

Sero-Prevalence of Bovine Brucellosis and its Associated Risk Factors in Becho District, South West Shewa, Oromia Regional State, Ethiopia

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Abstract: A cross-sectional study was conducted in selected areas of Becho district, Oromia regional state from December, 2018 to May, 2019 to estimate the sero-prevalence of bovine brucellosis in the study area and identify the associated risk factors for the seropositivity. Simple random sampling method was used for sampling each herd and animal during serum collection, and risk factors accessed through interviewee of the animal owners. A total of 384 serum samples were collected from cattle of above 6 months of age and sera were initially screened with Rose Bengal Plate Test (RBPT) and those samples found positive by RBPT were further tested by Indirect Enzyme-linked Immuno Sorbent Assay (I-ELISA) for confirmation. Based on the result, 13(3.39%) were positive using RBPT and the overall sero-prevalence of bovine brucellosis at animal-level estimated to be 4(1.04%) based on I-ELISA result. At herds' level, an overall sero-prevalence of 5.48% was obtained. Pearson's Chi-square revealed that the presence of abortion history, retained fetal membrane and breed type were significantly associated with the seropositivity of bovine brucellosis ($p < 0.05$) with Chi-square value of 38.2, 5.76 and 3.99 and P-value of 0.000, 0.016 and 0.046, respectively. The odds of having brucellosis increased by 12.4 times in cow with history of abortion compared to cow without the history, increased by 4.5 times in the animal suffered from history of retained placenta compared to cow with no history of retained placenta and 3.1 times in cross-breed than indigenous breeds. In conclusion, the study demonstrate that the prevalence of bovine brucellosis was low in Becho district. However, poor understanding of brucellosis among communities and a high level of risky practice might result in an increased transmission between animals and zoonoses in risky group personnels. Thus, awareness creation about impacts of the disease, zoonotic importance, risk factors and method of prevention were recommended.

Keywords: Bovine brucellosis, cattle, I-ELISA, RBPT, Risk factors, sero-prevalence

1. INTRODUCTION

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide and it is considered as one of the most widespread zoonosis in the world (Asgedom *et al.*, 2016). The disease affects domestic animals (cattle, sheep, goat, camel and pig), human and wildlife (Takele, 2016). Bovine brucellosis usually caused by *Brucella abortus* and occasionally by *B. melitensis* and *B. suis* (Megersa *et al.*, 2012; Kebede *et al.*, 2008), all of which are facultative intracellular, gram-negative, non-motile, partially acid-fast coccobacilli that lacks capsule or endospores.

The disease is characterized by trimester abortion at first gestation, weak calves, still birth, endometritis, infertility, reduced milk production and is mainly caused by biovars (mainly biotype-

1) of *B. abortus* (Geresu *et al.*, 2016; OIE, 2008), whereas bulls can develop orchitis, epididymitis, seminal vasculitis and sterility (Megid *et al.*, 2010; Kebede *et al.*, 2008). Furthermore, since brucellosis is an important cause of abortion especially in first calf heifers, the disease can also cause important economic losses in developing countries (Megersa *et al.*, 2012).

The most common route of transmission is ingestion of contaminated pasture, feed, fodder, water, and after birth; aborted fetuses, uterine discharges and new born calves, which contains large doses of infectious organism and constitutes a very important source of infection (Geresu *et al.*, 2016). However, infection through injured/intact skin, the mucosa at the respiratory system and conjunctiva frequently occurs (Tsfaye *et al.*, 2017; Radostits *et al.*, 2007).

Although the livestock sector in Ethiopia has a significant contribution to the national economy; productivity (meat and milk) per animal is very low, majorly due to technical constraints and disease like brucellosis (Shiferaw *et al.*, 2003). A large number of studies on bovine have been reporting brucellosis sero-prevalence ranging from 0 to 50% in intensive management systems (Lakew *et al.*, 2019; Yohannes *et al.*, 2013; Alem and Solomon, 2002; Belihu, 2002) and 0.05 to 15.2% in extensive management systems (Garoma, 2018; Degefa *et al.*, 2011).

Sero-prevalence of bovine brucellosis had been established in different parts of the country, but still there is no online information on status of the disease in Becho district. Further assessment of the status of disease and the associated risk factors has paramount importance that can be used for research, control and prevention of brucellosis in Ethiopia as general and specifically in the study site. Hence, the current study was undertaken to estimate the sero-prevalence of bovine brucellosis and to assess the risk factors

associated with bovine brucellosis in Becho district.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in Becho district, Oromia regional state, central Ethiopia, which is located at 80kms South-West of Addis Ababa, at an altitude of 2,193 meters above sea level. Its annual temperature is 17.2°C and the mean annual rainfall is 1,148 mm (NMSA, 2011). The livestock population of the area includes 101,282 cattle, 21,631 sheep, 19,521 goats and 20,954 equines according to 2010EC data from Office of Livestock Development and Resources of the Becho district. The town has human population density of 99,090 (CSA, 2013). The area was selected based on gap of no previous published literature on the prevalence of bovine brucellosis. Geographically, the study areas cover latitude and longitude ranges of 8°31' to 8°45' North and 38°7.5' to 38°24.5' East respectively. The district having the study area found is briefly sketched as illustrated in (Figure 1).

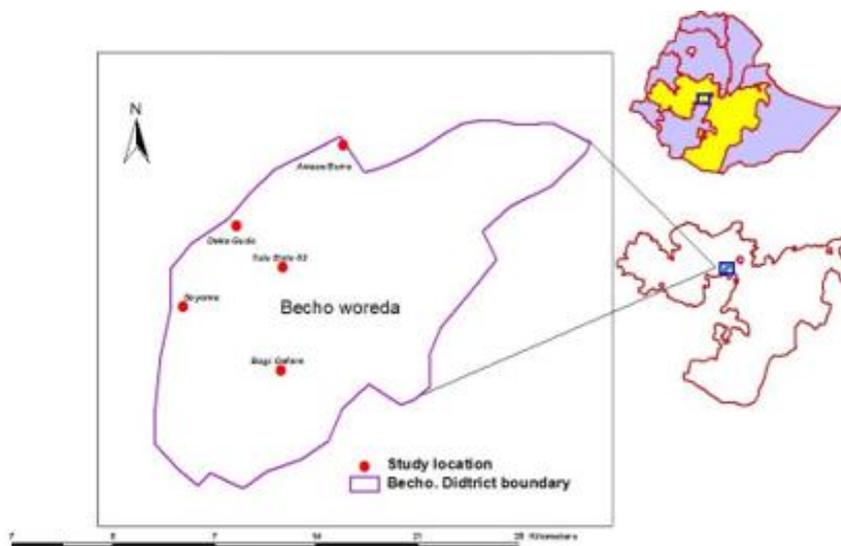


Figure1. Map of Ethiopian showing the study area

2.2. Study Populations

The target populations consisted of cattle that were managed under the extensive and semi-intensive (mixed-crop livestock) production system. The cattle under study comprised of the local breeds and cross breeds with no history of vaccination against brucellosis. Both sexes and different age group greater than six month were included in the study as the disease was not common in the cattle less than 6 months of age due to maternal antibody. Individual animals

were categorized as young (≤ 36 months) and adult (> 36 months) according to (Geresu *et al.*, 2016).

2.3. Study Design and Method

A cross-sectional sero-prevalence study and questionnaire survey of bovine brucellosis was conducted in selected areas of Becho district from December, 2018 to May, 2019 to determine the sero-prevalence of bovine *Brucella* infection in cattle and to estimate the associated risk factors for the seropositivity. Simple random

sampling method was implied to sample herds and individual animals.

2.4. Sampling Methodology and Sample Size Determination

The list of householders/animal owners was obtained from Office of Livestock Development and Resources of the Becho district. Since herds were regarded as the primary sampling units, 73 herds were selected randomly from one urban and four rural kebeles based on sampling frame. The herd sizes were classified in to small (1-15 animals), medium (16-30 animals) and large (> 30 animals) according to number of animals within the herd according to (Megersa *et al.*, 2011b). The individual animal sampling activities were done using the simple random sampling.

To determine the sample size, an expected prevalence of 50% was taken into consideration since there was no online information about the status the disease at Becho district beforehand. The desired sample size for the study was calculated using the formula given by (Thrusfield, 2018) with 95% confidence interval and 5% absolute precision.

$$n = \frac{Z^2 * P_{exp} * (1 - P_{exp})}{d^2}$$

Where n= required sample size, Z= reliability coefficient (1.96 at d= 0.05 or 95% CI), P_{exp} = expected prevalence (50%) and d= desired absolute precision (95% CI).

$$n = \frac{1.96^2 * 0.5 * (1-0.5)}{0.05} = 384$$

Therefore, the total numbers of animal to be sampled from the area were 384.

2.5. Sample Collection and Submission

2.5.1. Questionnaire Survey

Parallel to collection of serum samples, a structured questionnaire survey (Annex 2) was administered to respondents whose cattle were included in the study through interviewee by local language. The questionnaire was focused on herd-level covariates such as herd size, categorized as small, medium and large, the presence of separated calving pen, source of replacement stock (raise own farm, market or both), abortion history, history of retained fetal membrane, management system (extensive and semi-intensive) and ways of keeping livestock (keep all species together and separate all species

In different barn) were recorded. The method of disposing placenta and aborted fetus was categorized into deep bury, dispose into the environment and allow to eaten by carnivores. Awareness of the owners about brucellosis was also coded.

2.5.2. Sample Collection and Transportation

Blood samples were collected aseptically from jugular vein of individual animals selected during sampling for serological survey purpose. Thus, from each selected animal, approximately 10ml of blood was collected using plain vacutainer tubes and needle. Identification for each of the animal was labeled on corresponding vacutainer tube. The collected blood samples were kept overnight to allow clotting in slant position at room temperature at Becho wereda veterinary clinic. Then, the sera were carefully decanted into 1.8ml labeled cryovials without mixing with the clotted blood. The harvested sera were then taken to National Animal Health Diagnostic and Investigation Center (NAHDIC), bacterial serology laboratory using icebox, and stored at - 20°C until further processing.

Information regarding the individual animal attributes such as kebele, breed, sex, age, abortion history, pregnancy and parital status of each sampled animals was recorded along the blood collected (Annex 1).

2.6. Serological Diagnostic Tests

2.6.1. Rose Bengal Plate Test

All serum samples collected were screened using RBPT according the procedures described by the World Organization for Animal Health (OIE, 2004) and manufactures' instruction. RBPT contains *Brucella* antigen strain 99 (from ID.vet, RSA-RB-016, 0112 GB, 310, rue Louis Pasteur-Grables- FRANCE). Before performing the test, antigen and sera were taken out of refrigerator and left at room temperature for half an hour before the test commences to maintain to room temperature and processed following the recommended procedures. Briefly, 30 µl of sera samples were dispensed on to the plate, and the same volume of RBPT antigen were dropped alongside the sera. The antigen and the sera was then mixed thoroughly by using an applicator stick, the plate was shacked for about 4 minutes, and examined for agglutination immediately after the mentioned minute (Annex 3).

2.6.2. Indirect Enzyme-Linked Immuno Sorbent Assay (I-ELISA)

For further analysis of RBPT positive samples, I-ELISA was performed using a commercial I-ELISA kit (from ID.vet, BRUS-MS-5P, C35, 1014 GB, 310, rue Louis Pasture- Grables FRANCE) to detect antibodies directed against *brucella abortus* in cattle serum sample, and the protocol provided by the developers and (OIE, 2004) was followed precisely. Before performing the test, the serum and all reagents taken out of the refrigerator and left at room temperature for half an hour and homogenized by inversion. The serum and controls were added to microwells, were coated with *brucella abortus* LPS, at a diluted rate of 1/20. Following the addition of the a multi-species horseradish peroxidase (HRP) conjugate and substrate solution (TMB) at a recommended strength, the plate was incubated and examined for the intensity of reaction with an automated ELISA reader at 450 nm. The developed yellow color within a well after addition of stop solution indicates that the tested serum has antibodies to *Brucella*.

The test were valid if the mean OD value of positive and ratio of positive to negative controls were calculated as $OD_{PC} > 0.350$ and $OD_{PC}/OD_{NC} > 3$, respectively. The result was interpreted by Seropositivity percentage (S/P %) of $\geq 120\%$ as positive and $\leq 110\%$ as negative (Annex 4).

2.7. Data Management and Analysis

Data collected during the study period, risk factors believed to be associated with the occurrence of bovine brucellosis, serological and questionnaire survey were, entered into Microsoft Excel spreadsheet and analyzed using standard software programs (STATA version 9.0) and SPSS version 22. The sero-prevalence at individual animal level was calculated by dividing the number of positive animals by the total number of animals tested. Herd prevalence was calculated by dividing the number of herds with at least one reactor by the number of all herds tested. Pearson’s Chi-square test was utilized to measure the association between the potential risk factors and the seropositivity. After the association of exposure variables with *Brucella* seropositivity was analyzed at individual animal level by the Chi-square test, those variables significantly associated with *Brucella* seropositivity (breed type, abortion

history and RFM) were further analyzed by multivariable logistic regression. A multivariable logistic regression model was used to identify the potential risk factors associated with *Brucella* infection in animal and variables with a p-value of < 0.05 (in Chi-square analysis) were included in the multivariable logistic regression model. Odds ratio (OR) was utilized to measure the degree of association between significant risk factors and sero-prevalence of bovine brucellosis. For statistical inference, p-value < 0.05 (at 5% level of significance) was considered as statistically significant.

3. RESULTS

3.1. Sero-Prevalence of Anti-Brucella Antibodies at Animal and Herd Level

In the current study, a total of 384 cattle sera were collected from 145 males and 239 females, which includes 288 (75%) indigenous and 96 (25%) cross-breeds of indigenous zebu and Holstein Friesian were tested for bovine brucellosis. Of them, 13 (3.39%) and 4 (1.04%) were tested positive for *Brucella* antibody at individual level by RBPT and I-ELISA, respectively. The herd level sero-prevalence was 12 (16.44%) and 4 (5.48%) by RBPT and I-ELISA, respectively. The overall animal level sero-prevalence of bovine brucellosis of the present study was 1.04%. The sero-prevalence distribution of bovine *brucella* infection in RBPT and I-ELISA tests for female (dairy cattle) animas with the prevalence of 12 (5.02%) and 4 (1.67%), respectively were presented in the following (Table 1).

Table1. Sero-prevalence of bovine Brucellosis by RBPT and I-ELISA tests

Types of test	Sero-prevalence		
	Both sexes	Dairy cattle	Herd-Level
RBPT (positive)	13 (3.39%)	12 (5.02%)	12 (16.44%)
I-ELISA (positive)	4 (1.04%)	4 (1.67%)	4 (5.48%)

3.2. Chi-Square Analysis of Association of the Potential Risk Factors with Brucella Seropositivity Based on Sex

According to the results obtained from the present study, 3.13% and 0.0% of sero-prevalence were recorded in female and male animals, respectively. A Chi-square analysis revealed that breed type were significantly

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associated with the seropositivity of bovine brucellosis ($P < 0.05$). Herd size, sex, age, management system, source of replacement stock, ways of keeping livestock and awareness

about brucellosis were insignificantly correlated ($P > 0.05$) with the seropositivity of *Brucella* infection at individual animal level (Table 2).

Table2. Association of potential risk factors with *Brucella* seropositivity based on sexes

Variables	Level	No. tested	Prevalence %	[95% CI]	χ^2	P-value
Kebeles	Boji	54	1.85(1)	-0.0178 - 0.0541	5.492	0.240
	A/Guda	55	3.64(2)	-0.0137 - 0.0864		
	Soyema	121	0			
	A/Bune	142	0.71(1)	-0.0068 - 0.0208		
	T/Bolo	12	0			
Breed	Local	288	0.35(1)	-0.0033 - 0.0102	5.389	0.020*
	Cross	96	3.13(3)	-0.0038 - 0.0663		
Sex	Male	145	0		2.452	0.117
	Female	239	3.13(3)	0.0003 - 0.0330		
Age	Young	107	0.93(1)	-0.0090 - 0.0277	0.016	0.898
	Adult	277	1.10(3)	-0.0014 - 0.0230		
Herd size	Small	56	0		1.113	0.573
	Medium	130	0.77(1)	-0.0074 - 0.0228		
	Large	198	1.50(3)	-0.0019 - 0.0322		
	Extensive	289	1.4(4)	0.0003 - 0.0273		
Management system	Semi-intensive	95	0		1.328	0.249
Source of replacement stock	Own farm	38	0		2.384	0.303
	Market	147	2.1(3)	-0.0025 - 0.0434		
	Both	199	0.5(1)	-0.0048 - 0.0149		
Ways of keeping livestock	All together	297	1.34%(4)	0.0002 - 0.0266	1.184	0.277
	Separating all	87	0			
Awareness about brucellosis	Yes	12	0		0.131	0.718
	No	372	1.1%(4)	0.0002 - 0.0212		
	Total	384	1.04(4)	0.0002 - 0.0206		

*: indicates significant at $p < 0.05$; χ^2 - Pearson's Chi-square; **CI**: confidence interval

The following, table 3 shows the result of Chi-square test analysis of the association between risk factors and *Brucella* infection seropositivity in dairy cattle. It revealed that the presence of abortion history, retained fetal membrane and

breed type were significantly associated with the seropositivity of bovine brucellosis ($p < 0.05$) with Chi-square value of 38.2, 5.76 and 3.99 and P-value of 0.000, 0.016 and 0.046, respectively.

Table3. Association of potential risk factors with *Brucella* seropositivity in dairy cattle

Variables	Level	No. Tested	Prevalence%	[95% Conf. Interval]	χ^2	P-value
Breed	Local	168	0.59(1)	-0.005 - 0.017	3.9965	0.046*
	Cross	71	4.23(3)	-0.005 - 0.089		
Age	Young	75	1.30(1)	-0.012 - 0.039	0.0769	0.782
	Adult	164	1.82(3)	-0.002 - 0.038		
Parity	No parturition	67	0		1.6184	0.445
	Single parturition	49	1.10(1)	-0.019 - 0.060		
	Multi parturition	123	2.43(3)	-0.003 - 0.051		
Pregnancy status	Pregnant	94	2.07(3)	-0.002 - 0.044	0.3501	0.055
	Non-pregnant	145	1.06(1)	-0.010 - 0.031		
History of RFM	Present	28	7.14(2)	-0.026 - 0.169	5.7649	0.016*
	Not present	211	0.94(2)	-0.003 - 0.022		
Abortion history	Aborted	23	17.4(4)	0.014, 0.333	38.204	0.000*
	Non aborted	216	0			
Herd size	Small	33	0		0.8963	0.639
	Medium	74	1.35(1)	-0.013 - 0.040		

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	Large	132	2.30(3)	-0.002- 0.048		
Management system	Extensive	178	2.24(4)	0.001- 0.044	1.3941	0.238
	Semi-intensive	61	0			
Ways of disposing placenta/aborted fetus	Deep burry	10	0			
	Dispose to environment	66	3.00(2)	-0.011- 0.072	1.106	0.575
	Allow to eaten by carnivores	163	1.23(2)	-0.004- 0.029		
Source of replacement stock	Raise own farm	23	0		2.4909	0.288
	Market	90	3.31(3)	-0.0041- 0.070		
	Both	126	0.80(1)	-0.0076, 0.0235		
Calving pen	Yes	29	0	-	5.618	0.454
	No	210	1.92(4)	0.000- 0.037		
Ways of keeping livestock	All together	180	2.22(4)	0.000- 0.043	1.333	0.248
	Separating all	59	0			
Awareness about brucellosis	Yes	10	0		0.1776	0.678
	No	229	1.75(4)	0.001- 0.034		

*: indicates significant at $p < 0.05$; χ^2 - Pearson's chi-square; CI: confidence interval

3.3. Multivariable Logistic Regression Analysis of Risk Factors Associated with Brucella Seropositivity

The odds ratio indicated that cross-breed animals were 3.1 times more likely to develop brucellosis than indigenous animals. Regarding the effect of abortion, animals with the history of abortion had significantly higher prevalence (17.4%) and OR

indicated that 12.4 times more likely to develop brucellosis than animals without history of abortion. There was also a trend of increment in individual animal sero-prevalence with history of retained fetal membrane. The OR indicated that animals with the history of retained fetal membranes were 4.5 times more likely to develop brucellosis than animals without history of retained fetal membranes (Table 4).

Table 4. Degree of association of potential risk factors with the Brucella infection in dairy cow by multivariable logistic regression

Variables	Level	OR	[95% CI]
Breed	Local	0.331	0.061 - 1.809
	Cross	3.065	1.694 - 5.543
RFM	Present	4.519	1.590 - 12.848
	Absent	0.562	0.211 - 1.499
Abortion	Aborted	12.37	8.037 - 19.034
	Not aborted	-	-

OR: odds ratio; CI: confidence interval; RFM: retained fetal membrane

4. DISCUSSION

The overall sero-prevalence of *Brucella* antibodies determined with RBPT and I-ELISA in Becho district, Oromia regional state were 3.39% and 1.04%, respectively. Since I-ELISA is the recommended diagnostic test for brucellosis with higher sensitivity and specificity (Getachew *et al.*, 2016; Uzal *et al.*, 1995), the overall sero-prevalence of bovine brucellosis in the study area is 1.04%. This low sero-prevalence is in agreement with the earlier report of (Meles and Kibeb, 2018) with the same prevalence in the Chencha district, GamoGofa zone, South-Eastern Ethiopia, (Adugna *et al.* 2013; Kang'ethe *et al.*, 2007) with 1% in the Benishangul Gumuz region of North-Western Ethiopia and Nairobi, Kenya, respectively. It is comparable with other previous

reports from different part of Ethiopia; (Terefe *et al.*, 2017) with 1.3% in Eastern Ethiopia, (Degefu *et al.*, 2011) with 1.38% in Jijjiga Zone of Somalia regional state, (Geresu *et al.*, 2016; Gumi *et al.*, 2013) with 1.4% in Bishoftu and Asella, central Ethiopia and South-Eastern pastoral livestock of the country, respectively, (Mekonnen *et al.*, 2010) with 1.49% in Western Tigray Zone, North West Ethiopia, (Tesfaye *et al.*, 2011) with 1.5% in Addis Ababa dairy farms, (Asmare *et al.*, 2010) with 1.66% in Sidama Zone, Southern Ethiopia, (Yohannes *et al.*, 2012) with 1.97% in Guto-Gida district, East Wollega zone, (Alemu *et al.*, 2014) with 2% in Eastern Shewa Zone, central Ethiopia.

On the other hand, there were reports with a relatively higher sero-prevalence rate of bovine

brucellosis in other parts of the country; (Dinka and Chala, 2009) with 11.2% in pastoral and agro-pastoral areas of East Shewa Zone, (Kebede *et al.*, 2008) with 11.0% in Wuchale-Jida district, central Ethiopia, (Megersa, *et al.*, 2011a) with 10.6% in Borana, (Eticha *et al.*, 2018) with 9.87% in Asella organized dairy farm, South East Ethiopia, (Megersa *et al.*, 2012) with 8.0% in pastoral region of the country, (Haileselassie *et al.*, 2011) with 4.9% in Western Tigray, (Tibesso *et al.*, 2014) with 4.3% in Adami Tulu, central Ethiopia, (Berhe *et al.*, 2007) with 3.19% in the extensive cattle production system of Tigray region, Northern part of the country, (Ibrahim *et al.*, 2010) with 3.1% in Jimma Zone of Oromia region. Similarly, relatively higher sero-prevalence were reported in other African countries; (Angara *et al.*, 2004) with 24.5% in Sudan; (Mai *et al.*, 2012) with 24.0% in Nigeria, (Mensah *et al.*, 2011) with 21.9% in Ghana, (Matope *et al.*, 2011) with 5.6% in Zimbabwe, (Swai and Schoonman, 2010) with 5.3% in Tanzania.

On the other hand lower sero-prevalence was recorded in certain part of the country: (Garoma, 2018) with 0.73% in Jimma zone, Western Ethiopia, (Sarba *et al.*, 2016) with 0.49% in Western Shewa, (Asmare *et al.*, 2007) with 0.4% in urban dairy farms of Northern Ethiopia and Sebeta while (Asmare *et al.*, 2013) in Nazareth, Gondar, and Mekele (possibly due to low sample size in their study), (Bashitu *et al.*, 2015) with 0.2% in Debrebirhan and Ambo Towns, (Degefa *et al.*, 2011) with 0.05% in Arsi zone, and (Lakew *et al.*, 2019; Alem and Solomon, 2002) were unable to find even a single positive reactor in intensive dairying farms in Fafan Zone of Ethiopian-Somali and central Ethiopia, respectively.

In this study breed was supposed to be one of the risk factors, consequently sero-prevalence was found to be higher in cross-breed animals (3.13%) than local breeds (0.35%). This difference was statistically significant ($p < 0.05$) which is similar to (Ndazigaruye *et al.*, 2018; Eticha *et al.*, 2018; Minda *et al.*, 2016; Alemu *et al.*, 2014; Yohannes *et al.*, 2012; Jergefa *et al.*, 2009) reported significant variation on serological prevalence of brucellosis with higher prevalence in cross-bred than in local ones. In contrast to this finding, (Geresu *et al.*, 2016a; Deselegn and Gangwar, 2011) reported that, the significance of breed might be due to the origin of the animal from the previously infected or

exposed herds and limitation in sample sizes of local breeds.

Sex has been one of the risk factors affecting susceptibility of cattle to *Brucella abortus* infection (Yohannes *et al.*, 2012). Seropositivity in females (3.13%) was indicated in study area where as no seropositive in males; this finding is in agreement with previous report by (Garoma, 2018; Adugna *et al.*, 2013). This implies that female cattle were at higher risk than males. The lower prevalence of male reactors in this study could be due to smaller number of males tested as compared to female and it was also reported that the serological response of male animal to *Brucella* infection is limited (Mohammed, 2009; Berhe *et al.*, 2007). In addition, it has also been reported that infected male animals are usually found to show low antibody titers (Godfroid *et al.*, 2002).

It is well known that sexually mature animals are more susceptible to *Brucella abortus* infection (Garoma, 2018; Berhe *et al.*, 2007), which could be explained by the fact that susceptibility increased during sexual maturity and gestation period due to the influence of sex hormones and elevation of fetal fluid and erythritol sugar in the placenta. This stimulates the growth and multiplication of the bacteria in the reproductive organs (Asgedom *et al.*, 2016; Hileselassie *et al.*, 2008; Radostitis *et al.*, 2007). Accordingly a higher sero-prevalence (3.13%) in older age category (greater than 36 months) and seronegativity in younger age category (6 months - 36 months) was found in present study. This finding was in consistent with report of (Ibrahim *et al.*, 2010; Abebe *et al.*, 2008; Hailemeleket *et al.*, 2007).

The existence of previous history of abortion and retained fetal membrane was, as expected, significantly associated ($p < 0.05$) with *Brucella* seropositivity in the present study. A sero-prevalence of 17.4% and 7.14% were recorded for the occurrence of previous abortion and RFM in this study areas based on questionnaire survey. This finding was consistent with (Tolosa, 2004) who reported 17.6% in selected sites of Jimma Zones and (Geresu *et al.*, 2016) 17.39% in Asella and Bishoftu. This could be explained by the fact that abortions and/or retained placenta are typical outcomes of brucellosis (Eticha *et al.*, 2018; Geresu *et al.*, 2016; Alemu *et al.*, 2014). Other studies have also shown a significant association between seropositivity, and history of abortion and RFM (Alemu *et al.*, 2014; Adugna *et al.*,

2013; Ibrahim *et al.*, 2010; Tolosa *et al.*, 2008). Similarly, a number of studies in different African countries also show that individual animal brucellosis sero-prevalence correlates with the presence of abortions (Muma *et al.*, 2012; Schelling *et al.*, 2003; McDermott *et al.*, 2002; Kubuafor *et al.*, 2000).

On the basis of parity, the difference observed in sero-prevalence was statistically insignificant. Similar observations were made by (Geresu *et al.*, 2016a; Berhe *et al.*, 2007). Although there is insignificant association between parity and brucellosis seropositivity, the higher sero-prevalence was observed in multi-parturition (2.43%) than single-parturition cattle (1.10%) in the study areas. The higher sero-prevalence of brucellosis in the multi-parturition cattle of this study was in line with (Geresu *et al.*, 2016; Asmare *et al.*, 2013) who reported 2.41% and 2.5% in multi-parturition dairy cattle respectively.

Herd size is another risk factor that affects occurrence of brucellosis (Yohannes *et al.*, 2013). In this study significant differences of *Brucella* sero-prevalence among three herd size categories, which was 1.5%, 0.77% and 0% in the large (>30 animals), medium (16-30 animals) and small (1-15 animals) herds, respectively. This revealed that comparatively higher seropositivity in the larger herd categories, which agreed with the findings of (Geresu *et al.*, 2016; Berhe *et al.*, 2007). However, in contrary to this, (Kebede *et al.*, 2008) reported that the risk of *Brucella* seropositivity was independent of herd size in small holder farms from Wuchale-Jida district of east Wollega Zone of Ethiopia. Higher seropositivity in large herd size can be explained by the fact that an increase in herd size is usually accompanied by an increase in stocking density, one of the determinants for exposure to *Brucella* infection especially following abortion or calving (Crawford *et al.*, 1990; Geresu *et al.*, 2016).

5. CONCLUSION AND RECOMMENDATIONS

Bovine brucellosis has a major impact on human health, besides causing significant economical losses in dairy industry. The present study revealed that the overall sero-prevalence of bovine brucellosis in smallholders of Becho district was relatively low at individual animal and herd level. There was strong association between abortion, retained fetal membrane and breed seropositivity for bovine brucellosis in the study area. Moreover, the finding demonstrated a poor understanding of brucellosis among

communities and a high level of risky practice being undertaken that is inevitably, might result in an increased transmission between animals and zoonoses in risky group personnels. Therefore, on basis of the above conclusion the following points were recommended:

- Awareness creation among the people about the impact of the diseases, modes of transmission, risk factors and methods of prevention of the diseases should be undertaken in the study area.
- Collaboration as in one health approach needed to be introduced since neither veterinarian alone nor physician alone could not perform all approaches to control such important disease in the country.
- Further detail and fully fledged research is very important to have full and all round information regarding epidemiology of the brucellosis.

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