

## Sero-Prevalence of Bovine Brucellosis and its Associated Risk Factors in Three Districts of Arsi Zone Oromia Regional State, Ethiopia

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**Abstract:** This cross-sectional study was carried out from November 2016 to May 2017 to determine the sero-prevalence and associated risk factors of bovine brucellosis in three selected districts of Arsi zone, Oromia Regional State, Ethiopia. The present study involved testing of 600 animals from cattle of 6 or more months of age with no history of previous vaccination against brucellosis. The Rose Bengal plate test (RBPT) and complement fixation test (CFT) were used for the screening and the confirmation to detect *Brucella* seropositivity. Out of the 26 RBPT positive sera (N=10) were found to be positive to CFT. Accordingly, the overall sero-prevalence of bovine brucellosis was found to be 1.6%. The sero-prevalence of bovine brucellosis in the three districts in Asella, Bakoji and Sagurue were 2.5%, 1.5% and 1, respectively. The sero-prevalence of bovine brucellosis was statistically significantly ( $P < 0.05$ ) associated with the reproductive status and production system of the animal in the study districts. However, there was no statistically significant difference ( $P > 0.05$ ) in the sero-prevalence of bovine brucellosis and age, sex, breed, breeding system, districts. Result of this study indicated that the sero-prevalence of bovine brucellosis in the study area is low. However, there is probable risk of spread of the disease in the unaffected cattle population since there were no precaution measures taken in the areas that should have been practiced by farmers. The public in general and high risk group in particular should be made aware of the zoonotic importance of bovine brucellosis.

**Key words:** Bovine • Brucellosis • CFT • RBPT • Sero-Prevalence

### INTRODUCTION

Ethiopia is a resourceful country with estimated cattle population of 59.5 million [1]. The livestock subsector has an enormous contribution to the national economy and livelihoods of many Ethiopians and still promising to rally round the economic development of the country. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 40% of the agricultural GDP excluding the values of draught power, manure and transport of people and products [2]. It also contributes 15% of export earnings and 30% of agricultural employment [3]. However, trans-boundary and zoonotic animal diseases such as bovine brucellosis constrain the livestock sector of the country and affect livelihoods via their impact on animal health, animal food production, availability and quality.

Bovine brucellosis is primarily caused by *B. abortus*. It is also occasionally caused by *B. melitensis* whenever cattle and sheep or goats are staying together [4, 5]. Clinically bovine brucellosis is characterized by impaired fertility specifically with abortion, metritis, orchitis and epididymitis. Bovine brucellosis has a great impact on both animal and human health as well as tremendous socio-economic impact in developing countries where rural income relies largely on livestock breeding and dairy products [6]. Brucellosis is considered by Food and Agriculture Organization (FAO), World Health Organization (WHO) and World Organization for Animal Health (OIE) as one of the most widespread zoonoses in the world [7].

Brucellosis has been noted as one of the important livestock diseases in Ethiopia demonstrating that the disease is endemic [8-13]. Brucellosis is a public health

problem with adverse health implications both for animals and human beings as well as economic implications for individuals and communities. Management, animal movement, wide ranges of host, herd size, commingling of different animal species is risk factors for animal brucellosis. The possible risk factors for human brucellosis are feeding behavior, occupational exposure, contact with diseased animals or their products and discharges.

Currently, there is an increasing trend of establishment of a number of small and medium dairy farms that supply raw milk and milk products for the communities in Arsi zone. Increase in the demand for milk and milk products will exacerbate zoonotic diseases like brucellosis. In light of the above, the objectives of the current study were to estimate sero-prevalence of bovine *Brucella* antibody circulation in selected districts of Arsi zone of Oromia regional State and to assess the possible risk factors associated with *Brucella* antibody in the study area.

## MATERIALS AND METHODS

**Description of the Study Area:** The study was conducted in three purposely selected districts in central Ethiopia, Oromia regional state of Arsi zone (in and around Asella, Sagure and Bakoji town). These study areas were selected based on the accessibility and availability of high cattle population that constituted the known milk sheds [14]. Asella is located at 175 km southeast of Addis Ababa and the altitude and annual rainfall of the area ranges from 502-4130 meters above sea level, at 07° 57' 43.5''N and 039° 07' 49.0''E and 200-400 mm with mean annual temperature of 22.5°C, respectively. The second study district was Sagure which is located 198kms south east of Asella and climatic condition and topography is the same with Asella. The third study district was in Bakoji district which is located at 225 km south east of Addis Ababa. The area is characterized as bimodal rainfall pattern with mean average rainfall of 940 mm. Altitude and longitude of 7°35'N39°10'E with an elevation of 2810 m. The 2015 animal population data of the Zonal Livestock Development and Health Agency show that Arsi zone holds about 2, 528, 903 cattle, 1, 662, 797 sheep, 738, 729 goats, 240, 559 horses, 20, 337 mules, 421, 7337 donkeys and 1, 885, 492 poultry [1].

**Study Population:** The study animals were indigenous cattle breeds kept under extensive and semi intensive

management system in the area. All cattle in the study area with the age of 6 months or above were considered as the study animals. During sampling of the study animals data related to sex, age, breeding system, production system and reproductive status of all the sampled cattle from the study area was recorded. The status of the disease was determined in relation to categories of; age as young (6 months -2.5 years old) and adult (greater than 2.5 years old), breed (local and crossbred), production system (extensive small holder farming and semi intensive), breeding system (artificial insemination, natural breeding and both), reproductive status (pregnant, lactating and dry) and sex (male and female).

**Study Design:** This cross sectional study was conducted from November 2016 to May 2017 in three districts (Asella, Sagure and Bakoji), Arsi zone, Oromia Regional State to determine the sero-prevalence of bovine brucellosis both on indigenous and crossbred cattle using serological tests of Rose Bengal plate agglutination test (RBPT) and the complement fixation test (CFT) and the role of risk factors such as age, breed, sex, reproductive status of animal, breeding system and different production system systems. Information about these risk factors of all study cattle were recorded during blood collection.

**Sampling Method and Sample Size Determination:** The sampling method used was purposive for the three districts and simple random for study animals. First, the three study districts were selected purposively based on accessibility and availability of high cattle population from Arsi zone of Oromia Regional State [14]. The study extensive small holders and semi-intensive farms were selected through simple random sampling based on the willingness of the owners. The number of animals to be sampled from each extensive smallholder was also determined by the proportion of the livestock population within the herds. From each small holder herds, 2 to 5 study animals were sampled randomly from the three study districts. In addition, 12 farms having mainly crossbred and local dairy cattle were also selected randomly and included in the sample from Asella, Sagure and Bakoji town.

The sample size for each district was determined by the formula recommended by Thrusfield [15] as indicated below:

$$N = 1.96^2 \times PQ/D^2$$

where:

N = required sample size

P = expected prevalence

Q = 1-P

D is the level of precision (5%).

Since there was no previous study carried out on bovine brucellosis in the study area, a 50% expected prevalence as used in the formula. Accordingly, the total number of animals to be bled was 384. The sample size was increased to 600 in order to increase precision and reduce standard error. Hence, a total of 600 cattle (200 each from Asella, Sagure and Bakoji) were considered for this study.

**Collection of Blood Samples:** Approximately 10ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tube and needles. Each sample was labeled by using codes describing the specific animal. Serum was separated from clotted blood by centrifuging. Separated serum was collected in a screw capped sterilized plastic vial and stored at -20°C until tested.

**Serological Test:** The serological tests employed were RBPT for screening and the sera that tested positive to RBPT were further tested using complement fixation test [16]. A screening test with high sensitivity was used to increase the likelihood of detecting a seropositive animal. For practical and cost reasons, the CFT was used to confirm RBT-positive samples only. Only serum samples that were positive to both RBPT and CFT were considered as positive.

The Rose Bengal Plate Test (RBPT): Rose Bengal Plate Test was performed according to the standard procedure described by OIE [16]. The test was carried out at Asella Regional Veterinary Laboratory (ARVL). The antigen of RBPT consists of a suspension of *Brucella abortus* obtained from Institut Purquier 326 (Rue de la Galera, 34097 Montpellier Cedex5, France).

The test uses a suspension of *B. abortus* smooth cells stained with Rose Bengal dye (pink color) to detect Brucella agglutinins. The stained bacterial suspension agglutinates when mixed with samples containing specific IgG or IgM antibodies present in the serum. The results were read by examining the degree of agglutination in good light source and when necessary using magnifying glass deemed. Any visible agglutination was considered positive [16].

**Complement Fixation Test:** Sera, which reacted positive to RBPT, were retested by Complement Fixation Test (CFT) [16] as confirmatory test to eliminate any cross reaction at the National Veterinary Institute (NVI) at Bishoftu. Preparation of the reagents was performed according to the protocols recommended in the OIE [4]. Antigen, control sera and complement were obtained from the BgVV, Berlin, Germany.

The reading of results for the CFT was carried out as follows: When there was complete fixation (no haemolysis) with clear water supernatant, result was recorded as +++, nearly complete fixation (75% clearing) as ++, partial haemolysis (50%) as + and some fixation (25% clearing) as +. Complete lack of fixation (complete haemolysis) was recorded as 0. For positive reactions final titrations was registered (OIE, 2004). Interpretation: Serum with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1: 5 and at least with 50% fixation of complement (2%) at a dilution of 1:10 and at dilution of 1:20 were classified as positive [16].

**Data Analysis:** The collected data were entered and stored in Microsoft excel then transferred and analyzed using STATA [17]. Pearson chi-square was used to evaluate the statistical significance of different risk factors with the result of RBPT and CFT. Univariate and multivariable logistic regression analysis were performed to quantify crude and adjusted effect of pre-specified risk factors on the presence of Brucella antibody. P-value less than 5% ( $P < 0.05$ ) was considered statistically significant. In cases of estimating the effect of different risk factors in terms of Odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its value.

## RESULTS

**Sero-Prevalence of Brucellosis:** The overall sero-prevalence of bovine brucellosis was found to be 1.67% using CFT. Out of 26 sera samples positive for RBPT, 10 (1.67%) were confirmed to be positive with CFT. The sero-prevalence of brucellosis in the three district of Arsi zone ranged from 1% to 2.5%. There was no statistically significant variation in individual animal sero-prevalence of brucellosis among the three districts ( $P > 0.05$ ). Comparatively, the highest sero-prevalence of brucellosis was recorded in and around Asella district (2.5%) followed by Bakoji (1.5%) and Sagurae (1%) district (Table 1).

Table 1: Sero-prevalence of bovine brucellosis in the three selected districts of Arsi zone, Oromia Regional State Ethiopia

Study district	No examined	RBPT	CFT	X <sup>2</sup>	CFT P-value
		No. positive and Sero-prevalence	No. positive and Sero-prevalence		
Asella	200	12(6%)	5 (2.5%)	1.4237	0.491
Bakoji	200	8(4%)	3(1.5%)		
Sagure	200	6(3%)	2(1%)		
Total	600	26(4.33%)	10(1.67%)		

Table 2: Sero-prevalence of bovine brucellosis on the basis of various risk factors as detected by RBPT and CFT in the study areas, P-value based on CFT

Risk factors	No animals tested	RBPT positive	CFT positive	X <sup>2</sup>	P-value
<b>Breed</b>					
Cross	408	19(4.7%)	8(2%)	0.673	0.412
Local	192	7(3.6%)	2(1%)		
<b>Production system</b>					
Extensive	346	5(1.4%)	0(0%)	13.8529	0.000
Semi-intensive	254	21(8.3%)	10(3.9%)		
<b>Age</b>					
Young	151	3 (2.0%)	1 (0.7%)	1.2421	0.265
Adult	449	23 (5.1%)	9 (2%)		
<b>Reproductive status</b>					
Dry	378	10 (2.6%)	4 (1.1%)	7.9615	0.019
Lactating	67	9 (13.4%)	3 (4.5%)		
Pregnant	49	6 (12.2%)	3 (6%)		
<b>Sex</b>					
Female	494	25 (5.1%)	10 (2%)	2.1821	0.14
Male	106	1 (0.9%)	0 (0%)		
<b>Breeding system</b>					
AI	284	18 (6.3%)	9 (3.2%)	4.9151	0.086
Natural	131	6 (4.6%)	1 (0.8%)		
Both	91	1 (1.1%)	0 (0%)		

Table 3: Univariable and multivariable logistic regression analysis of the association between animal-related risk factors and prevalence of bovine brucellosis detected by CFT in the three study districts

Risk factors	CFT positive	Univariate Analysis		Multivariate Analysis	
		OR	P-value	OR	P-value
<b>Reproductive status</b>					
Dry*	4(1.1%)				
Lactating	3(4.5%)	4.38	0.057	4.07	0.104
Pregnant	3(6%)	6.1	0.02	7.9	0.019
<b>Breed</b>					
Cross*	8(2%)				
Local	2(1%)	0.53	0.42	0.44	0.330
<b>Age</b>					
Young*	1(0.7%)				
Adult	9(2%)	3.07	0.289	1.41	0.770

\* Refers to Reference

**Sero-Prevalence in Relation to Breed, Age and Production System:** The sero-prevalence of bovine brucellosis in semi-intensive and extensive production systems was (1.4% and 8.3%) and (0% and 3.9%) on the basis of RBPT and CFT, respectively (Table 2). The recorded difference in the sero-prevalence between the

two production systems was found to be statistically significant ( $P<0.05$ ). Furthermore, there was no statistically significant association ( $P>0.05$ ) between the sero-prevalence of bovine brucellosis and either breed or age of the animal. However, analysis showed that sero-prevalence was higher in crossbred (2%) than local

breed (1%). In case of age group, the sero-prevalence was higher in adult accounting for 2% compared to young with 0.7% (Table 2).

**Sero-Prevalence in Relation to Breeding System, Sex and Reproduction Status:** The sero-prevalence of bovine brucellosis was statistically significantly associated ( $P<0.05$ ) with the reproductive status of the examined animals. Both RBPT and CFT results showed that sero-prevalence of bovine brucellosis was higher in pregnant and lactating animals as compared to dry cows. In both tests, there was a statistically significant difference ( $P<0.05$ ) between the sero-prevalence of bovine brucellosis and reproductive status of examined animals. Concerning sex and breeding systems, there were no statistically significant difference ( $P>0.05$ ) in the sero-prevalence of bovine brucellosis. However, higher seropositivity of brucellosis was recorded in female (2%) than male animals (0%) using CFT. The sero-prevalence of bovine brucellosis was higher in artificially inseminated cows followed by both (natural and AI) and natural bull mated cows (Table 2).

Univariable and multivariable logistic regression analysis of the effect of different risk factors on the prevalence of brucellosis is presented in (Table 3). It was disclosed that there was a statistically significant difference ( $P<0.05$ ) in the sero-prevalence of bovine brucellosis and reproductive status of the examined animals. Accordingly, the sero-prevalence was higher in pregnant animals (6%) compared to lactating (4.5%) and dry cows (1.1%). The OR univariable logistic regression result also showed that pregnant animals were 6.1 times at higher risk than dry animals (OR=6.1). However, lactating animals were not statistically significant by taking dry animals as a reference. The other studied factors (breed and age) did not statistically significant difference ( $P<0.05$ ) in the seropositivity of brucellosis. Thus, further analysis of the association of risk factors with the prevalence using multivariable analysis showed that pregnant animals were 7.9 times at higher risk of infection with brucellosis as compared to dry animals (OR= 7.9). However, the effect of lactation on seropositivity of brucellosis was not important in the multivariate logistic regression analysis.

## DISCUSSION

The present study indicated that the overall sero-prevalence of bovine brucellosis in individual animal determined with RBPT and CFT were 4.33% and 1.67%,

respectively. Since CFT is the recommended confirmatory test for brucellosis with high specificity [18]. The overall sero-prevalence of bovine brucellosis in the study district was 1.67%.

This low sero-prevalence is in agreement with previous findings in different parts of Ethiopia: 1.38% in agro pastoral areas of Jijjiga zone of Somali Regional State [12], 1.92% in Sidama Zone [19], 1.97% in Jimma area [20] and 1.49% in Tigray Region [21]. Contradict to the current study, there were reports with a relatively higher sero-prevalence of bovine brucellosis in other parts of the country. For instance, 11.2 % from pastoral and agro-pastoral areas of East Showa zone [22] and 11% in Wuchale-Jida district [11]. Even a lower sero-prevalence of 0.61% was documented from Jimma [20]. The reason for variations in sero-prevalence of brucellosis in current finding and previous reports could be differences in agro-climatic condition of the study areas, types of livestock management systems, laboratory techniques used in diagnosis of the disease, study design, sample size and gradual development of hygienic practices, use of maternity pen or separation of cows during parturition.

The present study revealed that the overall sero-prevalence of bovine brucellosis was 1.67% in the three district of Arsi Zone and each districts of sero-prevalence was 2.5%, 1.5% and 1% in and around Asella, Bakoji and Sagure, respectively. However, there was no statistically significant association ( $P>0.05$ ) of infection between the districts. Similar to this Adugna [23] reported absence of any significant difference in cattle sero-prevalence between the Dibate and Wembera districts. The absence of significant difference in cattle sero-prevalence between the three districts could be due to the similarity of management practices and agro-climatic condition of the study districts.

Among the potential risk factors considered in the present study, reproductive status of the animal and production system had statistically significant association ( $P<0.05$ ) with seropositivity of bovine brucellosis. With respect to reproductive status of the animal, the significantly higher prevalence of brucellosis was encountered in the pregnant cows than lactating and dry cows. This current study was in agreement with the previously who reported higher sero-prevalence of brucellosis in the pregnant cows [24]. This could be due to erythritol sugar in the placenta and foetal fluid is elevated during gestation period. This stimulates the growth and multiplication of the bacteria in the reproductive organs [25]. The bacterial load often reduced in months following calving and abortion until the next pregnancy [26].

According to Adugna [23] and Tolosa [27], there was no statistically significant association between seropositivity of Brucellosis and reproductive status of the animal.

In case of production system, significantly a higher sero-prevalence was observed in cattle under semi-intensive than in those under extensive small holder farming. Current study was in agreement with previous finding that reported higher sero-prevalence of brucellosis was found among dairy cattle in semi-intensive production systems in highland areas of Ethiopia [24, 8]. On contrary to the present findings Tolosa [20] reported higher prevalence of brucellosis in extensive production system in Jimma Zone. The higher prevalence in the semi-intensive production systems could be due to the fact that there is a greater chance of contact between infected and healthy animals in these systems, or between healthy animals and infectious materials and larger number of animals per herd were being kept under semi-intensive farms with long period of time.

In current study, there was no statistically significant association ( $P>0.05$ ) between the prevalence of bovine brucellosis and sex of the animals. But only female animals were positive reactors to the disease in the present finding. This finding was in agreement with studies that reported there was no statistically significant difference of *Brucella* prevalence between sexes [29, 30]. These findings might be influenced by a small sample size of male animals ( $n=106$ ) as compared to female animals ( $n=494$ ) and males are also kept in the herd for shorter period which decrease their exposure to the disease. On the other hand, earlier studies statistically significant association between sex and sero-prevalence of brucellosis with higher prevalence being in female as compared to male animals [30, 31]. This was explained by male animals are less susceptibility to the *Brucella* infections due to lack of erythritol sugar [32].

Even though age was not significantly associated with *Brucella* seropositivity ( $P> 0.05$ ) in current study, a sero-prevalence of 2% was found among the adult age group where as 0.7% *Brucella* seropositivity was observed in the young age group of animal in the study sites. This was in agreement with the previous researchers where there was no statistically significant association between the sero-prevalence of the bovine brucellosis with age [28, 33, 34]. However, there is more chance of the aged animals to become infected with *Brucella* infection. This could be also explained by sexually mature and pregnant cattle are more susceptible to infection due to sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity [25].

There was no statistically significant association ( $P>0.05$ ) between breeding system and sero positivity of brucellosis in the present study. However, higher sero-prevalence was encountered in animals that used artificial insemination. In the same study, relatively higher sero-prevalence was noted among artificially inseminated cows. The reason could be due to lack of proportional sampling between the different breeding systems. The present finding was not in agreement with previous studies that reported significant association between sero-prevalence of brucellosis and breeding system [28, 35, 36, 37, 38]. The possible reasons could be poor hygienic practices before and after insemination and inappropriate techniques. The inseminators had only a few months of training and there is no regular monitoring or upgrading of their skills.

## CONCLUSION AND RECOMMENDATIONS

The sero-prevalence carried out in this study indicated that brucellosis as one of the important diseases of bovine in raising districts. Besides, the study also showed that pregnancy and semi intensive production system are important risk factors associated with the prevalence of the infection. The current finding also indicates the presence of foci of infection that could serves as source of the infection for the spread of the disease into the uninfected animals and herd. Knowledge of prevalence of brucellosis and effect of risk factor is essential for introduction of cost effective and efficient control program.

Therefore, based on the current finding the following the following remarks are recommended: Strict movement control of animal from one area to another in order to prevent the spread and transmission of the disease from infected cattle to the non-infected ones. Proper management of aborted cases and areas possibly contaminated with aborted materials like disinfection and regular cleaning of the area and animal premises. The implementation of test and slaughter policy with compensation payment to the farmers is required as the prevalence of the disease is low in the study area.

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