BOVINE BRUCELLOSIS: SEROPREVALENCE AND ITS ASSOCIATED RISK FACTORS IN CATTLE FROM SMALLHOLDER FARMS IN AGARFA AND BERBERE DISTRICTS OF BALE ZONE, SOUTH EASTERN ETHIOPIA

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ABSTRACT

A cross-sectional study was conducted to determine seroprevalence of bovine brucellosis and to assess potential risk factors that played a role for the existence of the disease in Agarfa and Berbere districts of Bale Zone, South Eastern Ethiopia from November, 2015 to June, 2016. A total of 768 sera from 76 herds were collected and examined by using Rose Bengal plate test (RBPT) and complement fixation test (CFT) as screening and confirmatory tests, respectively. The present study revealed that the seroprevalence of bovine brucellosis was 6.12% and 4.95% as detected by RBPT and CFT, respectively. Since CFT is the recommended confirmatory test for brucellosis with high specificity, the overall seroprevalence in the study area was 4.95%. A higher seroprevalence of 7.36% was observed in Agarfa compared to Berbere (3.17%) district. In the multivariate logistic regression analysis, female animals (AOR = 3.086, 95% CI = 1.229, 7.754, P< 0.05), cross breed (AOR=5.040, 95% CI=2.278, 11.150, P<0.001), pregnancy (AOR=3.601, 95% CI=1.711, 7.579, P<0.01), abortion (AOR=3.328,95% CI = 1.497, 7.397, P< 0.01), retained foetal membrane (AOR=3.418, 95% CI=1.339, 8.729, P< 0.05) and metritis/endometritis (AOR=6.842, 95% CI= 2.059, 22.737, P< 0.01) were identified as the major risk factors for individual animal seroprevalence. Most of the respondents had a habit of raw milk consumption without boiling or pasteurization, hence, it can be a potential risk for both susceptible animals and humans, therefore, there is a need to create general public awareness about the disease and design and implement appropriate control measures that will prevent further spread of a disease within and outside the study area.

Key words: Brucellosis, bovines, seroprevalence, RBPT, CFT, risk factors, Ethiopia.

INTRODUCTION

Brucellosis is an ancient and one of the world's most widespread zoonotic diseases affecting both, public health and animal production. It is endemic in many developing countries of Asia, Latin America and Africa including Ethiopia. Brucellosis is considered by Food and Agriculture Organization (FAO), World Health Organization (WHO) and Office International des Epizooties (OIE) as one of the most widespread zoonoses causing substantial morbidity in both livestock and human populations globally (Schelling et al*.,* 2003; Corbel, 2006; Lopes et al., 2010). According to OIE, it is the second most important zoonotic disease in the world after rabies. It is a highly contagious zoonotic infection affecting livestock and human beings (Ajay et al., 2016). The disease affects cattle, swine, sheep, goats, camels and dogs. It may also infect other ruminants and marine mammals (Mantur et al., 2007; Wadood et al., 2009).

Brucellosis is caused by bacteria of the genus *Brucella* species which are coccobacilli, Gram- negative, aerobic, non-spore-forming, non-motile, non-capsulated and facultative intracellular pathogen of many vertebrate species including man (Bargen et al., 2012). Currently ten species are recognized including the better known six classical species. Of these species, *B. melitensis* has the greatest risk for human infection followed by *B. suis* and *B. abortus*, however, several of the other species have been shown to be virulent for humans (Godfroid et al., 2011). Among the animal brucellosis, bovine brucellosis, caused by *B. abortus* is the most important disease in many countries around the world due to its economic importance (McDermott and Arimi, 2002; Silva et al., 2000; Taleski et al., 2002).

Clinically bovine brucellosis is characterized by impaired fertility specifically with abortion, metritis, orchitis and epididymitis (Radostits et al., 2007; Seleem et al., 2010). The target organs and tissues of *Brucella* spp. are placenta, mammary glands, and epididymis in animal reservoir host (Adams, 2002; Xavier et al., 2009; Neta et al., 2010).

The mode of transmission of the bacteria varies with the epidemiological area, the animal reservoir and the occupational exposed groups (Seleem et al., 2010; Pappas et al., 2006). Sources of infection for the transmission of the bovine brucellosis are aborted fetuses, the fetal membranes after birth, and vaginal discharges and milk from infected animals (Radostits et al, 2000; Tolosa, 2004). The most significant feature of bovine brucellosis epidemiology is the shedding of large

numbers of organisms during 10 days after abortion or calving of infected cows and the consequent contamination of the environment (FAO, 2003; OIE, 2009b).

Currently, brucellosis has been eradicated or severely curtailed in developed countries by a combination of strict veterinary hygiene measures, control programs and improved food safety measures (Greenstone, 1993; Whatmore et al., 2006). However, in Ethiopia, a low income country, the disease remains endemic in different localities of the country and is growing problem in cattle herds of the country (Ibrahim et al., 2010). Brucellosis seroprevalence in other Sub-Saharan countries follows a similar trend as that of Ethiopia (Mugizi et al., 2015). There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be due to brucellosis (Munir *et al*., 2010; Maadi *et al*., 2011).

Since the first report of brucellosis in the 1970s in Ethiopia (Domenech, 1977; Meyer, 1980), the disease has been noted as one of the important livestock diseases in the country (Asfaw et al., 1998; Eshetu et al*.,* 2005; Ibrahim et al., 2010; Kebede et al*.,* 2008). A large number of studies on bovine have been reporting individual brucellosis seroprevalence ranging from 1.1% to 22.6% in intensive management systems (Asmare et al*.,* 2007; Hailemelekot et al*.,* 2007; Tolosa et al., 2010; Tesfaye et al*.,* 2011) and 0.05% -15.2% in extensive management system (Berhe et al., 2007; Hunduma and Regassa, 2009; Asmare et al., 2010; Degefa et al., 2011; Megersa et al., 2011).

Though all the above study revealed that as bovine brucellosis is endemic in different cattle production system of Ethiopia, the disease has been insufficiently investigated and to date there is no published data on its status, magnitude and distribution in Bale Zone in general and in Agarfa and Berbere districts in particular. Furthermore, most studies on bovine brucellosis in Ethiopia have been carried out on state dairy farms in the central parts of the country, where dairy cattle production, using mainly exotic breeds, is intensive. Hence**,** the aim of this study was i. to establish seroprevalence of bovine brucellosis ii. to identify putative risk factors influencing *Brucella* seropositivity iii. to assess livestock owner's knowledge, attitude and practices (KAPs) regarding brucellosis.

MATERIALS AND METHODS

Description of the study area: The study was conducted in two selected districts of Bale Zone namely: Agarfa and Berbere districts of Oromia Regional State, South Eastern Ethiopia, based on accessibility to road, feasibility to sample collection and number of cattle they possess.

Agarfa district is located at a distance of 464km south east of Addis Ababa, the capital city of Ethiopia. The mean annual temperature of the district is 17.5 ^{o}c. The maximum and minimum temperatures are 25^{o}c and 10^{o}c, respectively. The annual rainfall of the area ranges from 400-1200mm with mean annual rainfall of 800mm. Agricultural production system of the study area is mixed crop and livestock farming. In the rural and lowland areas of the district, rearing and breeding is the main stay of the people. There are about 229,206 bovine, 63,485 ovine, 15,674 caprine, 33,777 equines and 40,150 poultry in Agarfa district (ADAO, 2015).

Berbere district is located at a distance of 530 km south east of Addis Ababa, the capital city of Ethiopia. The annual average temperature of the district is 16.5^{o}c whereas the minimum and maximum temperature is 9^{o}c and 23^{o}c, respectively. The annual average rainfall is 850mm whereas the minimum and maximum rainfall is 1060 and 1150mm, respectively. From early days, livestock rearing has played an important role in the life of district population. 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).

Study Population and animals: The target study population was comprised of cattle with the age of 6 months or above in the two selected districts of Bale Zone. The status of bovine brucellosis in Agarfa and Berbere districts was unknown being no study had been conducted in the districts. The study animals were consisted of 768 cattle of 6 months or above ages with history of no vaccination. The samples, cattle selected, were selected by simple random sampling method from both districts.

Study design: A cross-sectional type of study supported by laboratory tests and questionnaire survey was conducted to determine seroprevalence of bovine brucellosis that potentially affect cattle production system and associated potential risk factors that played a role for the existence of the disease in the two districts.

Sampling technique and sample size determination: The sampling was performed using a two level approach, selecting first a household with abortion history and then randomly selecting individual animals systematically inside each farm. In each study area, the approximate numbers of households were listed with the assistance of local veterinary/ agricultural office. A lottery system was used on the lists of the households that were obtained from the respective kebeles. A herd was defined as a total number of cattle that is kept by a household sharing the same grazing area and/or watering point. About 9.21% of the sampled cattle were from small herd size while the remaining 39.47% and 51.32% were from medium and large herd size, respectively. Since there is no reasonable research done in these areas so far; the sample size for

cattle was calculated using a method recommended by Thrusfield (2007), with 95% confidence interval, at 5% desired absolute precision and expected prevalence of 50%. Accordingly; the total numbers of sample required for this study were 384 cattle, but to increase the precision level a total of 768 cattle belonging to 76 herds were sampled and investigated. Six kebeles (lowest administrative structure) were randomly selected using a lottery system out of the 10 kebeles with high concentration of cattle in the district. Proportionality of incorporating cattle in the sample will be applied as per the population size of each district and kebeles.

Table 1. Proportional allocation and number of animals sampled from each districts.

Districts	No. of kebeles	No. of cattle population in the districts	No. of kebeles sampled	No. of cattle sampled (calculated sample size)
Agarfa	19	229,206	3	325
Berbere	17	311,881	3	443
Total	**36**	**541,087**	**6**	**768**

Source: Data obtained from Agarfa and Berbere "Woreda" Agricultural Office (2015).

Questionnaire survey: A structured questionnaire was prepared, pre-tested and administered by interviewer to the heads of the households to assess potential risk factors for the transmission of brucellosis from cattle to cattle and possibility of humans acquiring brucellosis in the study area. It was conducted after carefully explaining the purpose of the work to the interviewees. The questionnaire was focused on the awareness of the respondents and risk factors exposing to bovine brucellosis in the selected districts. Information was recorded on sex, age, breed, herd size, origin, pregnancy, cases of abortion, risk of infection by brucellosis to herd owners and family members. It was also used to find out knowledge on brucellosis and its impacts and control methods, whether they used any protective materials while handling the aborted materials and consumption of raw milk or not were included in the questionnaire survey format.

Serological blood sample collection: Blood samples (10 ml) were collected from the jugular vein of each animal, using sterile needles and plain vacutainer tubes. The blood samples were allowed to stand overnight at room temperature and centrifuged at 1500 × g for 10 min to obtain the serum. Sera were decanted into cryovials, identified and transported to the National Veterinary Institute, Ethiopia in ice packs and stored at -20°C until screened for antibodies against natural *Brucella* exposure using serological analysis.

Serological laboratory techniques

Rose Bengal plate test (RBPT): All sera samples collected were initially screened by RBPT using RBPT antigen (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) according to OIE (2004) and Alton et al. (1975) procedures. Briefly, sera and antigen were taken from refrigerator and left at room temperature for half an hour before the test to maintain to room temperature and processed following the recommended procedures.

Complement fixation test (CFT): Sera that tested positive to the card test, i-ELISA and RBPT were further tested using CFT for confirmation using standard *B. abortus* antigen S99 (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009b). Sera with a 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 were classified as positive and lack of fixation/complete hemolysis was considered as negative.

Case definition: Animals were considered as seropositive on the complement tests result, i.e., an animal was considered positive if tested seropositive on RBPT and CFT in serial interpretation. The test was regarded as valid if the negative control serum showed complete haemolysis and the positive control shows inhibition of haemolysis. Due to its high accuracy, complement fixation is used as confirmatory test for *B. abortus*, *B. melitensis*, and *Brucella ovis* infections and it is the reference test recommended by the OIE for international transit of animals (OIE, 2009a, b).

Data analysis: All data from laboratory investigations and questionnaire survey were entered into a Microsoft Excel spreadsheet and checked for accuracy. After validation, data were transferred to STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA) for analysis. The response variable considered in the analysis of our data was bovine brucellosis infection status and the potential risk factors considered were origin, age, sex, breed type, parity number, physiologic condition (pregnancy), history of abortion and retained placenta. The animal level seroprevalence was the proportion of positive animals out of the total number of animals sampled and analyzed, while the herd level

seroprevalence as the proportion of herds with at least one positive animal out of the total number of herds sampled. The association between the independent factors and the prevalence of brucellosis was evaluated using the Chi-square test ($\chi 2$). Multivariate logistic regression analyses were used to analyze the effects of different potential risk factors on the sero-prevalence of brucellosis. Odds ratio (OR) was utilized to measure the degree of association between potential risk factors with brucellosis sero-prevalence. The 95% confidence interval and a p-value <0.05 was considered statistically significant.

RESULTS

Knowledge, attitudes and practices (KAPs) of the respondents' about brucellosis: A 100% response was found when selected households were visited and the farmers were interviewed. A total of 76 households were interviewed to investigate the risk factors that played role in transmission of brucellosis from cattle to cattle and possibility of humans acquiring brucellosis (zoonotic aspect) in the study area. It was observed that100%, 90% and 87.18% of the herd owners in small, medium and large herd sizes responded as they have no awareness about brucellosis, respectively (Table 2). Regarding consumption of raw milk and milk products, 100% of the respondents of small herd size, 76.67% of medium size and 76.92% of large size herds consume raw milk daily immediately after milking while small proportion of respondents did not take raw milk (Table 2). Almost all of the households (100% of small herd size, 90% of medium herd size and 89.74 of large herd size) dispose after birth & aborted material by throwing elsewhere to open dump. No history of wearing protective gloves was recorded in small herd size respondents; 90% of medium size herd and 87.18% of large size herd respondents did not use protective materials during handling of aborted foetuses, afterbirths and parturient animals. It was observed that vast majority of owners retain their aborted cows while few number of them sale or cull as illustrated in Table 2.

Table 2. Knowledge, attitudes and practices (KAP) of herd owner's about *Brucella* infection in small, medium and large herd size in the study areas (n = 76).

Variables	Level	Percentage of respondents (n)		
		Herd size		
		Small (n = 7) n (%)	Medium (n = 30) n (%)	Large (n = 39) n (%)
Brucellosis awareness	No	7(100)	27 (90)	34 (87.18)
	Yes	0 (0)	3 (10)	5 (12.82)
Consumption of milk	Boiled	0 (0)	4 (13.33)	4 (10.26)
	Raw	7 (100)	26 (86.67)	35 (89.74)
Disposal of after birth	Burrying/ Burning	0 (0)	3 (10)	4 (10.26)
	Open dump	7 (100)	27 (90)	35 (89.74)
Protective clothes	No	7 (100)	27 (90)	34 (87.18)
	Yes	0 (0)	3 (10)	5 (12.82)
Fate of aborted animal	Retain	5 (71.43)	25 (83.33)	34 (87.18)
	Sale	2 (28.57)	4 (13.33)	3 (7.69)
	Cull	0 (0)	1 (3.33)	2 (5.13)
Level of education	Illiterate	5 (71.43)	21(70)	29 (74.36)
	Literate	2 (28.57)	9 (30)	10 (25.64)

Herd management characteristics: Of the 76 herds assessed by a questionnaire survey, it was observed that 71.43%, 83.33% and 89.74% of small size, medium size and large size herd owners responded natural service as breeding method, respectively. However, few of the respondents use artificial insemination for breeding purpose when their cows show estrus. It was found that 57.14%, 66.67% and 64.10% of small size, medium size and large size herds had bulls. No maternity pen was maintained by the small herd size, while 6.67% medium size and 7.69% large size herds possess it. From the questionnaire administered it was discovered that almost all of the households (85.71%, 90% and 84.62%) did not clean maternity pen with water or detergents. It was found that 57.14% of the small herd size owners, 66.67% of small herd size64.10% of large size herd had bulls in their herds. The study revealed that most of the herds in the study area had no frequent contact with other herds as presented in Table 3.

Seroprevalence of bovine brucellosis: In the present study, an overall seroprevalence was estimated to be 4.95% by CFT. The average herd prevalence (proportion of herds with at least one sero-positive animal) was 15.79% (12/76) on the basis of CFT. The higher

seroprevalence rate of *Brucella* antibodies was observed at Agarfa district, 7.36% (24/326) when compared to Berbere, 3.17%(14/442) as illustrated in Table 4.

The number of animals tested within each 6 study kebeles and proportions found to be positive for brucellosis are depicted in Table 5. Of the six 6 kebeles selected, Agarfa atvet was with the highest brucellosis seroprevalence (8.59%) while Ekata was the least (1.79%). There was no significant association between the selected kebeles of the studied districts and *Brucella* seropositivity.

Chi-square analysis of association of the putative risk factors with *Brucella* seropositivity: A Chi-square analysis revealed that prevalence of bovine brucellosis was significantly associated with the sex (P<0.004), breed (P<0.001), age groups (P<0.05), origin (P=0.008), parity (P<0.05), pregnancy (P<0.001) while its association with either herd size or body condition was not statistically significant (P>0.05) (Table 6).

Table 3. Proportion of variables related with management systems in the three herd sizes (n=76).

Variables	Level	Percentage of respondents(n)		
		Herd size		
		Small (n = 7) n (%)	Medium (n = 30) n (%)	Large (n = 39) n (%)
Breeding methods	Natural service	5 (71.43)	25 (83.33)	35 (89.74)
	AI	1 (14.29)	2 (6.67)	2 (5.13)
	Both	1 (14.29)	3 (10)	2 (5.13)
Bull	No	3 (42.86)	10 (33.33)	14 (35.90)
	Yes	4 (57.14)	20 (66.67)	25 (64.10)
Replacement stock	Raised in own herd	5 (71.43)	24 (80)	32 (82.05)
	Market	0 (0)	3 (10)	4 (10.56)
	Mixed	2 (28.57)	3 (10)	3 (7.69)
Frequent contact with other herd	No	5 (71.43)	24 (80)	30 (76.92)
	Yes	2 (28.57)	6 (20)	9 (23.08)
maternity pen	No	7 (100)	28 (93.33)	36 (92.31)
	Yes	0 (0)	2 (6.67)	3 (7.69)
Cleaning of calving pen	Flushing with water	0 (0)	2 (6.67)	4 (10.56)
	Disinfection with detergent	0 (0)	1 (3.33)	2 (5.13)
	None	7 (0)	27 (90)	33 (84.62)

Table 4. Results of RBPT and CFT of bovine brucellosis by origin/study area.

Origin	RBPT	CFT
	No. positive (%)	No. positive (%)
Agarfa	28 (8.59)	24 (7.36)
Berbere	19 (4.30)	14 (3.17)
Overall	47 (6.12)	38 (4.95)

Pearson $\chi2(1) = 7.019$; Pr = 0.008; N: Number of cattle tested.

Table 5. Seroprevalence of bovine brucellosis in selected kebeles of the study districts.

Selected kebeles	Positive samples (N)	Negative samples (N)	Total	Prevalence (%)
Agarfa				
Ali	7	75	82	8.54
Agarfa atvet	11	117	128	8.59
Elani	6	110	116	5.17
Berbere				
Sirrima	7	171	178	3.93
Galma	5	147	152	3.29
Ekata	2	110	112	1.79

Pearson $\chi2$ (5) = 9.536; Pr = 0.090; Pr=Precision value

Table 6. Association of bovine brucellosis seropositivity with animal-level risk factors.

Variables	Level	No tested	No positive (%)	χ2(P value)
Sex	Male	285	6 (2.11)	7.786(0.005)
	Female	483	32 (6.63)	
Breed	Local	653	20 (3.06)	32.951(0.000)
	Cross	115	18 (15.65)	
Age	< 5 years	368	11 (2.99)	5.764(0.016)
	≥ 5 years	400	27 (6.75)	
Origin	Agarfa	326	24 (7.36)	7.019(0.008)
	Berbere	442	14 (3.17)	
Parity	No parity	312	7 (2.24)	8.171(0.017)
	Primiparous	118	8 (6.78)	
	Pluriparous	338	23 (6.81)	
Pregnancy	No	654	24 (3.67)	15.305(0.000)
	Yes	114	14 (12.28)	
Herd size	≤ 5	72	3 (4.17)	0.113(0.945)
	[6 - 10]	304	15 (4.93)	
	> 10	392	20 (5.10)	
Body condition	Poor	296	15 (5.07)	0.019(0.990)
	Medium	270	13 (4.81)	
	Good	202	10 (5.95)	

Multivariable logistic regression analysis of risk factors associated with *Brucella* sero positivity: Logistic regression analysis of the effect of different risk factors on the prevalence is presented in Table 7. Accordingly, multivariate analysis revealed that; female animal (AOR=3.086, 95%CI: 1.229, 7.754), cross breed (AOR=5.040, 95%CI: 2.278, 11.150), Pluriparous cows (OR=4.091, 95%CI: 1.280, 13.075) and pregnant animal (AOR=3.601, 95%CI: 1.711, 7.579) were more likely to be infected with *Brucella abortus.*

Association of bovine brucellosis seropositivity with reproductive disorders: Table 8 shows association of reproductive disorders with prevalence of bovine brucellosis. The frequency of anti-*Brucella* antibodies was higher in cattle with history of reproductive disorders. Statistical significant difference in seropositivity was observed in cattle with history of abortion, retained foetal membrane, still birth, repeat breeding and metritis/endometritis (P<0.001).

Table 7. Multivariable logistic regression analysis of risk factors with *Brucella* seropositivity.

Variables	Level	No. tested	Prevalence (%)	COR (95% CI)	AOR (95% CI)	*p*-value
Sex	**Female**	**483**	**32 (6.63)**	**3.99(1.362, 7.991)**	**3.086 (1.229, 7.754)**	**0.016**
	Male	285	6 (2.11)	1	**1**	
Breed	Cross	115	18 (15.65)	5.873(3.000, 11.497)	5.040 (2.278, 11.150) 1	0.000
	Local	653	20 (3.06)	1		
Age	≥ 5 years	400	27 (6.75)	2.349(1.148, 4.804)	2.978 (0.889, 9.969)	0.077
	< 5 years	368	11 (2.99)	1	1	
Origin	Agarfa	326	24 (7.36)	2.430(1.237, 4.773)	1.217 (0.547, 2.711)	0.630
	Berbere	442	14 (3.17)	1	1	
Parity	No parity	311	7 (2.25)	1	1	
	Primiparous	118	8 (6.78)	3.181(1.345, 7.522)	1.721 (0.578, 5.125)	0.330
	Pluriparous	339	23 (6.79)	3.169(1.123, 8.943)	4.091 (1.280, 13.075)	0.017
Pregnancy	Yes	114	14 (12.28)	3.675(1.839, 7.342)	3.601 (1.711, 7.579)	0.001
	No	654	24 (3.67)	1	1	

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference.

Table 8. Association of bovine brucellosis seropositivity with reproductive disorders.

Risk factors	Level	Total No. tested	Number positive (%)	χ2(P- value)
History of abortion	Yes	79	12(15.19)	19.640(0.000)
	No	689	26 (3.77)	
History of retained foetal membrane	Yes	48	9 (18.75)	20.738(0.000)
	No	720	29 (4.03)	
History of still birth	Yes	32	6 (18.75)	13.525(0.000)
	No	736	32 (4.35)	
History of repeat breeding	Yes	43	7 (16.28)	12.435(0.000)
	No	725	31 (4.29)	
History of metritis/endometritis	Yes	39	10 (25.64)	37.408(0.000)
	No	729	28 (3.84)	

Multivariable logistic regression analysis revealed that cattle with history of abortion, retained foetal membrane and metritis/endometritis had a significant effect ($p < 0.05$) on the overall seroprevalence of bovine brucellosis in the study area as depicted in Table 9. Aborted cows were approximately 3.33 times more likely to be affected by brucellosis than non aborted ones. Furthermore, odds ratio indicated that animals with history of metritis/endometritis were 6.8 times more likely to be seropositive to brucellosis than those with no endometritis.

Table 9. Multivariable logistic regression analysis of *Brucella* seropositivity with reproductive disorders.

Risk factors	Level	Total No. tested	Number positive (%)	COR (95%CI)	AOR (95% CI)	P-value
History of abortion	Yes	79	12 (15.19)	4.567(2.204, 9.465)	3.328(1.497, 7.397)	0.003
	No	689	26 (3.77)	1	1	
History of retained foetal membrane	Yes	48	9 (18.75)	5.499(2.435, 12.416)	3.418(1.339, 8.729)	0.010
	No	720	29 (4.03)	1	1	
History of still birth	Yes	32	6 (18.75)	5.07(1.952, 13.203)	1.189(0.160, 8.842)	0.866
	No	736	32 (4.35)	1	1	
History of repeat breeding	Yes	43	7 (16.28)	4.353(1.795, 10.558)	0.593(0.081, 4.359)	0.608
	No	725	31 (4.29)	1	1	
History of metritis/endometritis	Yes	39	10 (25.64)	8.633(3.833, 19.444)	6.842(2.059, 22.737)	0.002
	No	729	28 (3.84)	1	1	

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference.

DISCUSSION

The present serological study revealed the presence of circulating antibodies of *Brucella abortus* among cattle sampled from smallholder farms in Agarfa and Berbere Districts of Bale Zone, South eastern Ethiopia. The overall seroprevalence of *Brucella* antibodies determined with RBPT and CFT in Agarfa and Berbere districts were 6.12% and 4.95%, respectively. Since CFT is the recommended confirmatory test for brucellosis with high specificity (Smith and Sherman, 1994; OIE, 2000; Radostits et al., 2007), the overall seroprevalence of bovine brucellosis in the study area was 4.95%. This seroprevalence is in close agreement with previous findings of 5.2% in Addis Ababa (Asfaw et al., 1998); 4.63% in Northwestern part of Amhara Regional State, Ethiopia (Mussie et al., 2007); 5.2% from Cameroon (Bayemi et al., 2015).

The current finding is slightly higher than the reports of various authors: Bisrat (2007), reported sero-prevalence of 1.9% in Debre-Zeit; Asmare et al. (2007) documented a 2.46% in Sidama Zone of southern Ethiopia. Tesfaye et al. (2011) reported seroprevalence of 1.5% in Addis Ababa; Tolosa et al. (2010) in Jimma area revealed 1.97% and Fekadu et al. (2014), 2.0% from Eastern Showa and Geresu et al. (2016) documented overall prevalence of 1.40% from Asella and Bishoftu towns.

Contrary to the present finding, higher seroprevalence rates were recorded in Ethiopia. For example, Meyer (1980) has indicated a 39% in western

Ethiopia; Asfaw et al. (1998), 8.1% in dairy farms in and around Addis Ababa; Jiksa (2002), 10% in different private dairy farms of Addis Ababa; Hunduma and Regassa (2009), 11.2% in east Shewa zone of the Oromia region; Kebede et al. (2008), 11.0% in Wuchale-Jida district; Ibrahim et al. (2010), 15.0% in Jimma zone of Oromia region.

This relatively low prevalence might be attributable to extensive grazing conditions; these could reduce both animal-to-animal contact and the contamination of pastures under dry climatic conditions (Crawford et al., 1990). Another explanation could be that, in the area studied, most of the farmers replace their animals from their own stock instead of buying animals from markets. Thus, the mixing of cattle from many herds, especially at watering points, is less marked than in the pastoral areas of the country. Furthermore, latent infections occur in some animals whose serological tests gave negative results. In addition, serological diagnosis is considered to be unreliable when applied during the period of 2 to 3 weeks before and after abortion or calving suggesting that false-negative results could occur (Muma et al., 2009; Haileselassie et al., 2010).

The apparent geographical variation in the sero-prevalence of bovine brucellosis might reflect differences in the management systems prevailing in different parts of the country, age, sex and breed among dairy farms, creation of awareness to dairy farm owners in which positive reactors could have been removed, levels of natural immunity and types of test used, that is, sensitivities and specificities of the diagnostic methods used among researchers might also influence the outcome (Kebede et al., 2008; Desalegn, and Gangwar, 2011; Sikder et al., 2012).

The present study revealed higher sero-prevalence rates in female than male animal and significantly associated (P=0.004) with *Brucella* seropositivity. Multiple logistic regression analysis revealed female (OR = 3.086, 95% CI=1.229, 7.754) had higher odds for acquiring brucellosis than male. This finding was consistent with Kebede et al. (2008) and Mergesa et al. (2011) who reported a significant difference ($p<0.05$) between sexes in traditional livestock husbandry practice in Wuchale-Jida district, Southern and Eastern Ethiopia, respectively. This was explained by Radostits et al. (2007) who stated clearly that sex has been one of the risk factors affecting susceptibility of cattle to *Bruella abortus* infection. It was also reported that serological response of male animals to *Brucella* infection is limited. It was indicated that the testes of infected male animals were usually observed to be non-reactors or showed low antibody titers (Crawford et al., 1990). Similarly, one research finding showed that male cattle are more resistant than females (Nicoletti, 1980). Moreover, possibility of venereal transmission being rare limits the extent of spread of brucellosis in males even when the prevalence is high in females (McDermott et al., 2002).

Among the potential risk factors considered in the present study, breed of cattle was shown to have a significant effect ($p<0.001$, OR=5.040, 95% CI = 2.278, 11.150) on the sero-prevalence of bovine brucellosis. The sero-prevalence was higher in cross breed (15.65%) than local or indigenous breed cattle (3.06%) which were endorsed by Omer et al. (2000); Salihu et al. (2011); Patel et al. (2014). The higher prevalence in cross breeds might be due to malnutrition, poor husbandry practices and tropical environmental stress. Moreover, it could be due to the origin of the animal from the previously infected or exposed herds (Deselegn and Gangwar, 2011).

The increased bovine brucellosis seropositivity with age was recorded in present findings with significantly associated differences in prevalence between animals of different age groups ($p<0.05$). It was also reported by others (Bekele et al., 2000; Tolosa, 2004; Berehe et al., 2007 and Kebede et al., 2008). It has been reported that susceptibility of cattle to *B. abortus* infection is influenced by age of the individual animal. Thus, sexually matured and pregnant cattle are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex (Radostits et al., 2000). Brucellosis is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus. On the other hand, younger animals tend to be more resistant to infection and frequently clear infections, although latent infections could occur (Walker et al., 1999). This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits et al., 2000).

The present study revealed that origin of cattle was significantly associated with brucellosis seropositivity ($P<0.05$) and the results showed higher individual animal seroprevalence in Agarfa (7.36%) when compared to Berbere (3.17%). The reasons for the variations in brucellosis seroprevalence among the study areas might be related to the difference in management practicesand agro-ecological factors in the two study areas.

Chi-square analysis of risk factors revealed association betweenparity and seropositivity of bovine brucellosis with P-value < 0.05. This is probably due to increased contact with fetal materials and vaginal discharge from infected cows there by increasing the chance of being infected by *B. abortus*. This is in agreement with the finding of other investigators (Dinka and Chala, 2009; Degefu et al., 2011).

The current finding also showed that antibodies to *Brucella* infection varied with pregnancy ($P<0.001$). The values of odds ratio indicated that pregnant animal were about 3.6 times more likely to be seropositive than

non pregnant and hence, pregnancy was one of the potential risk factors in the study area. This is comparable with the findings of Nahar and Ahmed (2009); Sikder et al. (2012). Infected reproductive tract of cows could act as a potential reservoir for the organisms to propagate and become active during pregnancy which might be the cause behind higher prevalence rate in pregnant cows.

Although the prevalence of bovine brucellosis was not associated with herd size in the present study, Asmare et al. (2007) reported increase of the bovine brucellosis prevalence with herd size. It is generally observed that large herds are characterized by increased stocking density and increased risk of exposure to infection especially following abortion (Nicoletti, 1980; Berehe et al., 2007).

Sero-prevalence of brucellosis was higher in cattle that had a history of reproductive disorders. Chi-square analysis indicated that history of abortion, retained foetal membrane, still birth, repeat breeding and metritis/endometritis were significantly associated with brucellosis seropositivity ($p < 0.05$). It was also statistically identified that reproductive disorders were the major risk factors for *Brucella* seropositivity. The prevalence rate of reproductive disorders in the current study was in agreement with other investigators (Berehe et al., 2007; Desalegn and Gangwar, 2011; Tesfaye et al., 2011; Azevedo et al., 2011; Sikder et al., 2012; Chand and Chhabra, 2013; Tebug, 2013; Patel et al., 2014). This could be explained by the fact that reproductive disorders are typical outcome of brucellosis (Swell and Brocklesby, 1990; Radostits et al., 2007). In addition, in highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is a cardinal feature of the disease (Radostits et al., 2007). Some scientists also found higher prevalence of brucellosis with reproductive disorders but their association with prevalence was non-significant (Kebede et al., 2008; Al-Majali et al., 2009; Ibrahim et al., 2010; Tedele et al., 2010; Makita et al., 2011).

The results of the questionnaire survey revealed that in all the study areas, almost all of the respondents were not aware of bovine brucellosis. Lack of knowledge and awareness about the disease and information on the zoonotic potential of brucellosis signify that farmers do not take required precautions when handling *Brucella* infected animals; and products and by products from infected animals thus jeopardizing their health. All of the respondents in this study with the small herd size dispose after birth & aborted materials by throwing elsewhere to open dump. In addition, most proportions of the households have habit of milk consumption without boiling or pasteurization. Moreover, majority of the respondents did not use protective clothes while handling aborted foetuses, afterbirths and parturient animals. With these results it is obvious that no precaution is taken to prevent spread of the disease to other herds within or outside the study area. Presence of strong association between brucellosis and abortion as well as retained placenta was indicative of risk to cattle attendants and professionals working in the area without precautions and protective clothes (Radostits et al., 2007). Since most of the owners were observed to consume raw milk, the risk from the disease could be high. These underline the risk of transmission of brucellosis to the owners in the area (Kebede et al., 2008; Tesfaye et al., 2011).These factors combined with the poor cleaning practice by the owners could pose a great risk of the spread of the disease to unaffected animals and humans (Tolosa, 2004; Geresu et al., 2016).

Conclusion: In the present study, bovine brucellosis was prevalent in smallholder cattle farms in the study districts. Female animals, older cattle, origin, local breeds, cattle with history of abortion, retained foetal membrane, still birth, repeat breeding, metritis/endometritis were the risk factors significantly associated with *Brucella* seropositivity in the study areas. Most respondents have no awareness about brucellosis and its zoonotic implications. In addition, almost all of the owners in the study area handled aborted foetuses and foetal membranes without precautions and protective clothes. Furthermore, they consumed raw milk from potentially infected cow. Moreover, aborted materials were disposed unsafely. All the above-mentioned factors could contribute to the wide spread occurrence of brucellosis both in animals and humans in the study areas. Therefore, more proactive measures should be taken to protect the cattle populations from *Brucella* infection and to reduce its economic impact to the dairy industry and the risk of zoonotic infection in exposed human population in the study areas. Furthermore, epidemiological study to investigate the link between bovine and human brucellosis in the present study area is recommended.

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