ORIGINAL ARTICLE

Seroprevalence of brucellosis in small ruminants, its risk factors, knowledge, attitude and practice of owners in Berbere district of Bale Zone southeast Ethiopia

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ABSTRACT

Brucellosis is an important infectious disease causes significant reproductive losses in sexually mature animals and zoonotic importance. A cross sectional study was conducted from November 2018 to November 2019 in Berbere districts with the objectives of determining seroprevalence of small ruminant brucellosis, identifying potential risk factors for small ruminant brucellosis and assessing knowledge, attitudes and practices of owners about brucellosis in Barber district of Bale zone South-Eastern Ethiopia. A total of 470 sera from 80 flocks were collected (Goat, n=306, Sheep, n=164) and screened for evidence of brucellosis using the Rose Bengal Plate Test (RBPT) with positive results confirmed by Complement Fixation Test (CFT). To this effect, the overall seroprevalence in both species found to be 2.97% (2.43% goats and 3.26% sheep), whereas 17.5% prevalence at flock level. Flock size, age, parity and history of retained fetal membrane found to be significantly associated with *Brucella* seropositivity in small ruminants ($P<0.05$). While at flock level flock size, abortion and retention placenta found also to be significantly associated with Brucella seropositivity ($P<0.05$). As to the results of questionnaire survey, the majority of the communities do not have sufficient knowledge about brucellosis and they are in risk of acquiring the infection. Most of respondent was consuming raw milk, milk by products, poor handling of aborted fetus and other aborted materials without protective clothes. In conclusion, brucellosis is moderately prevalent among small ruminants in the study area. Therefore, awareness creation for animal owners, amongst pastoralist and other stockholders about the disease through collaborative roles (One health) of both veterinary and public health professionals and conducting further research on the isolation and molecular characterization of circulating Brucella species in livestock in study areas.

Keywords: Berbere, Brucellosis, CFT, Goat, Seroprevalence, Sheep, RBP

INTRODUCTION

Brucellosis is zoonosis and the infection is almost invariably transmitted to people by direct or indirect contact with infected animals or their products and becoming public health importance in developing countries. The disease affects many domestic animals but especially those produce food: sheep, goats, cattle, camels and pigs and wildlife, yalks, bison and reindeer. It is caused by various Brucella species such as *B. melitensis*in small ruminants, *B.abortus* in cattle, *B.suis* in swine and *B. canis*in dogs, while all the species are known to zoonotic importance. Brucella species are slow-growing, Gram negative, small cocobacilli and intracellular bacteria that is capable to survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008). Brucella *melitensis* is considered to have the highest zoonotic potential followed by *B. abortus* and *B. suis.* According to the Office for International des Épizooties (OIE), the disease is also classified as one of the neglected zoonoses with a serious veterinary and public health importance throughout the world (WHO, 2006; OIE, 2009).

Globally, it is estimated that nearly 500,000 cases of brucellosis would occur in humans every year (Pappas *et al.,* 2006). Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs. It frequently persists in the poorest and most vulnerable populations and remains the concern of economic and public health impact in developing countries (FAO, 2003; Roth *et al.,* 2003). The disease creates a barrier to trade of animals and animal products, an impediment to free animal movement (Zinsstag *et al.,* 2011). It also causes losses due to abortion or breeding failure in the affected animal population, diminished milk production and in human brucellosis causing reduced work capacity through sickness of the affected people (FAO, 2003).

In Africa and central Asia, the incidence of brucellosis is generally considered higher in pastoral areas. However, because of the difficulty to access pastoral communities the occurrence and the control of brucellosis are poorly understood both in humans and their animals in the pastoral settings of the sub-Saharan Africa where the burden of the disease could be high (Mcdermott and Arimi, 2002). According to the Central Statistics Agency (CSA), Ethiopia is one of the developing countries with domestic small ruminant population estimated to be 27.35 million sheep and 28.16 million goats (CSA, 2014).

Small ruminants are the chief source of cash income to small holders (EPAIAT, 2003; Akabarmehr and Ghiyamirad, 2011). This is because sheep and goat provide rapid cash turnover (OIE, 2009; Godfroid *et al.,* 2011). Most of the sheep and goat populations in Ethiopia are raised under pastoral conditions. These small ruminants and their milk/meat products represent an important export commodity, which significantly contributes to the national economy. At optimum off take rates, Ethiopia can export 700,000 sheep and 2 million goats per year and at the same time supply 1,078,000 sheep and 1,128,000 goats for the domestic market (Alemu and Markel, 2008). Even though these animals contribute much to the national economy, however, there production is hampered by different constraints in Ethiopian pastoral areas. Among many factors that limit economic return from small ruminants, reproductive diseases including brucellosis are the major disease affects pastoral areas (ILRI, 2006).

Brucellosis in sheep and goats due to *Brucella melitensis* is the most important zoonosis in terms of presenting a serious hazard to public health. The reports from different parts of Ethiopia are indicating that the occurrence of livestock and human brucellosis is increasing. Studies on the prevalence of brucellosis have been carried out in many parts of Ethiopia by different researchers.Previous reports on the overall prevalence of small ruminant prevalence range between 0.4% according to a study conducted in and around Bahir Dar (Ferede*et al.,* 2011), 13.7% as a pooled prevalence for a study conducted in the district of Tellalake in Afar Region (Tadeg, *et al.,* 2015) and overall prevalence of small ruminant 16% according to report of (Yibelta, 2005) in selected sites of Afar and Somali Regions, Ethiopia. The Flock-level seroprevalence among the small ruminant population of Ethiopia has been reported by different researchers (Asmare *et al.,* 2013; Teklue *et al.,* 2013; Tegene*et al.,* 2016; Chaka *et al.,* 2018).

Study on risk factor about brucellosis is lacking in much of the previous studies. However, understanding the risk factors, community perception of the disease is critical, thus consideration of the baseline survey level of infection is therefore essential for the formulation of appropriate control strategies (Hotez *et al.,* 2012). Moreover, the identification of risk factors for infection and spatial heterogeneities in the disease distribution could allow control efforts. However, no research has been done to quantify and document the actual prevalence of brucellosis in the present study areas. So that, there is an urgent need to know the status of the disease both in humans and animals (small ruminants) for better response to the impact of the disease. Furthermore, livestock keepers in the study area are likely to be more prone to that disease due to close cohabitation, handling animal cases and their eating habit. The knowledge of the community regarding of these disease, their attitude and practice predispose them to zoonootic diseases has not been studied previously in the study area, but it is important for future public health education and training. Therefore, the study was undertaken to determine seroprevalence of small ruminant brucellosis, identify potential risk factors for small ruminant brucellosis and assess knowledge, attitudes and practices of owners about brucellosis in Barber district of Bale zone South-Eastern Ethiopia.

MATERIALS AND METHODS

The Study area

The present study was conducted in Berbere district. Berbere is one of the districts in Bale Zone of Oromia Region, Ethiopia.Bale zone is found at 6°.44′ to 59°.99′ latitude and 40°14′ to 60°.00′ longitude Berbere is bounded on the south by Mennaa, on the northwest by Goba, on the north by Sinana, on the northeast by Goro, on the east by Guradhamole and Somali regional state. The administrative center of the Woreda is HaroDumal which is located at a 530 km south east of Addis Ababa, the capital city of Ethiopia. The annual average temperature of the district is 27°c whereas the minimum and maximum temperature is 16°c and 38°c, respectively. The annual average rainfall is 730mm whereas the minimum and maximum rainfall is 600 and 855mm, respectively. The study was carried out in five randomly selected peasant association of Berbere District namely Sirima, Walta'I Darasa, Galma, Haro Dumal and Gabe. Livestock rearing has played an important role in the life of district population specially in the rural and lowland areas of the district, rearing and breeding is the main stay of the people. There are about 311,881 Bovines, 14,931 Sheep, 155,265 Goats, 46,011 Equines and 132,755 Chickens, (BDAO, 2015)

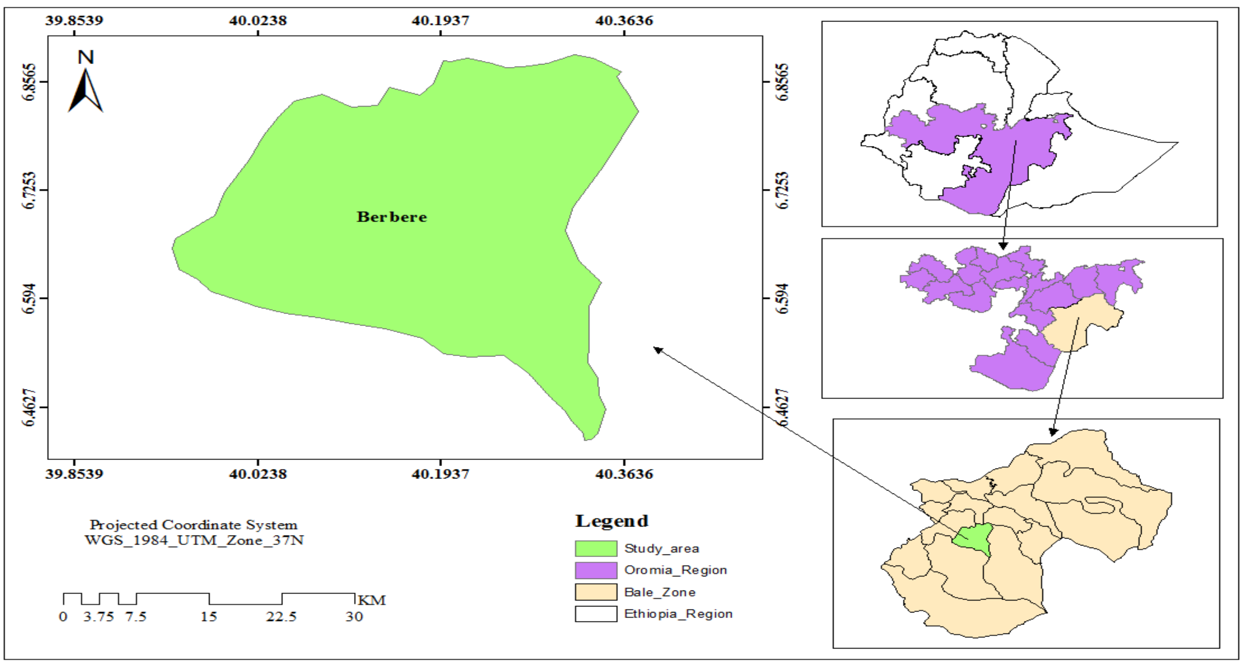

Figure 1. Map of the Study District

Study Design

A cross-sectional study was conducted from November 2018 to November 2019 in small ruminant under extensive production system to estimate the overall prevalence and flock prevalence of brucellosis and structured questionnaire survey was used to collect data on factors believed to influence the spread and dissemination of brucellosis.

Study Animal

The study animals are small ruminants in study area kept under extensive management system above 6 months. Those factors conceded as risk factor for brucellosis was collected before the blood sample was collected. There are namely, peasant association, species, sex, age, body condition, reproductive status, parity, history of abortion, stage of abortion and retained fetal membrane.

Sample Size Determination

The sample size for this study was determined as described by Thrusfield (2007) as follows.

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

where: n = required sample size, Pexp= expected prevalence, d = desired absolute precision

There is no report on prevalence of brucellosis in small ruminant in Bale Zone. Therefore, the average expected prevalence rate was assumed to be 50% for the area within 95% confidence interval (CI) at 5% desired precision. According to above formula the minimum sample size was 384, however, a total of 470 small ruminants (306 goats and 164 sheep) were involved to increase the precision of the study. A questionnaire survey was administered to 80 animal owners/attendant respondents whose animals were included in the study.

Sample and Data Collection

Questionnaire

A pre-tested structured questionnaire was used to collect information about potential risk factors associated with Brucellosis in individual and flocks Sero-positivity and knowledge, attitude and practices of pastoralists towards Brucellosis.

Operational definition

- Attitude**:** The way the community views and behaves on brucellosis. The importance of brucellosis as a zoonotic disease, as well as its spread and control.
- Knowledge**:** It is the community's awareness of brucellosis' zoonotic importance, including its causes, transmission, symptoms, and signs in both humans and animals.
- Practice: The routine involvement of the community in preventing brucellosis transmission during assisting abortion, including the use of gloves when handling an aborted fetus, washing after contact with animals and animal products, and methods of disposing of aborted fetuses and placenta.

Data on small ruminant owners' and attendants' knowledge, attitude, and practice (KAPs) were gathered through interviews with individuals using a pre-tested structured questionnaire. The respondents' verbal consent was obtained, and the survey's purpose was clarified to them before the interview began. The interviews were conducted in the participants' native languages (Afaan Oromo). Risk factors, socio-demographic flock characteristics, and data on brucellosis knowledge, attitude, and practice were all part of the questionnaire. Individual animal and flock attributes were collected, including peasant association, species, sex, age, physical condition, reproductive state, parity, abortion history, abortion stage, and retained fetal membrane.

Blood Sample collection

Sheep and goats selected for sample collection were individually restrained and approximately 5ml of blood was collected from the jugular vein following standard procedures by using plain vacutainer tubes. Identification of each animal was labeled on the corresponding vacutainer tube the collected blood sample allowed to stand overnight in order to get the serum. Serum was collected from the vacutainer using a disposable plastic Pasteur pipette dispensed to cryovial tube and stored in the freezer at -20°C until used for serological testing.

Serological tests

Rose Bengal plate test (RBPT)

The test procedures were done at the regional veterinary laboratory (RVL) in Asela, Ethiopia. The protocol of RBPT as recommended by OIE is used as screening test for the presence of Brucella antibody in the sampled sera. This test is generally considered to be as a sensitive test which reported as 97.9% sensitive for RBPT (OIE, 2009). The test is performed according to manufacturer's manual. Before performing test, antigen and sera are brought to room temperature. 30µl of serum was mixed with an equal volume of antigen suspension on a glass plate. After four minutes of rocking, any visible agglutination was considered a positive result. The screened positive sample and the sera were preserved at -20^{o}c and subjected to CFT test.

Complement fixation test (CFT)

All sera which tested positive to the RBPT were further tested using CFT for confirmation. The CFT was performed at the National Veterinary Institute, Bishoftu, Ethiopia. For confirmation using standard *B. abortus* antigen S99(Veterinary Laboratories Agency, New Haw, Addle stone, Surrey KT15 3NB, United Kingdom), preparation of the reagent is evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009) (Appendix 4). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above is classified as positive and lack of fixation/complete hemolysis is considered as negative. An animal was considered positive if the serum specimen tested positive on both RBPT and CFT whereas a flock was considered positive if at least a single serum specimen from an animal within the flock tested positive on both RBPT and CFT.

Data management and statistical analysis

The data were entered into a computer on a Microsoft Excel spreadsheet and Statistical analysis using SPSS 20 window version. All samples were tested for brucellosis using RBPT and CFT. The sample was reported positive if it is positive in both tests. All analyses were based on the CFT serological test results. Two epidemiological parameters were generated namely individual animal and flock level prevalence. Individual animal prevalence was computed by dividing the number of test positives by the total number examined multiplied by 100.In the same way flock level prevalence was also calculated by dividing the number of flocks' has at least one brucellosis positive animal by the total number of examined flocks multiplied by 100. Univariate logistic regression was used to test the significance of the effect of different risk factors on sero-prevalence of brucellosis. All risk factors that had non-collinear effect and p-value ≤0.25 in the univariable logistic regression analysis were subjected to multivariable logistic regression analysis. The multiple effect between predictor variables and outcome variable was assessed by Odds ratio (OR) and 95% CI values in logistic regression model. In all the analyses, a 95% confidence interval and P-value (P<0.05) was set for significance of statistical associations between the dependent and independent variables.

RESULTS AND DISCUSSION

Seroprevalence of Brucellosis

In the present study, a total of 470 small ruminants (306 goats and 164 sheep) sera were collected out of those 17(3.61%) were positive in a RBPT and 14 (2.97%) of them were confirmed to be seropositive for brucellosis using CFT, an overall animal level seroprevalence of 2.97 % (CFT) and 17.5 % flock level seroprevalence were recorded. At individual animal level the prevalence of small ruminant brucellosis was significantly higher in Large flock size (p = 0.031) and not significantly different when compared animals from household introduction new animal and those who do not (P >0.05). However, higher proportion of seropositivity was observed in those introduced new animal in the flock (4.12%) when compared to those not introduced (1.98%), (Table 1).

Similarly, despite females having a slightly higher proportion of infection (4.12 %(n=291) compared to males 1.11 %(n=179), gender was found to be an insignificant factor of brucellosis infection in the study area (P =.068). Among 291 females, 63 (21.64%) had retained fetal membranes, 89 (30.85%) had a history of abortion, and among those 89 had a history of abortion. Based on reproductive status, 47 (52.8 %) of 3-month fetuses were aborted, while 42 (47.1%) of >3-month fetuses were aborted. 28.26% were pregnant, 20% were lactating, 19.31% were dry, and 32.06 percent were lamb/kid. Similarly, a statistically significant difference in brucellosis seroprevalence was observed among parity (P =.033), with > 3 parity having a higher proportion of infection 41.37 % (n=291) compared to null parity and 1-3 parity having a lower proportion of infection 24.8 % (n=290), (Table 1).

Small ruminant flock size, age, parity and history of retained fetal membrane were having a significance effect on seroposativity of small ruminant brucellosis in the study area and introduction of new animal, sex, reproduction status; abortion and gestation of abortion are those variables were not significantly associated with animal level seropositivity as it is indicated in Table 1 and 2. The flock level Multivariables logistic regression analysis revealed that flock size, history of abortion and retention fetal membrane in flock was found to be strongly associated with flock seropositivity to *Brucella* (p-value < 0.05) in (Table 1).

Animal level risk factors analysis

In Table 1, the results of Multivariable logistic regression analysis showing important risk factors for individual animal *Brucella* seropositivity recorded. Small ruminant in flock size, were large flock size (5.29%) revealed a statistically significant variation (p<0.05) with the odds of seropositivity being at least 3.8 times more likely to be infected with *Brucella* organisms than shoat have small flock size. Accordingly, the odds of brucellosis seropositivity were found to be 8.3 times higher among older shoat compared to those of the younger one. Correspondingly, parity and history of retained fetal membrane status in females were to be significantly associated with seropositivity. Brucellosis was significantly (p=.033) higher in small ruminant with more than three parities with 8.4 times more likely to be seropositive than animals with null parity. There was a significantly high sero-prevalence (P= 002) of small ruminant brucellosis in those have a history of retained fetal membrane when compared to small ruminant not have history of retained fetal membrane. Accordingly, the odds of brucellosis seropositivity were found more than 12.8 times higher among those have a history of retained fetal membrane from those not have history of retained fetal membrane. The rest risk factor showed no statistically significant associations regardless of the seropositivity recorded (Table 1)

Table 1. Potential risk factors of brucellosis at animal level based on Multivariable logistic regression

Factors	Category	No animal	CFT positive (%)	CI 95%	OR	P-value
Flock Size	Small	78	-			
	Medium	203	4(1.97%)	0.13 - 39.3	2.28	
	large	189	10(5.29%)	1.287 - 11.401	3.830	.016
Introduction	No	252	5(1.98%)			
	Yes	218	9(4.12%)	0.427 - 4.617	1.404	.577
Age	Young	153	-			
	Adult	219	4(1.82%)	0.73- 40.78	5.47	
	Old	98	10(10.2%)	2.786 - 25.170	8.374	.000
Sex	Male	179	2(1.11%)			
	Female	291	12(4.12%)	. 900 - 20.149	4.258	.068
Parity	Zero parity	98	-			
	1-3 parity	72	1(1.38%)	.032 - 43.1	6.122	
	>3 parity	121	11(9.09%)	1.187 - 60.880	8.499	.033
Reproduction status	Heifer	93	-			
	Lactation	84	5(5.95%)	.338 - 26.4	.562	
	Pregnant	14	7(6.14%)	.301 – 1.424	6.55	.285
Abortion	No	201	3(1.49%)			
	Yes	89	9(10.11%)	.018 - 13.033	. 490	.670
GS abortion	<3 month	41	5(12.19%)			
	>3 month	48	4(8.33%)	0. .984-4.541	2.113	.055
History of RP	No	227	2(0.88%)			
	Yes	64	10(15.62%)	2.575-64.585	12.896	.002

No = Number.OR = Odds Ratio, CI = Confidence Interval

Flock level risk factors analysis

Out of 80 flocks studied, 17.5% (14/80) were positive using CFT. The flock level univariable regression analysis revealed that flock size, abortion in heard and placenta retention in flock were found to be strongly associated with flock seropositivity to *Brucella* (p-value ≤ 0.25), Table 2.

Table 2. Potential risk factors of brucellosis seropositivity in flock level based on univariate logistic regression

Factors	Category	Number of Flock	CFT (%)	p-value
Flock Size	Small	35	-	
	Medium	23	4(17.4%)	
	Large	22	10(45.45%)	.000
Abortion in Heard	No	50	3(6%)	
	Yes	30	11(36.6%)	.000
Placenta Retention	No	60	6(10%)	
	Yes	20	8(40%)	.002

In Table 3, the results of Multivariable logistic regression analysis showing important risk factors for *Brucella* seropositivity of flocks. Accordingly, in univariate logistic regression model those risk factors with p-value (≤0.25) where included in the Multivariable logistic regression model. Therefore, flock size, abortion and retained fetal membrane was fitted for multivariable logistic regression model and all of them were significantly associated with flock level *Brucella* seropositivity (p<0.05) Multivariable logistic regression analysis depicts that large flock size were more than 11 times more likely to become *Brucella* positive compared to that small flock size.

Questioner Survey

Socio-demographic characteristics of the respondents

The socio-demographic characteristics of the respondents are presented in Table 4. The age of most of respondents was between. 41-50 years old. Majority of the respondents in the study areas were Male (68.8%). Significant number of the community are Illiterate (41.3%) and 6.3% of them are college Graduate.

Table 3. Potential risk factors of brucellosis at flock level seropositivity based on Multivariables logistic regression

Factors	Category	Number of Flock	CFT positive (%)	CI 95%	OR	P-value
Flock Size	Small	35	-	-	-	-
	Medium	23	4(17.4%)	1.985 – 23.102	5.102	
	Large	22	10(45.45%)	2.582 - 47.023	11.018	.001
Abortion	No	50	3(6%)			
	Yes	30	11(36.6%)	.017 - 0.627	.102	.014
Placenta	No	60	6(10%)			
Retention	Yes	20	8(40%)	.021 - 0.759	.127	.024

No = Number OR = Odds Ratio, CI = Confidence Interval

Table 4: Socio-demographic composition of study population (n=80)

Parameter	Category	Number of respondents	Percentage
Age (years)	18-30 Years	7	8.80
	31-40 Years	24	30.00
	41-50 Years	32	40.00
	Above 51 Years	17	21.30
Sex	F	25	31.30
	M	55	68.80
Education level	Illiterate	33	41.30
	Primary	25	31.30
	Secondary	17	21.30
	College Graduate	5	6.30

Knowledge, Attitude and practice of community about brucellosis

Knowledge, attitude and practice of community about brucellosis of the studied population are presented in Table 5. Livestock reared in the area was camel, cattle, goat, sheep, chicken, and donkey. All of studied households were rearing small ruminants. 46.2%, 27.5% and 26.3% of them holds small (1-29), medium (30-50) and large (> 51) flock size respectively. Livestock are retained inherited generation to generation; however, flock size increases naturally and through selling of old animals and the practice of buying younger ones was observed in 48.8% of the households. Thirty-seven point five (37.5%) of heard have abortion history and 94.4% of study population were not support during abortion. Majority of community (82.5%) not using protective glove when assisting of animal during calving, working with abortion animal and retention placenta. 77.1% of community in study area is never know prevention and control method of brucellosis in animal and human. The three main practices for management of aborted material and fetus in the study area were giving to dogs, dispose it in the ground and burying in 39.6%, 33.3% and 27% of the cases respectively. Furthermore 55% of the respondents explained that they were in contacts with fetal membrane and/or fetal fluids in one way another. Only 33.8% and 16.3% of them was washing their hands after contact with animal and animal products respectively. 76.3% of respondent explained they consume raw milk and milk by product. 56.3% of the respondents participated in this study had never heard of a disease known as brucellosis (Table 5).

The present study revealed that the overall seroprevalence of small ruminant brucellosis is 2.97% in the Berbere district of Bale Zone South East Ethiopia. The prevalence in this study is closely in agreement with the findings of 2.7% (Nigatu *et al.*, 2014) in Selected Export Abattoirs Addis Ababa. 2.25 % (Bezabih and Bulto 2015) in Werer Agricultural Research Center Afar, 3.5% (Teklue*et al.*, 2013), Southern Zone of Tigray (3.7%), (Melese, 2016) in Ethiopia. In contrast, it is lower than the previous reports of seroprevalence of small ruminant brucellosis reported elsewhere in Ethiopia including 12.35% reported in Afar region by (Anteneh*et al.*,2014), 9.6% in Yabello pastoral Area (Yohannes *et al.*, 2013) and 9.11% in Dire Dawa (Negash *et al.*, 2012). However, the current prevalence obtained in this study is higher than the prevalence of (Teshale*et al.*, 2006) who reported a seroprevalence of 1.7% in goat and 1.6% in sheep in Somali Pastoral Area. Other studies revealed seroprevalence of 1.3% in goats and 1.5% in sheep (Teklay and Kasali, 1990) in central highlands of Ethiopia.

These differences could be due to variations in sensitivity and specificity imparted by the various tests, agro-ecological location and amount of sampled study population, management, production systems and husbandry condition in the study areas those conditions could facilitate the rate of transmission of the disease (Radostits *et al.*, 2007).

Table 5. Knowledge, Attitude and practice of community about brucellosis (n=80).

Parameter	Category	Number of respondents	Percentage (%)
Rearing of sheep and goat	Yes	80	100
	No	0.0	0
Flock Size	Small	35	43.75
	Medium	23	28.75
	Large	22	27.5
Introduction of new animal	Yes	39	48.80
	No	41	51.20
Have you ever had a retained placenta	Yes	44	55.
problem in your sheep/goat heard	No	36	45.
How Manage the aborted Fetus	Bury	13	27.08
	Give to dogs	19	39.58
	Dispose on the ground	16	33.3
Abortion History of flock	Yes	30	37.50
	No	50	62. 50
Have a contacts with aborted fetuses	Yes	44	55.
	No	36	45. 0
Have a contacts with fetal membrane and/or fetal fluids	Yes	47	58.8
	No	33	41.3
Assisting during abortion(30)	Yes	13	43.33
	No	17	56.6
Who is assist (13)	Veterinary professionals	3	23.07
	Traditional healers	6	46.15
	By owner	4	30.76
Do you use gloves while assisting (13)	Yes	6	46.15
	No	7	53.84
Have a contacts with animal product	Yes	80	100.00
	No	-	-
Hands wash after contact animal	Yes	27	33.80
	No	53	66.30
Hands wash after contact animal	Yes	13	16.30
products.	No	67	83.80
Do you consume raw milk and/or	Yes	61	76.30
milk by products	No	19	23.80
Have you ever heard brucellosis	Yes	35	43.80
	No	45	56.30
Which animals affected by	Shoat	6	17.14
Brucellosis(35)	Wild animal	10	28.50
	Human	3	8.57
	I don't know	16	45.70
Brucellosis can affect human(35)	Yes	10	28.57
	No	25	71.42
Do you know prevention and control	Yes	8	22.85
measures(35)	No	27	77.14

In the present study significant effect in small ruminant seropositivity of flock size was categorized in three larger flock sizes the chance of being seropositive was approximately more than three times higher than small and medium (3.8, CI: 1.287 - 11.401). It agrees with the report of (2.7, CI: 1.4-5.1) by (Asmare *et al.*, 2013) and (3.45, CI: 1.12-10.27) by (Melese, 2016).

Age as a factor is supposed to have association with occurrence of brucellosis, because sexual maturity is very important for the rapid multiplication of *Brucella* organism (Mohammed, 2009). In present finding old age (above three years) category were eight times more likely to be seropositive than young animals (less than one year of age) (OR=8.374; 95% CI: 2.786 - 25.170) and in agreement with report from Afar (Ashenafi *et al.*, 2007), Borana (Megersa *et al.*, 2011), South omo (Ashagrie *et al.*, 2011), Jigjiga (Bekele *et al.*, 2011) South region and (Asmare *et al.*, 2013) Oromia region.

This increased susceptibility with increased sexual maturity is due to the influence of sex hormones and erythritol on the pathogenesis of Brucellosis (Radostits*et al.,* 2007). Similarly, multivariable logistic regression revealed that the risk of seropositivity was more than eight times higher in (>3) parity compared to (1-2) and null parity group. Higher parity was also significantly associated with the disease which agrees with the finding of (Ashagrie *et al.* 2011; Asmare*et al.,* 2013). It is because the prevalence of brucellosis continues to rise with repeated exposure to parturition and other physiological stresses during pregnancy (Matope *et al.,* 2011; Hadush *et al.,* 2013). In present study, statistically high significance difference (P=0.000) was recorded with high seroprevalence of brucellosis in small ruminants having history of retained fetal membrane than those without these problem (Radostits *et al.,* 2007).

In the present study there is not significant association between male and female however the smaller reactor was recorded in male than female. Some studies reported that serological response of male animals is limited and thus infected animals are usually observed to be non-reactors or show low antibody titer (FAO/WHO, 1989). Furthermore, male animals are known to be less susceptible to Brucella infection due to the less amount of carbon 4-sugar erythritol (Hirsh and Zee, 1999). History abortion and gestation of abortion was also no significant at individual animal level in the present study. This finding disagrees with finding of (Muluken, 2016) who reported the significance of stated risk factors. This difference is due to a difference in the number of samples collected from animals with a history of abortion (Muluken, 2016).

However, high seroprevalence were recorded in those animal have history of abortion than those not have abortion history and more than two-time high prevalence were recorded in those have history of abortion in late stage of gestation than in early stage. This could be explained by the presence of higher concentration erythritol (2R, 3S) - butane- 1, 2, 3, 4, tetraol, a low calorie sugar alcohol produced naturally by the developing fetus may favors multiplication of Brucella where it causes degeneration and necrosis of the cotyledons leading to abortion from about the last months of gestation (Coetzer and Tustin, 2004; Radostits *et al.,* 2007).

There was no significantly association observed in seropositivity in small ruminant body condition score. Nutrition plays great role in immunity against various infectious diseases. Underfed animals are expected to have a decreased immunity that is manifested by poor body condition (Kamili*et al.,* 2006; Radostits *et al.,* 2007). Therefore, body condition of the small ruminant was considered during the study to see the distribution of the infection in different body condition scores good, moderate and thin. The overall flock level seroprevalence of small ruminant brucellosis was 17.5% which is comparable to flock level seroprevalence report of 14.14% (Lone *et al.,* 2013 and 19.9% (Addis *et al.,* 2018), under extensive management systems. However, the present result is higher than the reports of previous studies in different parts of Ethiopia in which a disease prevalence ranging from 1.5 to 4.9 % were reported (Adugna *et al.,* 2013; Megersa *et al.,* 2011; Asmare *et al.,* 2010; Berhe *et al.,* 2007).The higher prevalence in extensive small holder production may be due to the fact that, in this production system, there is free animal movement and aggregation of animals within common pastures and watering points which may increase the transmission of brucellosis from animal to animal or from contaminated environment (Muma *et al., 2001;* Haileselassie *et al.* 2010; Makita *et al., 2011*).

Small ruminant with a history of abortion was significantly affects flock seropositivity. The flock seroprevalence of brucellosis was higher in flocks that had a history of abortion compared with no history of abortion. This could be explained by the fact that abortion is typical outcomes of brucellosis.The present study showed that participants engaged in this study had poor information of brucellosis. The brucellosis known through ''Gatachisa'' in Afan Oromo which means, a disease known to cause abortion. The present finding also showed that most of the respondents had never heard of the disease brucellosis similar to studies in Kenya and Tajisktan (Kang'ethe, *et al.,* 2008; Lindahl *et al.,* 2015) but in contrast to studies carried out in Egypt and Jordan which showed a high awareness of the disease (Holt *et al.,* 2011; Musallam *et al.,* 2015). The authors of those studies explained this high awareness by an endemic situation of brucellosis in the study area. The low awareness in this study could therefore in part be explained by a lower flock seroprevalence compared to Egypt and Jordan. Of the participants who had heard of the disease, knowledge about the cause, transmission routes controlling and prevention was still poor, among participants heard brucellosis about half was not knew even if which animals affected by brucellosis. Rearing of Small ruminant is common in study area even all of my respondent were rear Small ruminant.

Direct contact with animals and their secretions are miss practice on the study area. A community observed in the current study was assisting animals during normal delivery or abortions they touch the animal with bare hands and there after they wash their hands with water and soap. Lim *et al.*(2005) reported a similar case, where touching calves and/or placenta of infected animal was a risk factor for brucellosis transmission (Mishal *et al.,*1999).

Regarding knowledge to zoonotic disease risks, majority of people were not aware that humans could become infected with brucellosis from animals and from those heard brucellosis two third of them was not have information (knowledge) about prevention and control method of brucellosis in animal and human. The majority was aware of the risks through raw milk however 76% of them were consuming raw milk.

Unsurprisingly, this study found that all farmers were engaged in at least one risky practice conducive to transmission of Brucella to other animals and humans. Knowledge about the disease and preventive herd/flock management practices have previously been identified as the most important factors needed for minimizing the disease risk in animals (Díez and Coelho, 2013). Infected female animals excrete high concentrations of organism in their milk, placental membranes and aborted fetus (Radostits *et al*., 2007).

Most respondents were not washed their hands with soap after dealing with such Aborted material, but only one third of them was reported to thoroughly disinfect the area and use antiseptic for them self with disinfectant and antiseptic alcohol or other. In the rural areas, the most common practice appeared to be using just a brush without use water to clean the area. The practice of study cleaning the area with just a brush leaves a very high risk of contamination and bacteria could easily survive in the environment leading to transmission to other animals or humans. Brucella in aqueous suspensions are readily killed by most disinfectants (The Center for Food Security and Public Health, 2009), so use of disinfectants and protective gloves should be considered as part of a future control program by encourage farmers to use commonly. Only 27% farmers in this study reported disposing of placental membranes by burying, which is one of the most effective methods of reducing disease risks and with most reporting to discard them into the open environment, outside the boundaries of their home or even feed them directly to dogs. The pathogen has been recovered from fetuses that have remained in a cool environment for over 2 months; this is also could present a transmission risk to both other animals and humans in the area (Kahn and Line, 2010).

Similar results were found in Jordan and Pakistan, but in contrast, a study in Tajikistan found 94% or respondents would bury the placenta and aborted materials (Lindahl *et al*., 2015; Musallam, *et al*., 2015; Arif *et al*., 2017). It is interesting to note that often the placenta and aborted fetus are not disposed of in the same way; among farmers who commonly bury the placenta, many would still discard aborted material either to dogs or into the open environment rather than bury. This is perhaps because of the larger size of fetuses making them more difficult to bury and suggests that those who bury the placenta may not be doing it due to awareness of disease transmission risks but rather for other reasons such as practicality.

Direct contact with placental membranes and aborted fetuses is a major route of human infection (Corbel, 2006). This lack of knowledge could explain the fact that the majority did not use protective gloves when assisting with calving, when caring with aborted animal or aborted materials. This could also in part be due to lack of access to protective gloves, which would have to be bought at the farmer's expense. Similar results have been reported from Tajikistan, Egypt and Jordan, suggesting that the use of gloves is not common practice in many lower income countries (Holt *et al*., 2011; Lindahl *et al*., 2015; Musallam *et al*., 2015).

To sum up with current study, the seroprevalence of small ruminant brucellosiswas found to be 2.97% and 17.5% at animal and flock level respectively, this shows small ruminant brucellosis is prevalent in Berbere District of Bale Zone of Oromia Region, South East Ethiopia. The flock size, age,parity status and history of retained membranes of the animals are found to be significantly associated with seropositivity at animal level and flock size. There is low awareness of livestock owners on zoonotic importance of brucellosisand prevailing habit of consumption of raw milk, assisting parturition and handling of aborted materials; those are a factors contributing for human brucellosis. Hence, it is very important to create awareness amongst pastoralists, farmer and other stakeholders about transmission, economic and public health importance of Brucellosis in the study area. It is also very important to consider collaborative role of both veterinary and public health professionals through One Health approach in implementing prevention and control strategies of zonootic diseases and conducting further research on the isolation and molecular characterization of circulating Brucella species in livestock in study area.

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