present study, significant association between *Brucella seropositivity* and breed of sheep was found; in that Afar breed of sheep was 2.23 times more likely to acquire brucella infection as compared to Horro breed of sheep. Breed of an animal may affect susceptibility in sheep. The milking breeds seem to be the most susceptible to *B. melitensis* [29,30].Variation in susceptibility between different breeds of sheep has been noted [31].

Previous studies from Ethiopia reported that there was significant association (P<0.05) between seropoistivity to brucellosis and history of previous abortion in sheep [32]. The detection of 3.57% seropositivity in the current study is a good evidence for the presence and circulation of brucella infection among indigenous sheep flocks and that seronegative animals are at high risk of acquiring the infection or are within the incubation period of the disease. Muscle tissue usually contains low concentrations of brucella organisms but liver, kidney, spleen, udder and testis may contain much higher concentrations. In Ethiopia, dishes prepared from liver and kidney, which may contain much higher concentration of *Brucella sp* [30] are eaten raw or undercooked in some places while consumption of raw sheep milk is practiced among pastoral communities. Moreover, close contact between animals and humans is common, and adequate care is not taken by farmers while handling aborted fetuses and discharges. In Ethiopia, *Brucella* sp. has never been isolated from specimens and attempt to contain the disease is very much limited. On the other hand *Brucella abortus biovar* 6 was isolated recently in Kassala State (Eastern Sudan) from a mRBPT seropositive ewe suffering from pyometra [32]. From the questionnaire survey it was evident that majority of sheep herders and owners have poor knowledge about the importance of hygiene and good husbandry practices as a cheapest means of prevention of brucellosis. Thus, brucellosis might pose considerable public health problems in the study areas.

The short-comings of the study include failure to address some risk factors the way brucellosis is spread between flocks. These factors includes movement of sheep flock, frequency of contact with other sheep flocks at pasture and watering points, introduction of new animals into herd and handling of abortion material.

In conclusion, ovine brucellosis is endemic at moderately high level. History of still birth is independent predictor of the disease at animal level. Regular surveillance system, well organized educational program to the livestock owners about the transmission of the disease between animals and from animals to humans are suggested before the infection spreads. Studies that include the definitive diagnosis of brucellosis through isolation and identification, as well as molecular studies to determine the species and *biovar* of *Brucella* sp. deserves consideration as it gives valuable epidemiological information.

Acknowledgements

The financial support of Oromia regional state Rural Capacity Building highly acknowledged. The authors would like to extend their gratitude to the district assistant veterinary technicians and the farmers for their collaboration in the sampling and supplying necessary information respectively.

References

1. Cenci-Goga BT, Rossitto PV, Sechi P, McCrindle CM, Cullor JS (2011) Toxoplasma in animals, food, and humans: an old parasite of new concern. Foodborne Pathog Dis 8: 751-762.
2. Central Statistical Authority (CSA) (2008) Federal democratic republic of Ethiopia Central Statistical Agency agricultural sample survey, Report on livestock and livestock characteristics. Statistical Bulletin Addis Ababa, Ethiopia 417: 9–35.
3. Kusiluka L, Kambarage D (1996) Diseases of small ruminants in Sub-Saharan Africa: A Handbook, Oversea Development Administration Animal Health Programme, VETAID, pp: 28 -30.
4. OIE (2009) Ovine and caprine Brucellosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris, France: Office international des Epizooties, Paris.
5. Radostitis OM, Gay CC, Hinchcliff KW, Constable PD (2006) Veterinary Medicine. A Text book of diseases of cattle, sheep, pigs, goats and horses. W.B. Saunders (Ed) London pp: 963 -985.
6. Seleem MN, Boyle SM, Sriranganathan N (2010) Brucellosis: a re-emerging zoonosis. Vet Microbiol 140: 392-398.
7. Corbel MJ (1997) Brucellosis: an overview. Emerg Infect Dis 3: 213-221.
8. Yumuk Z, O'Callaghan D (2012) Brucellosis in Turkey -- an overview. Int J Infect Dis 16: e228-235.

9. Negash E, Shimelis S, Beyene D (2012) Seroprevalence of small ruminant brucellosis and its public health awareness in selected sites of Dire Dawa region, Eastern Ethiopia. Journal of Veterinary Medicine and Animal Health 4: 61-66.
10. Dohoo I, Martin W, Stryhn H (2003) Veterinary Epidemiologic Research. Charlottetown, Canada pp: 39–120.
11. Thrusfield M (2005) Veterinary Epidemiology. (3rd Edn) Oxford, UK, Blackwell Science Ltd pp: 228–246.
12. Shively MJ (1987) Veterinary Anatomy: Basic Comparative and Clinical. Texas, USA press, pp: 390-391.
13. OIE (2004) Caprine and Ovine brucellosis (excluding Brucella ovis infection). In Manual of Standard for Diagnostic Test and Vaccine, (4th edition) Paris, pp: 475-489.
14. Nielsen K, Dunkan JR (1990) Animal Brucellosis. Boca Raton, Florida, CRS Press Inc, pp: 173-179.
15. Yesuf M, Alemu S, Temesgen W, Mazengia H, Negussie H (2010) Seroprevalence of ovine brucellosis in south Wollo, north eastern Ethiopia. American-Eurasian Journal of Agricultural and Environmental Sciences 9: 288-291.
16. Yosef D, Nardos T (2010) Sero-prevalence of ovine brucellosis in and around Debre Birhan region of Ethiopia. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 31: 44-47.
17. Ferede Y, Mengesha D, Mekonen G, Hailemelekot M (2011) Study on the seroprevalence of small ruminant brucellosis in and around Bahir Dar, North West Ethiopia. Ethiopian Veterinary Journal 15: 35-44.
18. Bekele M, Mohammed H, Tefera M, Tolosa T (2011) Small ruminant brucellosis and community perception in Jijiga District, Somali Regional State, Eastern Ethiopia. Trop Anim Health Prod 43: 893-898.
19. Bekele TB, Kasali OB (1990) Brucellosis in sheep and goats in Central Ethiopia. Bulletin of Animal Health and Production in Africa 38: 23–25.
20. Bekele M, Mohammed H, Tefera M, Tolosa T (2011) Small ruminant brucellosis and community perception in Jijiga District, Somali Regional State, Eastern Ethiopia. Trop Anim Health Prod 43: 893-898.
21. Teklue T, Tolosa T, Tuli G, Beyene B, Hailu B (2013) Sero-prevalence and risk factors study of brucellosis in small ruminants in Southern Zone of Tigray Region, Northern Ethiopia. Trop Anim Health Prod 45: 1809-1815.
22. Tsegay A, Tuli G, Kassa T, Kebede N (2015) Seroprevalence and risk factors of Brucellosis in small ruminants slaughtered at Debre Ziet and Modjo export abattoirs, Ethiopia. J Infect Dev Ctries 9: 373-380.
23. Teshale S, Muhie Y, Dagne D, Kidanemariam A (2006) Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia: the impact of husbandry practice. Revue de Médecine Véterinaire 157: 557-563.
24. Tschopp R, Bekele S2, Moti T3, Young D4, Aseffa A5 (2015) Brucellosis and bovine tuberculosis prevalence in livestock from pastoralist communities adjacent to Awash National Park, Ethiopia. Prev Vet Med 120: 187-194.
25. Megersa B, Biffa D, Abunna F, Regassa A, Godfroid J, et al. (2012) Seroepidemiological study of livestock brucellosis in a pastoral region. Epidemiol Infect 140: 887-896.
26. Yesuf M, Alemu S, Temesgen W, Mazengia H, Negussie H (2010) Seroprevalence of ovine brucellosis in south Wollo, north eastern Ethiopia. American-Eurasian Journal of Agricultural and Environmental Sciences 9: 288-291.
27. Ashenafi F, Teshale S, Ejeta G, Fikru R, Laikemariam Y (2007) Distribution of brucellosis among small ruminants in the pastoral region of Afar, eastern Ethiopia. Rev Sci Tech 26: 731-739.
28. Girmay A, Hussien D, Afera B (2013) Seroprevalence of Ovine Brucellosis in a sheep export farm, Ethiopia. Global Veterinaria 11: 325-328.
29. Yosef D, Nardos T (2010) Sero-prevalence of ovine brucellosis in and around Debre Birhan region of Ethiopia. Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases 31: 44-47.
30. WHO (2006) Brucellosis in humans and animals. Produced by the World Health Organization in collaboration with the Food and Agriculture Organization of the United Nations and World Organization for Animal Health. WHO/CDS/EPR/2006.7, Geneva, Switzerland pp:1-89.
31. Acha PN, Szyfres B (2001) Zoonoses and communicable diseases common to man and animals. (3rd Edn) Washington, DC pp: 40-66.
32. Gumaa MM, Osman HM, Omer MM, El Sanousi EM, Godfroid J, et al. (2014) Seroprevalence of brucellosis in sheep and isolation of Brucella abortus biovar 6 in Kassala State, Eastern Sudan. Revue Scientifique et technique (International Office of Epizootics) 33:957-965.