

# Detection of Antibodies Against Brucellosis and Associated Risk Factors in Cross Breed Dairy Cattle in Smallholder Farmers, Southern Ethiopia

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**Background:** A cross-sectional study was conducted in selected districts of Gamo zone, southern Ethiopia over a period of November 2019 and September 2020 to estimate seroprevalence and associated risk factors and assess knowledge and practices of smallholder farmers about bovine brucellosis. Two districts and four kebeles from each district were purposively selected, and individual animals were sampled using a simple random sampling technique. A total of 384 sera samples were collected, and concurrently, 236 dairy cattle owners were interviewed. The samples were tested for antibodies against *Brucella* using both Rose Bengal Plate test and Complement Fixation test following OIE standard protocol. Risk factors associated with bovine brucellosis were analyzed using univariate and multivariate logistic regressions.

**Results:** The survey result has shown that 95% and 97% of the small holder farmers did not know the cause and symptoms of bovine brucellosis, respectively, and the majority have engaged in risky practices. Eight (2.08%) of the collected serum samples were positive with screening test (RBT) and only six (1.5%) were positive with confirmatory test (CFT). Multivariable logistic regression analysis showed a statistically significant association between herd sizes and the disease. The seropositivity of the disease is higher in small-sized herds followed by medium-sized herds.

**Conclusion:** The seroprevalence of bovine brucellosis was found to be at a low percentage with confirmatory tests even if there was a presence of associated risk factors for the disease in the study area. Again, the results suggest that smallholder farmers have poor knowledge and risky practices, which expose them to the disease. Awareness creation about the disease is of paramount importance even if the prevalence was low in this serological study. The implementation of a test and slaughter program before the disease becomes widespread, along with the testing of new stock before introduction to the farms is recommended.

**Keywords:** brucellosis, seroprevalence

## Introduction

Ethiopia has the largest livestock population in Africa with an estimate of 60.9 million cattle, 31.3 million sheep, and 32.7 million goats.<sup>1</sup> However, livestock are not performing well as food producers in Ethiopia due to wide spread animal diseases. In particular, brucellosis has a significant socioeconomic and zoonotic impact, but has a low mortality rate, and has received little attention.<sup>2</sup>

Brucellosis in cattle affects reproductive performance and is an infectious disease of sexually mature cattle in Africa.<sup>3</sup> The infection is mainly caused by *Brucella abortus*, to a lesser extent by *Brucella melitensis* and rarely by *Brucella suis*. Abortion and retained fetal membrane in cows and orchitis and epididymitis in bulls are characteristic signs of the disease.<sup>5</sup> Animals with a high susceptibility to the disease contract it directly from aborted cows and fetuses, or indirectly via contaminated fomites.<sup>6</sup>

The disease remains a major public and animal health concern in many developing countries.<sup>7</sup> Yet, *Brucella* infections are common in cattle populations in sub-Saharan Africa, with seroprevalences ranging from 0.3% to 45%<sup>8</sup> and the prevalence of each of the countries associated with a risk of factors related to production systems and environmental factors.<sup>5</sup>

Despite their tight eradication programme, most developed countries have made significant progress but know the disease in Africa exerts the most serious problem.<sup>9</sup> A number of serological reports have shown that *Brucella* infection among cattle is common in Ethiopia, particularly in pastoral areas.<sup>10</sup> In Ethiopia, the previous seroprevalence rates stated were 7.62%,<sup>11</sup> 8–11%,<sup>12</sup> and 1.8%.<sup>13</sup> A seroprevalence of 50% for *Brucella* was reported by<sup>14</sup> in the Borena Zone. Furthermore, Tolosa et al<sup>15</sup> reported 0.77% in Jimma zone and Haile Selassie et al<sup>16</sup> reported 1.2% in Tigray region. The results of another investigation in south and east Ethiopia revealed that 3.5% of cattle were positive for *Brucella* antibodies.<sup>17</sup> Smallholder farmers in the study areas probably raised a small number of crossbreed dairy animals under a zero grazing system to produce milk for their own consumption and sale. However, reproductive failure due to abortion is a critical issue and creates serious problems in the present study area. In addition, information on the extent of the disease is not well known in the recent study area. Therefore, the present study aimed to estimate the serological prevalence of bovine brucellosis, identify its associated risks, and assess informant knowledge and practices related to bovine brucellosis as well as reproductive disorders associated with seropositive cows.

## Materials and Methods

### Study Area Profile

The study was undertaken in two purposely selected districts, namely, Chench and Bonke of Gamo zone, Southern Ethiopia from November 2019 to September 2020 (Figure 1).

Chench District is situated between 1300m and 3250m above sea level. The location of Chench district in astronomical terms is between 37° 29' 57" East to 37° 39' 36" West and between 6° 8' 55" North and 6° 25' 30"

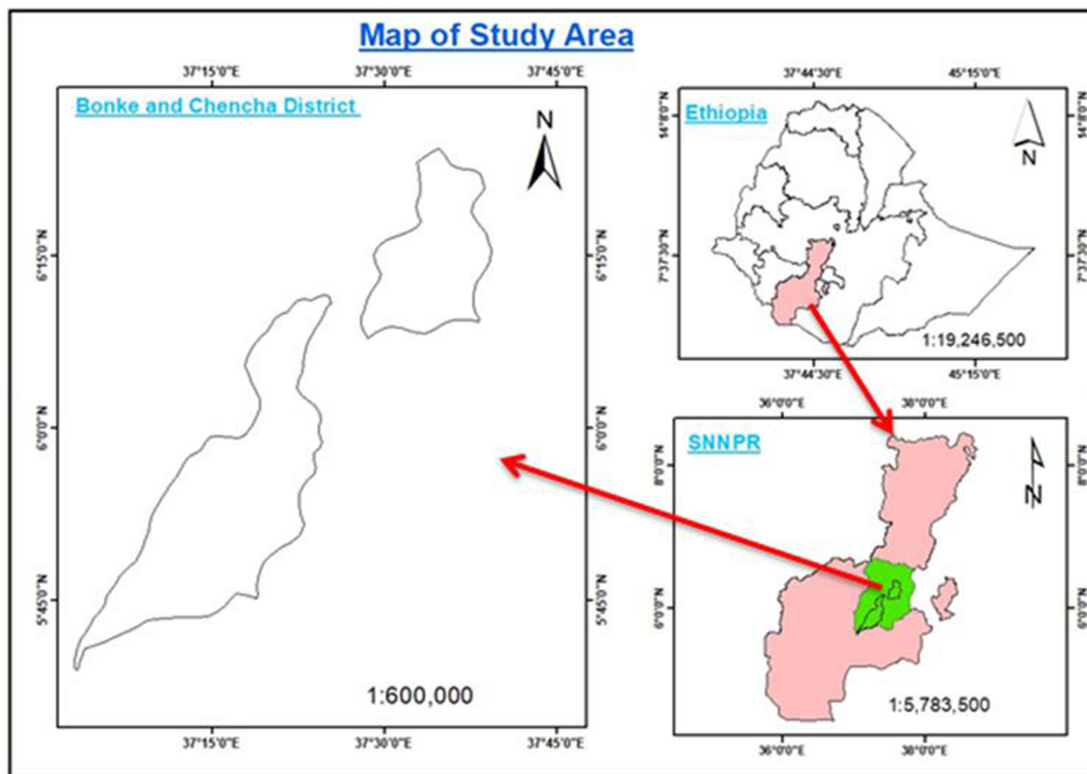


Figure 1 The study area landscape.

South. The district has two agroecological zones, namely, Dega and Weyna Dega, which account for approximately 82% and 18% of the total area, respectively. The rainfall regime in the district is bimodal. The first round of rain occurs between March and April. The second round of rain occurs from June to August. The annual rainfall distribution in the district varies between 900 and 1200 mm. The minimum and maximum temperatures in the district range from 11°C to 13°C and 18°C to 23°C, respectively. In the district, crop and livestock subsystems are practiced. Chenchha has 67,269 cattle, 106,594 sheep, 11,870 goats, and 22,554 equines.<sup>18</sup>

Bonke is one of the 15 districts in Gamo Zone and lies between 5°55'N latitude and 37°15'E longitude with an altitude ranges from 600 to 4200 masl. The land area of the district is estimated to be 85,940 km<sup>2</sup> and bordered on the south by the Derashe and Alle district, on the west by the Weito River, which separates it from Kemba, on the northwest by Deramalo, on the north by Dita, and on the east by Arba Minch Zuria district. The agro-ecology of the district is classified into three zones: Dega (covers 46% of the land area), Woina Dega (30%), and Kola (24%). The mean annual average rainfall and temperature of the district are 1400 mm and 13.05°C, respectively. The estimated livestock populations of the district are 137,171 cattle, 189,557 sheep, 65,758 goats, 36,566 equines, and 226,026 poultry.<sup>19</sup>

## Study Design

A cross-sectional type of study was employed between November 2019 and September 2020 to screen dairy cattle for bovine brucellosis. Furthermore, smallholder farmers were surveyed regarding their knowledge and practice concerning bovine brucellosis using semi-structured questionnaires.

## Study Animal

A study was conducted in purposively selected districts of the Gamo zone on cross-breeds of Jersey and Holstein Friesian exotic dairy cattle managed either under an extensive or semi-intensive management system. The animals in the study were no any previous vaccination history against bovine brucellosis. All crosses of jersey and Holstein Friesian aged more than 2 years were included in the study. In the sample collection period, age, parity, breed, and pregnancy status were collected and recorded as animal-level risk factors. The age of each study animal was determined by consulting the owners of the cattle.

## Inclusion Criteria

All cross-breed dairy cattle with jersey and Holstein Friesian greater than two years old, as well as cattle owners who expressed a willingness to participate in the study, were included.

## Sample Size and Sampling Method

Two districts, Chenchha and Bonke, and five kebeles from each district were selected based on their potential for cross-breed dairy cattle. The study animals were then randomly selected from each kebele using simple random sampling. The sample size was calculated based on the formula given below as described by Thrusfield.<sup>20</sup>

$$n = \frac{1.96^2 \times P_{\text{exp}} \times X(1 - P_{\text{exp}})}{d^2}$$

Where n = sample size, P<sub>exp</sub> = expected prevalence, and d = absolute precision.

Based on the expectation that disease prevalence would be 50% with 95% confidence level, and 5% absolute precision, a sample size of 384 exotic crossbreed dairy cows was calculated. A proportionate number of animals were then sampled from each district based on its crossbreed dairy cattle population. Thus, 236 and 148 individual animals were sampled from Bonke and Chenchha districts, respectively.

Smallholder farmers' knowledge and practices related to bovine brucellosis were surveyed in two districts along with blood sample collection. Thus, 236 dairy cattle owners were interviewed with semi-structured questionnaires following a pretest. Accordingly, 163 dairy cattle owners from Bonke and 73 from Chenchha districts were interviewed.

## Study Methodology

### Questionnaire Survey

An assessment of farmers' knowledge and practice of bovine brucellosis was conducted through a semi-structured questionnaire. A pre-tested semi-structured questionnaire was designed and administered to individual dairy cattle owners to assess the informants' knowledge of bovine brucellosis, its symptoms and the unhygienic practices that lead to the spread of the disease.

### Sample Collection and Preparation

Blood samples were collected from individual animals using plain 10mL vacutainer tubes from each animal's jugular vein. The collected samples were labeled and left at room temperature for a moment to allow coagulation, then transferred to another sterile cryovial tube and transported respecting the cold chain and finally stored at  $-20^{\circ}\text{C}$  until serological testing. The seropositivity of individual animals was determined using both screening (Rose Bengal plate test) and confirmatory test (Complement fixation test).

## Screening and Confirmatory Testing

### Rose Bengal Plate Test (RBPT)

The collected serum samples were screened for antibodies to brucellosis using the Rose Bengal plate test following the OIE protocol.<sup>21</sup> The sera and antigen were removed from the refrigerator 30 min before the test and allowed to reach room temperature. Equal volumes of test sera were added to 30  $\mu\text{l}$  of RBPT antigen. The mixture was thoroughly mixed, shaken for 4 min and finally agglutination was observed for positive sera. In the event of ambiguous results, a repeat test was conducted. The complement fixation test was performed on all positive RBPT reactor sera to confirm the diagnosis.

### Complement Fixation Test (CFT)

RBPT positive samples were confirmed with a complement fixation test using the OIE protocol<sup>22</sup> at the National Veterinary Institute (NVI) in Bishoftu, Ethiopia. Standardizing the antigen was done with a dilution (strength) of 1:20. *Brucella* antigen, complement, and 3% sensitized sheep red blood cells are added to serially diluted test sera on microtitre plates after serial dilution. The plates were incubated at  $37^{\circ}\text{C}$  for 30 min. At 1:10 dilution, positive results are considered for partial fixation (50% hemolysis) or complete fixation (no hemolysis). The negative control serum exhibited complete hemolysis, while the positive control serum exhibited inhibition of hemolysis.

## Case Definition

Samples positive for the Rose Bengal plate test and Complement fixation test were considered positive for brucellosis. Because there had been no history of brucellosis vaccination in Ethiopia over the past few years, the observed seropositive animals were considered to be natural infections.

## Associated Risk Factors

During the study period, age, breed, parity, and pregnancy status of study animals were considered intrinsic risk factors for bovine brucellosis, but management system, herd composition, herd size, history of previous abortions, history of previous retained fetal membranes, breeding practices, and source of replacement stock were considered as extrinsic risk factors. A data sheet was prepared for each animal containing this information, and individual animal biodata and management practices were recorded in line with blood sample collection from respective animals. Herd sizes can be divided into three categories: small herds under 10, medium herds between 10 and 50 animals, and large herds over 50 animals.<sup>4</sup>

## Statistical Data Analysis

A Microsoft Excel spreadsheet for Windows 2010 (Microsoft Corporation) was used to record and code data generated by laboratory investigations and the questionnaire survey and STATA version 14.0 was employed to analyze the data (Stata Corporation, College Station, TX, USA). The survey results and the proportion of bovine brucellosis-related risk

factors were presented using descriptive statistics. A seroprevalence calculation was conducted by dividing the number of seropositive samples by the total number of samples. Univariate and multivariate logistic regression analyses were used to show the degree of association between those risk factors and the disease (brucellosis). An odds ratio (OR) was calculated to measure the relationship between risk factors and brucellosis. Analysis output was interpreted as statistically significant ( $p \leq 0.05$ ) at 95% confidence level and 5% precision.

## Results

### Knowledge and Practice of Smallholder Farmers About Brucellosis

Approximately, 95% (225/236) and 97% (97/236) of small holder farmers surveyed did not know the cause and symptoms of bovine brucellosis, respectively. In this survey, 70% of respondents (166/236) reported poor management of their farming operations. As a result of lack of knowledge about the disease, a comparable number of smallholder farmers introduce naive animals without further investigation. It was reported that 77% of respondents disposed of dead fetuses in open dumps in the setting, and 23% burred aborted material immediately after release from dairy animals. Most smallholder farmers performed assisted parturition without using safety materials. In addition, there were limited provisions for separate rooms for delivery and aborted dairy animals. Furthermore, 86% of smallholder farmers consume raw milk without pasteurization or boiling (Table 1).

### Overall Seroprevalence of Bovine Brucellosis in Cross Breed Dairy Cattle

In the serological tests, 8 (2.08%) animals were found to be positive by RBPT, which were then subjected to CFT. On the basis of the confirmatory CFT test, the overall seroprevalence rate was 1.5% (95% CI: 0.70–3.44) at the individual animal level (Table 2).

**Table 1** A Survey of Smallholder Farmers' Knowledge and Practices Regarding Bovine Brucellosis

Variables	Number of Respondents	Response (%)
Hygienic practice of farm		
Clean	70	30
Not clean	166	70
Knowledge about cause of abortion		
Yes	11	5
No	225	95
Awareness about brucellosis		
Yes	6	3
No	230	97
New animal introduction in the farm		
Yes	107	45
No	129	55
Disposal of aborted fetus/fetal membrane		
Open dump	182	77
Burying	54	23
Act of assistance in parturition		
With protective material	74	31
Without protective material	162	69
Farm have separate parturition pen		
Yes	22	9
No	214	91
Habit of milk consumption		
Raw milk	203	86
Boiled milk	33	14

**Table 2** An Overview of the Associated Risk Factors for Bovine Brucellosis

Variable	Categories	No. of Examined	No. of Positive	Prevalence
Age	≤5 year	246	3	1.22
	>5 year	138	3	2.17
Parity	Few	243	4	1.65
	Many	141	2	1.42
Breed	Jersey	152	1	0.66
	Hoistain	232	5	2.16
	Friesian			
Management system	Extensive	98	3	3.06
	Semi intensive	286	3	1.05
Breeding practice	Natural mating	164	5	3.05
	Artificial Insemination	220	1	0.45
Herd size	Small	365	4	1.09
	Medium	19	2	10.52
Herd composition	Mixed	165	1	0.61
	Not mixed	219	5	2.28
Pregnancy status	Pregnant	116	3	2.59
	Not pregnant	268	3	1.12
Source of replacement stock	Off farm	207	2	0.97
	On farm	149	3	2.01
	Both	28	1	3.57

**Abbreviations:** Few, give birth less than or equal to three times; many, give birth greater than three times.

## Risk Factors Associated with Bovine Brucellosis Seropositivity

Out of nine potential risk factors, four were statistically significant ( $P \leq 0.05$ ) by univariable logistic regression analysis and subjected to multivariable logistic regression.

The management system of the study population was categorized into two groups: extensive and semi-extensive. Higher seroprevalence of bovine brucellosis was seen in extensively managed animals (3.06%), followed by semi-extensively managed animals (1.05%). A multivariate logistic regression revealed that extensively managed dairy animals have a 68.7% (OR = 0.31, CI 0.05–1.82) lower probability of showing seropositivity than semi-extended managed dairy animals (Table 3).

**Table 3** Analysis of Risk Factors for Bovine Brucellosis Using Univariate and Multivariate Logistic Regression

Risk Factors	No	Positive No (%)	Univariate			Multivariate		
			CI (95%)	OR	P- value	CI (95%)	OR	P- value
Age								
≤5 year	246	3 (1.22)						
>5 year	138	3 (2.17)	0.36–9.04	1.8	0.475			
Parity								
Few	243	4 (1.65)						
Many	141	2 (1.42)	0.16–4.75	0.86	0.862			
Breed								
Jersey	152	1 (0.66)						
Hoistain freisian	232	5 (2.16)	0.38–28.8	3.32	0.275			
Management system								
Extensive	98	3 (3.06)						
Semi intensive	286	3 (1.05)	0.07–1.69	0.34	0.186	0.05–1.82	0.31	0.194
Breeding practice								
Natural mating	164	5 (3.05)						
Artificial Insemination	220	1 (0.45)	0.02–1.25	0.15	0.080	0.02–1.65	0.18	0.129
Herd size								
Small	365	4 (1.09)						
Medium	19	2(10.52)	1.81–62.06	10.62	0.009	1.18–62.5	8.59	0.034
Herd composition								
Mixed	165	1 (0.61)						
Not mixed	219	5 (2.28)	0.44–33.11	3.83	0.222	0.85–93.3	8.89	0.069
Pregnancy status								
Pregnant	116	3 (2.59)						
Not pregnant	268	3(1.12)	0.08–2.14	0.43	0.301			
Source of replacement stock								
Off farm	207	2(0.97)						
On farm	149	3 (2.01)	0.35–12.8	2.12	0.418			
Both	28	1 (3.57)	0.33–43.3	3.80	0.283			

**Abbreviations:** CI, confidence interval; OR, odds ratio.

One of the other hypothesized extrinsic factor was the breeding practices of dairy animals, which were divided into two categories, natural and artificial mating. The prevalence of bovine brucellosis is higher on farms mating dairy animals through natural methods (3.05%) than on farms, which use artificial insemination (0.45%). An analysis of multivariable logistic regression revealed that the odds of being seropositive are 82% (OR = 0.18, CI 0.02–1.65) less likely in artificial insemination practice than in natural breeding practice.

An analysis of multivariable logistic regression was conducted to determine the association between seropositivity and hypothesized extrinsic risk factors like herd sizes and herd composition. Study dairy farms with less than 10 animals were 8.59 times more likely to be seropositive for bovine brucellosis than study dairy farms with between 10 and 50 animals (OR=8.59, CI 1.18–62.5). The highest seroprevalence (10.52%) was observed in small-sized herds followed by medium-sized herds (1.01%). Moreover, study dairy animals not mixed with other herds of animals in the home were 8.89 times more likely to be seropositive (OR=8.89 CI 0.08–93.3) for bovine brucellosis than herds composed of other animals (Table 3).

## The Association Between *Brucella* Seropositivity and Abortion and Retained Fetal Membranes in Cattle

A one-sided Fisher's exact test result revealed a positive and significant correlation between abortion and seropositivity for brucellosis ( $P < 0.05$ ). Conversely, occurrence of retained fetal membranes was not significantly ( $P > 0.05$ ) associated with seropositivity of bovine brucellosis (Table 4).

**Table 4** An Overview of the Association Between Seropositivity to Bovine Brucellosis and Abortion and Retained Fetal Membranes in Cattle

Risk Actors	No. Tested	Prevalence (%)	One Sided Fisher's Exact
Abortion history			0.011
Yes	34	3 (8.82)	
No	350	3(0.86)	
Previous retention of fetal membranes			0.060
Yes	27	2 (7.41)	
No	357	4 (1.12)	

## Discussion

### Farmers' Knowledge and Practices Concerning Brucellosis

Smallholder dairy farmers can greatly reduce the number of zoonotic infections, including brucellosis, if they change their habits and practices. Most respondents (97%) were not aware of the disease bovine brucellosis based on the results of the present study. The findings are consistent with those from studies in Tajikistan conducted by Lindahl et al<sup>23</sup> and Kenya conducted by Kang'ethe et al,<sup>24</sup> in which most respondents had never heard of brucellosis. In contrast to this finding, the findings of Kansime et al<sup>25</sup> and Holt et al<sup>26</sup> suggest that community members in Uganda and Egypt, respectively, are highly aware of bovine brucellosis. The low seroprevalence of bovine brucellosis in dairy cattle resulting in low awareness of smallholder farmers about brucellosis in the current study area compared to study conducted in Egypt and Uganda in endemic situation of brucellosis.

A number of high-risk behaviors were prevalent among smallholder farmers. Participating in animal parturition without personal protective materials, disposing of aborted fetuses in an open setting, and consuming raw milk are very common practices posing a high risk of contracting *Brucella* infection. The reason may be lack of understanding of the disease, the risks of transmission, and insufficient resources, which may be contributing factors. The results of the study are in agreement with the findings of Edao et al,<sup>27</sup> who reported that most farm workers in the study areas handled parturition animals in an unhygienic manner, disposed of aborted fetuses in an open setting, and consumed raw milk frequently. Furthermore, similar results were reported by Holt et al.<sup>26</sup>

### Seroprevalence and Associated Risk Factors of Bovine Brucellosis

The current study result revealed that the overall animal-level seroprevalence based on the confirmatory CFT test was 1.5% (95% CI: 0.70–3.44). This finding is in agreement with those of other studies in the country,<sup>3,28</sup> whose overall prevalence was 1.5% and 2.2%, in Addis Ababa dairy farms, respectively. A similar study by Geresu et al<sup>29</sup> reported 1.4% in Asella and Bishoftu towns using the card test (CT), RBPT, indirect Enzyme-Linked Immuno Sorbant Assay (i-ELISA), and Complement Fixation Test (CFT). Furthermore, comparable overall seroprevalence reports presented in the Jigjiga zone of Somali region (1.92%),<sup>30</sup> Sidama zone (1.92%),<sup>31</sup> South eastern Ethiopia (3.5%),<sup>17</sup> Guto Gida district of East Wollega zone (1.97%), Central Oromia (2.9%),<sup>32</sup> Jimma (0.77%),<sup>15</sup> Tigray region (3.19%),<sup>10</sup> Tigray region (1.2%),<sup>16</sup> and Jimma (0.61%)<sup>33</sup> have been documented. Elemo and Geresu,<sup>34</sup> on the other hand, observed 4.95% seroprevalence levels in smallholder farms in Agarfa and Berbere districts of the Bale zone using RBPT and CFT tests. Furthermore, the seroprevalence rate was reported to be 11.0% by Kebede et al,<sup>35</sup> in Wuchale Jida district, 4.9% by Alehegn et al<sup>36</sup> in and around Gondar Town, and 13.14% by Desalegn et al<sup>37</sup> in Assela government dairy farms and 4.6% by Hailemeleket et al<sup>38</sup> in selected Ethiopian sites. A lower sero prevalence of bovine brucellosis may be attributed to semi-intensive milking of dairy cows, which limits the exposure of dairy cows to disease risks.

Only herd sizes had statistically significant associations with brucellosis occurrence among the associated factors considered in the current study. Other extrinsic risk factors subjected to multivariate logistic regression including management system, breeding practice, and herd composition had no statistically significant association with the disease. It is in agreement with Mussie's<sup>39</sup> research concerning the effects of age, pregnancy status, stage of pregnancy, parity

number, and management system on mortality in extensive systems in northwest Ethiopia. A different finding was reported by Berhe et al,<sup>10</sup> who reported a higher sero-prevalence in transhumance systems compared to sedentary systems.

The present study found that seroprevalence varied statistically significantly from farm to farm with different herd sizes. These findings are consistent with those reported by Berhe et al<sup>10</sup> and Ahmad et al,<sup>40</sup> but differ from those reported by Kebede et al.<sup>35</sup> The significant statistical variation among herd sizes was attributed to the fact that maintaining a hygienic condition of the farm becomes more difficult to handle efficiently by smallholder farmers as the stocking density of the farm increases.

An analysis of one-sided Fisher's exact test results showed that abortion likelihood was positively and significantly correlated with brucellosis seropositivity ( $P < 0.05$ ). Nevertheless, bovine brucellosis seroprevalence was not significantly related to retained fetal membranes ( $P > 0.05$ ). This finding is consistent with Kebede et al<sup>35</sup> regarding retained fetal membranes, but differs in terms of abortion's effect. More likely, Ahmed et al,<sup>40</sup> in Jordan and Muma et al<sup>41</sup> in Zambia both found that abortion was associated with brucellosis seroprevalence. A possible reason is that abortion is often associated with *Brucella* infections.<sup>42</sup>

## Conclusion and Recommendations

The results of a survey showed that most surveyed small holders dairy farmers did not know the cause of bovine brucellosis and were unaware of its symptoms. Smallholder farmers have limited knowledge of bovine brucellosis and have engaged in risky practices. The study results also revealed that the introduction of naive cattle to their farm without further investigation was common. A low level of individual animal-level seroprevalence of bovine brucellosis was found in the study area. Only one of the four risk factors associated with the disease in univariable logistic regression has become significantly associated with it in multivariable models. Multivariable logistic regression analysis revealed that herd size was a statistically significant risk factor. A small herd has a higher seropositivity to the disease, followed by a medium herd. Therefore, creating awareness about the disease is of paramount importance to mitigate its growing trend even if the prevalence was low in the current study. Testing new stock before introduction to farms and implementing a test and slaughter program before the disease spreads and becomes more prevalent are recommended steps. Moreover, the smallholder dairy farmers maintain the hygienic condition of the farm standard level and if not reduce the stock, which makes the dairy animals risk for the occurrence of bovine brucellosis.

## Data Sharing Statement

On the author's request, all information is available.

## Ethical Approval and Consent to Participate

The study was carried out in accordance with animal welfare guidelines, and ethical approval was obtained from Arba Minch University's Ethical Review Committee (Certificate Ref. No.: AMU/AREC/8/2015). Also, the informed verbal consent was obtained from cattle owners to include their animals in the study. However, informed written consent was waived by the ethics committee as the majority of livestock owners could not read or write.

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## Author Contributions

All the stated authors have made a significant contribution to conception, study design, execution, and acquisition of data, analysis and interpretation. Again, all the authors took part in drafting, revising, or critically reviewing the article together with giving final approval of the version to be submitted to the agreed journal. Moreover, they all agreed to be accountable for all aspects of the work.

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## Disclosure

The authors confirm that they have no competing interests.

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