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Original Research Paper

Small animal brucellosis: associated risk factors, seroprevalence and Characterization of Brucella isolates in two districts of South Omo Zone, Ethiopia

Authors:
Feyera Gemeda Dima[1,2*], Maryam Dadar[3]

[1]*College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia.*

[2]*Addis Ababa University, Aklilu Lemma Institute of Pathobiology, Addis Ababa, Ethiopia.* [3]*Razi Vaccine and Serum Research Institute (RVSRI); Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.*

Corresponding Author:
Feyera Gemeda Dima

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ABSTRACT:
Brucellosis is one of the highly contagious zoonotic bacterial diseases, with a significant impact on the livestock industry. It is caused by Gram-negative bacteria from the Genus *Brucella*, and distributed worldwide including Ethiopia. However, there was a scarcity of epidemiological data on its occurrence in pastoral areas. A cross-sectional investigation was conducted from September 2018 to June 2019, to estimate the seroprevalence of brucellosis and to characterize main infecting small ruminants in two randomly selected pastoral districts of the South Omo Zone, Ethiopia. A pre-tested questionnaire was used and collected data were subjected to statistical analyses (multivariate logistic regression). For the serological test, blood samples were drawn from a total of 124 small ruminants with a history of abortion. Subsequently, 30 vaginal swabs were sampled from seropositive animals for *Brucella* isolation. All collected sera were first screened serologically using the modified Rose Bengal Plate Test (mRBPT) and *Brucella* seropositivity was further confirmed by the Complement Fixation Test (CFT). The seroprevalence of brucellosis among small ruminants with a history of abortion was 21% (26/124; 95% CI: 0.14 - 0.28). Multivariable logistic regression analysis showed that the main risk factors related to *Brucella* spp. infections were history of abortion (OR: 0.28, 95% CI: 0.18 - 0.43) and parity numbers (OR: 0.20, 95% CI: 0.059 - 0.72). *Brucella* spp. were also isolated from 5 (16.7%) of the 30 vaginal swabs cultured on *Brucella* Selective Agar. The isolates were identified as *B. melitensis* based on biochemical and bacteriological culture results. In conclusion, the present study showed that brucellosis is highly prevalent in small ruminants in the studied area. Therefore, regular testing of breeding animals is necessary to reduce brucellosis and its economic impact in the region.

Keywords: Abortion; Risk factor; B. melitensis; seroprevalence; small ruminants

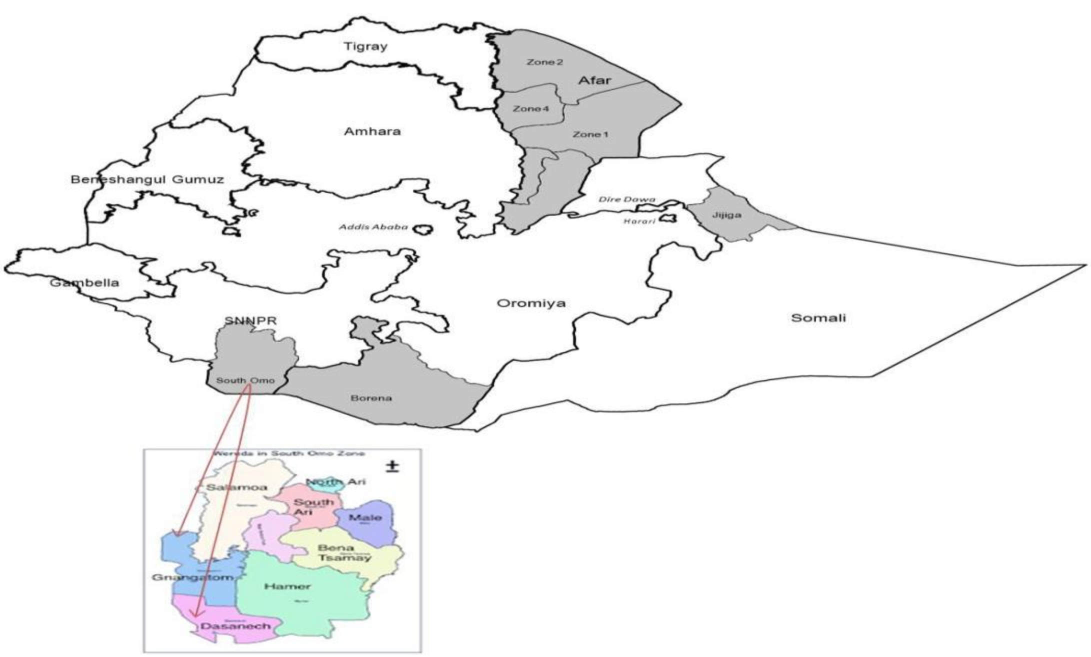
INTRODUCTION:

Brucellosis is a multi-species infectious and contagious bacterial disease causing economic losses for livestock production industry in many developing countries worldwide(Corbel, 1997; Nielsen, 2018). It is the most common zoonosis world wide with over 500,000 cases every year, often considered as a neglected hazard for the public health(Pal *et al.*, 2017; Dadar *et al.*, 2019b).Despite efforts made to establish brucellosis control program in different countries, it still represents an endemic disease in several regions worldwide, including the Central Asia, Middle East, Mediterranean region, and parts of Africa, Latin America (Franc *et al.*, 2018). Brucellosis is responsible for reproductive losses in livestock animals, that are commonly caused by *Brucella melitensis*, *Brucella abortusin cattle, Brucella suis* in pigs, and *Brucella canis in dogs*. Small ruminant brucellosis is the most frequent bacterial zoonosis in low-income countries including Ethiopia where the disease is a major cause of direct economic losses and an impediment to trade and exportation (Tewodros and Dawit, 2015). *Brucella melitensis* is the main species infecting small ruminants. Despite the economic losses incurred and the wide spread distribution of small ruminant brucellosis in Ethiopia, especially in South Omo Zone, less attention has been paid to the spread of the disease in pastoral areas. The accuracy of the diagnostic tests of brucellosis is an essential component in the success of test, eradication and control strategies. The isolation and identification of *Brucella* species in small ruminants are essential in these areas where livestock and pastoralists have close contact in their daily life. This could help authorities and decision makers to plan disease control and appropriate prevention strategies (Tewodros and Dawit, 2015). This represents an important challenge as brucellosis is endemic in Ethiopia and commonly causes retained fetal membrane, abortion, as well as infection of the accessory sex gland and orchitis in males (Hirsh and Zee, 1999). It is a widely distributed neglected zoonotic disease, with poor awareness among the community, and cause serious economic losses in small ruminants' production industry (Corbel, 1997; Ashagrie *et al.*, 2011; Bugeza *et al.*, 2019). The incidence of brucellosis is generally considered higher in pastoral settings of Africa. However, because of the difficulty to access pastoral communities, the occurrence and the control of brucellosis is 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). In Ethiopia, small ruminants are the main source of livelihood for small holders under extensive pastoral production system (Ashagrie *et al.*, 2011). However, Ethiopia fails to optimally utilize this resource and brucellosis significantly affects livestock productivity. In Ethiopia, several studies showed individual seroprevalence ranging from 0.1–15.2% in different parts of the country (Asmare *et al.*, 2010; Haileselassie *et al.*, 2010; Ashagrie *et al.*, 2011; Mohammed *et al.*, 2017) that most of them are largely confined to serological surveys. Although, isolation of *Brucella* species is the gold standard for the identification and confirmation of animal brucellosis, there is little research done to isolate and identify causative agents in Ethiopia. A recent study showed poor community's knowledge about brucellosis and high risk for *Brucella* infection among pastoral list communities of South Omo Zone (Ashagrie *et al.*, 2011). Therefore, the isolation and identification of the prevailing *Brucella* species and the assessment of potential risk factors are necessary. Accordingly, the aim of this study was to use a combination of serological, biochemical and morphological characterization methods to evaluate the prevalence of *Brucella* infections among small ruminants as well as to identify the associated risk factors in two districts (Nyangatom and Dassenech) of South Omo Zone.

METHODS:

Study area:

The study was conducted in two districts (Nyangatom and Dasenech) of South Omo Zone, in the Ethiopian Southern Nations, Nationalities and Peoples' Region (SNNPR) and it is located at750 km south of Addis Ababa (Figure1). This Zone has eight districts and two districts were selected for the purpose of this study. These were selected because of several factors like: the absence of enough data on the status of brucellosis, large population of small ruminants in the areas and source of the export animals, poor livestock management practices (no constructed houses for small ruminants), seasonal mixing off locks of different origins. The two districts (Nyangatom and Dassenech) mainly rely on small ruminant production for their livelihood. Nyangatom and Dassenech have an animal resource which is estimated at 415,292 cattle, 55,100 goats, 48,260 sheep, 11,218 donkeys and 5,474 chickens (Ethiopia, 2006).



Using GPS 7.10

Figure 1: Map showing the two study Districts of South Omo Zone, Ethiopia .

Sampling Methods:

The target animals for this study were small ruminants of the Nyangatom and Dassenech districts in South Omo Zone. The sample size for serological study was estimated in accordance with previous reports on the seroprevalence of *Brucella* infection in aborted small ruminants (Ashagrie *et al.,* 2011). The sample size was calculated using the below formula with defined precision of 5%,at 95% confidence interval as follows (Thrusfield, 2018). n= $[1.96^2 * p\ (1-p)]/\ d^2$ (*n*=required sample size, *P*=reported prevalence, *d*=desired absolute precision) A total of 124 small ruminants (sixty two small ruminants from each district) were considered for this study, while parallel to this, a number of milk and vaginals swabs were sampled from sheep and goats for bacteriological culture. From each of the two districts, three sub districts were included in this study. These sub districts were selected using simple random sampling technique. Blood, milk and vaginal swab samples collection Blood samples (86 goats and 38 sheep), milk (8 specimens) and vaginal swab (24 specimens) were collected from selected districts in South Omo zone. The sera were gently decanted into sterile tubes and all samples transported in cold chain to Add is Ababa University, Aklilu Lemma Institute of Pathobiology, Immunology Laboratory, Addis Ababa, Ethiopia and stored at -20^oC until further studies. Swab samples were collected with sterile applicator stick in Ames with Charcol Transport Medium (HiMedia, Mumbai, India). Similarly, milk sample were collected aseptically after washing, drying and disinfecting the whole udder and teats. Ten to 20 ml mid-stream milk samples were collected from each teat into sterile 50 ml screw capped falcon tubes.

Questionnaire survey A structured questionnaire survey was made to assess the degree of association between potential risk factors and of *Brucella* seropositivity. A pre-tested questionnaire on the risk factors was handled to 124 interviewees for 124 small ruminants (86 goats and 38 sheep) and the potential risk factors for brucellosis considered in this study included animal species, age, body condition, abortion history, frequency of abortion and parity number. The awareness and the way of prevention and control of the diseases were interviewed and the knowledge regarding the ways of the disease transmission was also evaluated among animal owners.

Laboratory Diagnosis:

Serological Tests:

All serum samples were screened using modified Rose Bengal Plate Test (mRBPT) and Complement Fixation Test (CFT) according to the described procedures (Alton *et al.,* 1988). Form RBPT, the agglutinations were recorded as 0,+,++ and +++, according to the degree of agglutination (Nielsen, 2018). *Brucella* positive and negative controls era were also tested along with the test sera to guide in the reading of the results. Then, positives era were further tested using CFT for confirmation using standard *B. abortus* antigen S99 according to the proposed procedure of the World Organization for Animal Health. (https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.04_Brucellosis.pdf).

Bacteriological test for milk and vaginal swab samples Bacteriological tests were carried out under Bio safety level three (BSL3) with high personal protections at the Brucellosis laboratory. All individual milk and vaginal samples from serologically positive by mRBPT were subjected to bacterial culture. Primary isolation of *Brucella spp.* was done by inoculating the milk and vaginal samples on a *Brucella* selective supplement (Hi Media, Mumbai, India) with selective antibiotics supplement (FD005) (HiMedia, Mumbai, India) and inactivated 5% horse serum in *Brucella* agar (Himedia, Mumbai, India) and incubated for 10 days with or without CO2 in 37°C with 5 and 10% CO_2 anaerobically. Milk samples were centrifuged at 6000 rpm for 15 minutes and the cream and deposit were spread on *Brucella* selective agar base with supplement (Himedia Mumbai, India). After two weeks of incubation, the bacterial cultures were discarded if no growth was visible(Dadar *et al.,* 2019a). *Brucella*-suspected colonies were characterized by their typical round and honey drop-like appearance according to Alton (Alton *et al.*, 1988). Typical colonies of *Brucella* spp. were subject to further analysis to determine full identification. Also, suspected *Brucella* colonies were stained by Stamp's modification of the Ziehl–Neelsen's and Gram staining method for subsequent micro scopic identification of the organisms. *Brucella* spp. Were identified based on Gram negative, very tiny appearance and coccobacilli shape that were arranged mostly in single but some in pairs and also in clusters according to Alton (Alton *et al.*, 1988).

Biochemical Tests:

Further biochemical characterization of the organism was done using (Oxidase test, catalase test, urea hydrolysis, nitrate reduction test, hydrogen sulphide (H2S) production and hemolyisis on blood agar),growth in the presence of thionin and basic fuchsindyes incorporated at 20 to 40µ g/ml concentrations, Carbon dioxide requirement according to Alton (Garin-Bastuji *et al.*,

2006; Dadar *et al.*, 2019a).

Ethical Consideration:

Ethical clearance was obtained from Institutional Review Board of Add is Ababa University, Aklilu Lemma Institute of pathobiology (ALIPBIRB/019/2011/2019). The protocol for field studies and collection of samples from animals was approved by Nyangatom and Dassenech District's agricultural and veterinary authorities of South Omo Zone and verbal consent from Livestock owners was approved by ethics.

Data Quality Assurance:

All data used for this study were primary data, and were collected by the principal investigator.

Data Analysis:

Statistical analysis (multivariate logistic regression) was done using Stataversion14. Prevalence was computed by dividing the number of test positives by the total number examined multiplied by 100. The Chisquare ($\chi 2$) and logistic regression tests was employed to identify possible association between risk factors and reproductive characteristics with seropositive to *Brucella* infection. The degree of association was considered significant when a P value of less than 0.05 is obtained or when the 95% confidence intervals of the odds ratio in the multivariable logistic regression analysis, which did not include were considered as significant (Thrusfield, 2018).

RESULTS:

Seroprevalence of brucellosis:

The seroprevalence of brucellosis was 24% (95%CI: 0.17-0.32) using mRBPT while it was 21 %(95%CI: 0.14-0.28) by CFT tests. Thus, the overall seroprevalence of *Brucella* infection in aborted small ruminants in Nyangatom and Dassenech districts of South Omo Zone was 21% by the combined mRBPT and CFT tests. Association of risk factors with *Brucella* seropositivity

Analysis for association between environmental factor and *Brucella* infection on the basis of the combined mRBPT and CFT was done using Pearon's chisquare and Fisher's exact tests (Table1). There was a significant association between majority of the kebeles *(*pastoral associations) and seroreactivity to *Brucella* infection (*P*<0.05). Among kekeles, high percentages of seropositivity were observed in charrii (32%), while lobot (0.2%) and nikiya (0.2%) were affected at lesser extent. Age, body conditions, frequency of abortion and parity status were significantly associated to *Brucella* seropositivity, but no association was shown with animal species and gender (*P*<0.05). The difference in seropositivity in chi-square and logistic regression among the variables indicates, there were confounding factors (age, body condition, and frequency of abortion) in this study, as they were only significant with chi-square, but not when compared with Multivariate logistic regression analysis (confidence interval of their Odds ratio included 1).

Table1: Association of risk factors with *Brucella* seropositivity in small ruminants, Nyangatom and Dassenech districts of south Omo Zone.

Variables	N/Tested	Seropositive	Prevalence(%)	χ^2- value	*P-Value*
PastAssoc (Kebeles)			27.3	1.298[b]	0.255
Kakuta	22	6			
Lorekacho	20	2	10	0.782[a]	0.676
Charrii	22	7	32	13.367[b]	0.000
Lobot	20	4	20	5.971[b]	0.015
Nikiya	20	4	20	3.856[b]	0.050

Trongole	20	3	15	2.599[b]	0.107
Species				1.152[b]	0.283
Caprine	86	20	23.3		
Ovine	38	6	16		
Age(Years)				3.19[b]	0.026*
≤3	18	3	17		
>3-5	38	3	7.9		
> 5	68	18	26.5		
Body condition				14.410[b]	0.001*
Good	28	11	39.3		
Medium	33	1	3		
Poor	63	14	22.22		
Frequency ofabortion				1.115[b]	0.003
Once	86	2	2.33		
>Onetimes	38	24	63.2		
Parity no				6.15[b]	0.046*
Monoparous	7	4	57.14		
Pluriparous	117	22	19		
Total	**124**	**26**	**21**		

From the above table (Table1); χ2: Chi-Square; [a]Fishe's exact value; [b]Pearson's chi-square value, Significant, N/tested: number of animals tested; Past Assoc: pastoral association According to the multivariable logistic regression model fitted (Table: 2) many of the putative risk factors considered: district (animal location), species, age, body condition, frequency of abortion and parity status were found to be significantly associated with *Brucella* seropositivity. Small ruminants in age group ≤3years (OR=0.06, 95%CI: 0.07-0.44) were likely to be at higher risk for *Brucella* infection than animals in>3-5and>5years'age groups. The multivariate analysis also revealed that increased parity of sheep and goats was more likely to be associated with an increasing risk of getting *Brucella* infection when evaluated collectively with other factors. Thus, animal with multiple parturition were at higher risk of encountering *Brucella* infection (OR=0.50, 95%CI: 0.399–0.63) than monoparous animals.

Table2: Multivariable logistic regression analysis of factors associated with *Brucella* Seropositivity

Variables	N/Tested	Infected	Crude OR (CI 95%)	Adjusted OR(CI 95%)	*P-Value*
PastAssoc (Kebeles)					
Kakuta	22	6(27.2%)	1a	1.5(0.67- 3.34)	0.321
Lorekacho	20	2(0.1%)	1a	0.999(0.45 -2.23)	1.000
Charrii	22	7(32%)	1a	0.087(0.02- 0.037)*	0.001*
Lobot	20	4(0.2%)	1a	0.062(008 - 0.471)*	0.007*
Nikiya	20	4(0.2%)	1a	0.067(0.009-0.50)*	0.009*
Trongole	20	3(0.15%)	1a	0.07(0.009 - 0.54)*	0.011*
Species				**0.52(0.15-1.74)**	**0.287**
Caprine	86	20(23.3)	1a	0.32(0.198-0.528) *	0.000*
Ovine	38	6(16)	1a	0.43 (0.28–0.67)*	0.000*
Age (Years)				**2.01(0.97-4.17)**	
≤ 3	46	3(17%)	1a	0.06(0.07-0.44)*	0.006*
> 3-5	10	3(7.9%)	1a	0.52 (0.35–0.76)*	0.001 *
> 5	68	18(26.5%)	1a	0.69(0.58-0.83)	0.000*
Body condition				**0.63(0.36-1.12)**	
Good	28	11(39.3%)	1a	0.75(0.35 - 1.56)	0.451
Medium	33	1(3%)	1a	0.17 (0.06 - 0.47)*	0.001*
Poor	63	14(22.2%)	1a	0.66(0.54 - 0.81)*	0.000*
Frequency of abortion				**0.67(0.22-2.01)**	
Once	86	2(2.33%)	1a	0.32 (0.19 - 0.52)*	0.000*
> One times	38	24(63.2%)	1a	0.45(0.29 - 0.69)*	0.000*
Parity no				**0.2(0.059-0.72)**	
Monoparous	7	4(57.14%)	1a	1.33 (0.298–5.96)	0.706
Pluriparous	117	22(19%)	1a	0.50(0.399 - 0.63)*	0.000*
Total	**124**	**26(21%)**	1a	0.50(0.399 - 0.63)*	0.000*

***Significant; OR: Odds Ratio; CI: Confidence Interval, aReference. N/tested: number of animals tested; Past Assoc: pastoral association.**

<u>Isolation of Brucella</u>:

From a total of 124 seropositive clinical samples, 8 milk and 22 vaginal swabs were subjected to Brucella selective culture and the further characterization of Brucella isolates by biochemical tests. The result showed that 16.7% (5/30) of samples were positive for the Brucella spp. selective culture. All isolates, were from vaginal swabs of 4 goats (13. 3 3 %) and 1 sheep (3. 33 %), while no isolates were found from milk samples, as it may be spoiled, during transportation from long distance (Table 3).

The isolates were initially recognized on the basis of colony morphology which was characteristic of Brucella growth with very small, smooth, glistening, pin-point and round like colonies with honey like appearance.

Microscopic examination was performed and Gram stained cultures revealed small Gram-negative coccobacilli arranged singly and inpairs and on Modified Ziehl-Neelsen (MZN) stain, the Brucella organisms were stained red on a blue background. On different biochemical reactions, the present Brucella species were found to be positive for catalase, oxidase, urea hydrolysis, nitrate reduction tests and all the colonies were grown without 5-10% CO2. The isolates were further differentiated biochemically using parameters such as CO2 requirement, H2S production, and growth on thionin and basic fuchsin dyes incorporated into trypticsoyagarat different concentrations were tested.

Table3: *Brucella* isolates recovered from seropositive sheep and goats' vaginal swabs and milk.

Type of sample	No of sample cultured	No. of isolates	Percentage (%)
Vaginal swab	22	5	22.7
Milk	8	0	0
Total	**30**	**5**	**16.7**

Accordingly, biochemical test, CO2 requirement and sensitivity to dyes (Table: 4) of culture isolates recovered from seropositive goats and sheep vaginal swabs sample revealed t h a t animals were infected by *B. melitensis.*

Table 4: Staining and biochemical test results of Brucella from seropositive sheep and goat's vaginal swabs.

Brucela isolates	Animal species	Gram's staining	MZN	Cat.	Oxd	Ure	CO2	H2S	Nitr.Red	Thionin	Basic Fuchsin
Vaginal swab 1	Caprine	Gram negative	+	+	+	+	-	-	+	+	+
Vaginal swab 2	Caprine	Gram negative	+	+	+	+	-	-	+	+	+
Vaginal swab 3	Caprine	Gram negative	+	+	+	+	-	-	+	+	+
Vaginal swab 4	Caprine	Gram negative	+	+	+	+	-	-	+	+	+
Vaginal	Ovine	Gram	+	+	+	+	-	-	+	+	+
swab 5		negative									

Cat, Catalase; Oxi, Oxidase; Ure, Urea hydrolysis; MZN, Modified Zeihl Neelsen stain; CO2 Req, Carbon Dioxide requirement; Agg, Agglutination; H2S pro, Hydrogen Sulphide production; Nitr.Red, Nitrate reduction.

DISCUSSION:

Given the important role of small ruminants and reliable data on the infection prevalence, several risk factors were described for brucellosis. Among them, animal species, age, body condition, frequency of abortion, and parity were reported as the most important risk factors (Kebede *et al*., 2008; Tsegay *et al*., 2015). This study showed that the overall seroprevalence of brucellosis in small ruminants with history of recent abortion reached 24% (n=30) and 21% (n=26) with mRBPT and CFT, respectively. This report was higher as compared to the seroprevalences of small ruminant brucellosis reported elsewhere in Ethiopia including 7.52% reported in Afar Region (Tekle, 2016), 9.6% in Yabello pastoral Area (Yohannes *et al.,* 2013) and 9.11% in Dire Dawa(Tekle, 2016). It was also higher than the report of Mugizi and his colleague, who reported 8% and 11% prevalence in sheep and goats with a history of abortion and retained placenta, respectively in Soroti towns of Uganda (Mugizi *et al.,* 2015). This could be due to difference in animal husbandry, communal grazing of range lands and watering areas as well as the influence of climatic conditions (Teshale *et al.,* 2006). The prevalence reported in this study using CFT was higher than the prevalence of 4.2% in small ruminants reported in the same study area (Ashagrie *et al.,* 2011). Teshale also reported a seroprevalence of 1.7% in goat and 1.6% in sheep in Somali pastoral a reas(Teshale *et al.,* 2006). Another study also reported seroprevalences of 1.3% in goat and 1.5% in sheep in central highlands of Ethiopia (Tekle, 2016). However, it is important to note that the current study was carried out on animals that had history of recent abortion, which could increase the chance of the seropositivity for brucellosis, since *Brucella* is a major cause of abortion in small ruminants (Asmare *et al.,* 2010; Franc *et al.,* 2018; Bugeza *et al.,* 2019). The higher prevalence reported in our study could be also due to variation in sensitivity and specificity imparted by the various tests, agroecological location and the number of sampled animals, management and production systems. Most of the prevalence studies used standard Rose Bengal Plate Test (RBPT) for screening, but this study used mRBPT. This simple modification is achieved by increasing the amount of sera for the test dose from 25 to 75 µl, while maintaining the antigen volume at 25 µl. This may significantly increase the sensitivity of the test without affecting the specificity (Blasco *et al.,* 1994; Ferreira *et al.,* 2003). All other risk factors considered in this study including district, age groups, body conditions, frequency of abortion and parity numbers were found to be associated with *Brucella* infection in small ruminants as observed by using multivariable logistic regression analysis. Brucellosis is a disease mainly affecting sexually matured animals. Our results showed that adult age (above three years) categories were more likely to be seropositive than young animals (less than one year of age). This study also showed that the frequency of abortions and parity was significantly associated with seropositivity for *Brucella* infection in the studied animals. This indicates that abortions or stillbirths and retained placenta are typical outcomes of brucellosis in the region (Mugizi *et al.,* 2015; Tsegay *et al.,* 2015; Tekle, 2016). Regarding the distribution of brucellosis in small ruminants having history of recent abortion among the different pastoral Kebeles, the highest seroprevalence was recorded in Charrii followed by Kakuta, Lobot, Nikiya and Trongole and Lorekacho.

There is a significant difference (*P*<0.05) in the brucellosis prevalence among the four past oral *kebeles* of the district. Age, frequency of abortion and parity status remained significant in multivariable logistic regression analysis. Seropositivity for *Brucella* infection increases approximately 6 times in animals with age > 5 years old when compared to ≤ 3 and >3–5 years old. This is in accordance with several previous studies showing a higher seroprevalence of brucellosis in adult age groups of small ruminants (Ashenafi *et al.,* 2007; Bekele *et al.,* 2013). Sexually mature animals are more susceptible to *Brucella* infection than sexually immature animals which is due to the fact that sex hormones and erythritol, stimulating the growth and multiplication of *Brucella* spp., tend to increase in concentration with age and sexual maturity (Berhe *et al.,* 2007). The multivariate analysis also revealed that increased parity of sheep and goats was associated with an increasing risk of *Brucella* infection when evaluated collectively with other factors. Thus, animals with multiple parturition were at higher risk of encountering *Brucella* infection (OR=0.50, 95% CI: 0.399-0.63) than monoparous animals. Interestingly, multivariable logistic regression revealed that the risk of seropositivity was approximately 5.5 times higher in pluriparous animals when compared to monoparous animals. Higher parity was also significantly associated with the disease which is in agreement with the findings of Ashagrie (Ashagrie *et al.,* 2011). Generally, animals abort once during the mid-third of gestation but re- invasion of the uterus occurs in subsequent pregnancies with shedding in fluids and fetal membranes (Fensterbank, 1987). Isolation of *Brucella* species is the gold standard for identification and confirmation of animal brucellosis. Previous studies in various parts of Ethiopia indicated that the disease is widespread among small ruminant populations. However, most surveys of brucellosis in Ethiopia rely on serological tests only, and there is little evidence for bacteriological isolation of *Brucella* species. To the best of our knowledge, only one study reported the isolation of *B. meltensis* from aborted goats in Afar Region (Tekle, 2016). In present study, all isolates were obtained from vaginal swabs, while no isolates were recovered from milk. Shedding of *Brucella* organisms through body secretion was an important source of infection in humans (Dadar *et al.,* 2019b). *Brucella* isolates were harvested form vaginal swabs which appeared to be very important in disease epidemiology since farmers assist delivery without any personal protection which aggravates the disease circulation. This stresses the need to coordinated brucellosis prevention strategies for human and animals. This is particularly the case for retained placenta, as the pastoralists usually use their bare hands to pull the placenta out of the vulva. These habits and practices expose them to high risk of contracting brucellosis (Habtamu et al., 2015). Based on biochemical characterization, *B. melitensis* was recovered from 22.7% (5/22) of vaginal swabs. This is in accordance with results obtained by Tekle *et al.* (2016), in Afar Region (Ethiopia), where 6 out 28 (21.43%) vaginal swabs were infected by *B. melitensis*. In goats, about two thirds of acute infections which were acquired naturally during pregnancy lead to infection of the reproductive organs, like vagina, and excretion of the bacteria in vaginal secretions during subsequent lactation and there is also evidence that indicated reproductive tract environment favored the growth of this bacteria (Tekle, 2016).

CONCLUSION:

This study revealed that among risk factors for brucellosis in small ruminants, the history of abortion and parity numbers were remarkably associated to *Brucella* infection in pastoral areas of Ethiopia. Animal owners have poor awareness on brucellosis and proper management of the animals could considerably help to control the spread of the disease in these regions. Among, key features that may aid to improve farmer safety and reduce the transmission of the disease, regular cleaning of the housings and the safe disposal of aborted materials appeared to be essential. In order to prevent brucellosis transmission within the flock or to the other healthy flocks, the regular screening of small ruminants for brucellosis along with the vaccination of animals after birth should be implemented in pastoral areas.

ABBREVIATIONS:

CI: Confidence Interval

OR Odds Ratio

B.melitensis BrucellaMelitensis mRBPT modified Rose Bengal Plate Test CFT Complement Fixation Test

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