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RESEARCH PAPER

Bovine brucellosis: seroprevalence, risk factors and assessment of knowledge, attitude, and practice of cattle owners in Lare and Jikawo districts of Gambella Region, Ethiopia

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Abstract

Bovine brucellosis is a zoonotic disease that causes substantial economic losses and strongly impacts public health. Though it has been eradicated in many developed countries, it is still endemic in developing countries like Ethiopia: The study's objectives were to estimate the seroprevalence of bovine brucellosis, determine the risk factors, quantify and assess the knowledge, attitude, and behavior of cattle owners in a few selected areas of Ethiopia's Gambella region. Lare and Jikawo were the two districts of the Gambella Region selected purposively. Kebeles, study animals and peasant associations were randomly chosen. A total of 384 serum samples from 70 herds were collected and screened using the Rose Bengal Plate Test and confirmed using the Complement Fixation Test. A semi-structured questionnaire survey was used to assess the risk factors for the seroprevalence of bovine brucellosis and the knowledge, attitude and practice of farmers in the study areas about the disease. The seroprevalence of brucellosis was summarized using descriptive statistics, and the association between risk factors, and seroprevalence of brucellosis was evaluated using logistic regression. The principal findings of the current study showed that individual and herd level seroprevalence of brucellosis using the Rose Bengal Plate test was 6.8% (26/384) and 24.3% (17/70), respectively, and the respective confirmation by complement fixation test3.1% (12/384) and 12.9% (9/70). Among the risk factors, herd size and the presence of other species had statistically significant associations ($p<0.05$) with Brucella seropositivity. Female cattle with more parity and those with abortion history had higher odds of Brucella antibodies compared to their counterparts. Although the overall respondents' knowledge, attitude, and practice were 66.4%, most were unaware that the disease was zoonotic, the ability of the disease to cause abortion, and the mode of the disease's transmission. Most respondents also had a poor attitude toward the mode of disease transmission, and they have been practicing risky practices that predisposed them to brucellosis. In conclusion, the overall seroprevalence of brucellosis and cattle owners' knowledge, attitude, and practice in the current study were low. However, being a contagious disease, brucellosis can easily spread among cattle herds and poses a public health risk. Therefore, improvement of cattle owners' knowledge, attitude, and practice and characterization of circulating Brucella species in the study areas are needed to design evidence-based disease control measures.

Keywords: Bovine, Brucellosis, Ethiopia, Gambella, Prevalence

Introduction

Brucellosis has been eradicated in many developed countries; however, it is still endemic in developing countries because of a lack of control programs and/or resources

(Akinseye *et al.*, 2016). It is caused by species of gram-negative, facultative intracellular bacteria that can infect many species of animals. The disease has been reported in many countries around the world, including Ethiopia. In cattle, brucellosis is predominantly caused by *B. abortus*, less frequently by *B. melitensis* and occasionally by *B. suis* (OIE, 2016). Direct contact with infected abortion materials, inhalation, and the consumption of infected milk and milk products are significant means of transmission of the disease to humans (Onunkwo et al., 2011). However, infection through injured/intact skin, the mucosa of the respiratory system, and conjunctiva occur frequently (Kebede *et al.*, 2008). Transmission to animals occurs mainly by ingestion of contaminated feed and water (Mukhtar and Kokab, 2008).

Brucellosis is endemic in most African countries (Mugizi *et al.*, 2015). It is considered to be an occupational disease that mainly affects abattoir workers, farm laborers, animal keepers, butchers, veterinarians and laboratory workers from a public health point of view (Moti and Jatinder, 2011). However, abattoir workers are more prone to acquire brucellosis than other occupations, because they are more exposed to carcasses, viscera, and organs of infected animals (Mukhtar and Kokab, 2008). The economic significance of brucellosis results from production losses associated with abortions, retained placenta, metritis, impaired fertility, and arthritis. Milk production losses in infected dairy cows can be up to 20% and the inter-calving period can be prolonged by several months (Mugizi et al., 2015). The spread and maintenance of brucellosis is influenced by risk factors that are related to management systems, the genetic content of susceptible animal populations, the biology of agents causing the disease, and environment (McDermott and Arimi, 2002; Radostits *et al.*, 2006). These factors also include the size and composition of the herd, age of the animals, contact between infected herds, poor farm biosecurity and climate change (Boukary *et al.*, 2013).

Various serological tests have been developed and are being used to provide rapid results (Zeng *et al.*, 2017). The standard Rose Bengal and Complement Fixation tests are the main serological tests used to detect antibodies against *B. abortus* and *B. melitensis* (Di Bonaventura *et al.*, 2021). Both tests have been used for several years for the eradication of bovine brucellosis in some countries (Al Dahouk *et al.*, 2007). Different authors have reported evidence of *Brucella* infection in Ethiopian cattle using various serological tests. Accordingly, relatively high seroprevalence of brucellosis (above 10%) has been reported from smallholder dairy farms in central Ethiopia. In comparison, low seroprevalence (below 5%) in cattle under crop-livestock mixed farming (Ibrahim *et al.*, 2010). Asmare *et al.*, (2014) and Tadesse, (2016) on the other hand reported a pooled national estimate of brucellosis of dairy cattle in Ethiopia as 3.3% and 2.9%, respectively.

Brucellosis is a zoonotic disease that leads to considerable morbidity. The economic and public health impact of brucellosis remains a concern in developing countries (Bagheri Nejad *et al.*, 2020). It is among the top five priority zoonotic diseases in Ethiopia (Pieracci *et al.*, 2016). In pastoral societies, where close intimacy with animals, raw milk consumption and low awareness of zoonotic diseases facilitate its transmission between livestock and humans, brucellosis constitutes significant public health importance. More importantly, traditional management systems of pastoral communities, such as communal grazing, purchase/entrance of animals from infected herds, intermixing their livestock at water points and using single bulls for breeding purposes without testing, indicate the need for the study of brucellosis in pastoral communities. There is no published literature about the prevalence of cattle brucellosis, the level of awareness of cattle owners about brucellosis, and the risk factors for the occurrence of brucellosis in the Gambella Region, Ethiopia. Therefore, the present study aimed to estimate the seroprevalence of bovine brucellosis, identify its risk factors and assess the knowledge, attitude, and practice of cattle owners in selected districts of the Gambella region, Ethiopia.

Materials and methods

Description of Study Areas

A study was conducted in the Lare and Jikawo districts of Nuer Zone, Gambella National Regional State, Southwest Ethiopia (Fig 1). Nuer is one of the four zones of Gambella region and it has a total cattle population of 276,876. The zone has more than 85% of the cattle population of the region (CSA, 2018). Traditional livestock production system prevails in the entire region and the major livelihood comprised of cattle rearing.

Jikawo and Lare districts are located 120 km and 45 km away from Gambella town. The majority of the community in both districts are agropastoral and pastoralist (CSA, 2008) and most animals are managed under an extensive system by smallholders (Dika, 2018).

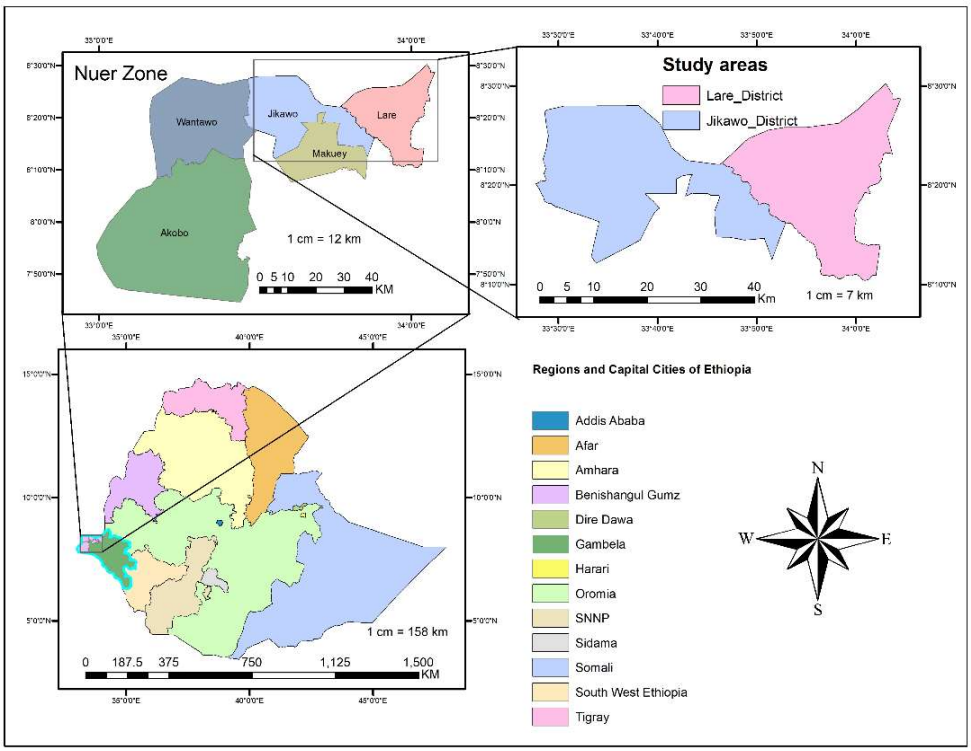

Figure 1. Map of study areas (ArcGIS 10.2.0.3.3348 ESRI).

Study design and study population

A cross-sectional study design, consisting of a questionnaire survey was conducted from October 2019 to April 2020 in the Lare and Jikawo districts of the Nuer Zone of the Gambella region, Southwest Ethiopia. All cattle found in the Lare and Jikawo districts were considered as study populations. There are 24 kebeles in the Lare district and 22 kebeles in Jikawo. Kebele is the smallest administrative unit in the district. Ten kebeles each were chosen based on their proximity to transportation. Two kebeles from each of the ten kebeles that are near transportation were chosen at random using a lottery technique. The target populations were cattle (both male and female), over six months of age and reared under an extensive management system in the study areas. The herd size was categorized into small (>=15 animals), medium (between 16 and 30 animals), and large (> 30 animals) (Megersa *et al.*, 2011). Based on parity, female bovine were grouped into no parity (heifers), 1-3 parity (animals which gave birth up to 3 times) and > 3 (animals which gave birth greater than three). The individual animal was

classified as young if it was under 24 months old and as adult if it was equal to or greater than 24 months old.

Sample size determination

The sample size for this study was calculated by the formula described by Thrusfield, (2007) using an acceptable error of 5% and at a 95% confidence interval. As there is no reported seroprevalence of brucellosis in the study areas, a 50% predicted prevalence and a 95% degree of confidence was employed. Accordingly, the calculated sample size was 384.

$$n = \frac{z^2 x\, P_{\exp}(1 - P_{\exp})}{d^2}$$

Where n = required sample size

Z=reliability coefficient (1.96 at d=0.05 or 95% CI)

Pexp=expected prevalence (50%)

d= desired absolute precision (95% CI)

For a questionnaire survey, the sample size was calculated using the formula given by Arsham (2002) which is as follows:

N = 0.25/SE2,

Where N = sample size and SE (standard error) = 5%.

Thus, the calculated sample size was 100, but 10% of the calculated sample size was added to compensate for non-response rates, which makes the total sample 110.

Sampling Technique

A multi-stage sampling technique was used to collect representative samples. The Gambella region is divided into three zones, and each zone is divided into districts. Each district is grouped into kebeles, and each kebele is also categorized into different peasant associations also called villages. Accordingly, two districts, Jikawo district and Lare district, were selected purposively based on accessibility and high cattle population. The two kebeles, eight Villages and households/herds from each district were selected using simple random sampling. Study animals were also selected using simple random sampling. The study animals were stratified according to their age and sex. From each stratum, animals were selected proportionally. Animals below six months of age were excluded from sampling. The present estimation of livestock and human population was obtained from the respective study districts. Accordingly, the total number of samples required from cattle was distributed according to the cattle population in each district and a total of 229 and 155 cattle from the Lare district and the Jikawo district, respectively, were considered as study animals. In the same manner, from the total population of the respective districts, a total of 50 and 60 households in Jikawo and Lare districts were selected and considered for the questionnaire survey.

Sample collection and interview data

Age, sex, herd size, parity, presence of other species, history of abortion and retained fetal membrane were recorded by interviewing the animal attendants or owners while collecting samples. From each study animal, about ten milliliters of blood was aseptically collected from the jugular vein using plain vacutainer tubes and sterile needles. After collecting, each vacutainer tube that had a blood sample was placed in an upright position at room temperature for 10 hours to obtain a serum sample. Then sera were decanted into cryovials and labeled. The serum samples were placed in an icebox and transported to the Animal Health Institute (AHI), Sebeta, Ethiopia, and kept in a refrigerator at -20 °C until laboratory examination was conducted.

Questionnaire Survey

A pretested KAP questionnaire survey consisting of 30 questions was prepared as the data collection tool. It was divided into four sections: (1) socio-economic characteristics of respondents (2) knowledge of brucellosis (3) attitudes toward brucellosis and (4) practices relating to cattle husbandry, disposal of aborted

material and dairy product consumption. The questionnaire survey was closed-ended and contained binary and multiple choices. Cattle owners aged at least 15 years, residents in selected kebeles and able to communicate verbally in the local Nyuer language were interviewed face to face. Cattle owners were randomly selected for a questionnaire survey.

Serological Tests

Rose Bengal Plate Test

All serum samples collected were screened using RBPT according to the procedures described by the World Organization for Animal Health (OIE, 2004) and manufacturers' instructions. The serum samples were screened using the RBPT antigen (VLA Weybridge, UK). The test serum and antigen were kept at room temperature for half an hour before the test. Then equal volumes (30 µl each) of RBPT antigen and test serum were placed alongside the plate and mixed thoroughly on the clean plate. Both certified reference positive and negative sera were used in each plate for the quality assurance of the result. The plate was manually rocked and rotated for 4 minutes, and the degrees of agglutination reactions were recorded. The result was interpreted as Negative if no agglutination and rimming were observed. If barely perceptible agglutination and/or some rimming was considered as 1+ a positive sample, fine agglutination, and definite rimming were considered as 2+ positive and clear clumping with definite clearing was considered as 3+ positive.

Complement Fixation Test (CFT)

A serum sample tested positive by the RBPT was further tested using CFT for confirmation using the standard *B. abortus* antigen (Cenogenics Corporation, USA). The standard B. abortus antigen was used to detect the presence of anti-Brucella antibodies in a serum sample. Preparation of the reagent was evaluated by titration and performed according to protocols recommended by the World Organization for Animal Health (OIE, 2009). A certified positive and negative control sera were run together with the samples on each plate as a quality control of the test. A serum sample 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, was classified as positive. If there was a lack of fixation or complete hemolysis, it was considered a negative.

Data management and analysis

The data from the laboratory investigation and the questionnaire survey were entered into a Microsoft Excel spreadsheet, coded, and analyzed with STATA version 14.0 software (Stata Corp, College Station, USA). For the questionnaire survey, descriptive statistics were used to describe the study variables. The overall score was obtained by summing responses from each question and categorizing them into groups, i.e., 50% correct responses to indicate low level, 50-75% correct responses to indicate medium level, and > 75% correct responses to indicate high level for knowledge, practice, and attitude. The seroprevalence of brucellosis was calculated as the number of seropositive samples divided by the total number of samples tested. Similarly, the herd level prevalence was calculated by dividing the number of herds with at least one animal positive for brucellosis by the total number of herds tested (Alehegn *et al.*, 2017). Descriptive statistics were used to summarize seroprevalence, whereas logistic regression was used to assess the association of risk factors with seroprevalence of Brucella antibodies. Potential risk factors considered for statistical analysis include age, sex, parity, herd size, abortion history, presence of other species, and district. For all risk factors, the level with the lowest prevalence was used as a reference category. All variables having a p-value of <0.25 in the univariable logistic regression analysis were further analyzed by multivariable logistic regression after checking for confounders. In all the tested variables, $p<0.05$ was set for significance, and the variables with $p<0.05$ in the multivariable model were concluded as predicting factors for seropositivity of brucellosis.

Results

Serological Analysis

A total of 384 sera samples were collected from 70 herds of cattle and screened with RBPT and confirmed with CFT. Out of 384 serum samples, 6.8% (26/384) and 3.1% (12/384) were found to be RBPT positive and CFT positive, respectively, at the animal level. The CFT result showed that the Jikawo district had a higher seroprevalence of bovine brucellosis at both the individual animal (5.2%) and herd level (16.7%) than the Lare district (Table 1).

Table 1. Seroprevalence of bovine brucellosis in Lare and Jikawo districts, Gambella, Ethiopia

Variable	**Animal level**			**Herd level**		
Study area	No of animals examined	RBPT	CFT	No of herds examined	RBPT	CFT
Lare	229	12(5.2)	4(1.8)	40	9(22.5)	4(10.0)
Jikawo	155	14(9.0)	8(5.2)	30	8(26.7)	5(16.7)
Total	384	26(6.8)	12(3.1)	70	17(24.3	9(12.9)

The univariable logistic regression analysis was performed for the variables namely district, age, sex, herd size and presence of other species. Parity and abortion history were analyzed separately as only female and mature animals are considered for these variables. Accordingly, the univariable analysis showed that the risk of bovine brucellosis in the Jikawo district is 3.1 times higher than in the Lare district. Adult cattle are more likely to be affected by brucellosis (OR = 4.4) than young cattle. Similarly, cattle kept mixed with small ruminants had a higher probability of being infected by brucellosis than cattle kept alone (OR = 4.4). The multicollinearity matrix result revealed that all independent variables were not collinear with each other ($r<0.5$). Thus, considering univariable p-value < 0.25, non-collinearity, and frequency of variable categories, the variables namely district, herd size, and presence of other species were selected for entry into the multivariable model:. The multivariable logistic regression model revealed that herd size (OR= 4.7; 95% CI: 1.6-13.3, $p<0.05$) and presence of other species (OR= 4.9; 95% CI: 1.0-23.8, $p<0.05$) were potential risk factors for cattle seropositivity to circulating Brucella antibodies and independent predictors of bovine brucellosis in the study areas (Table 2).

Table 2. Univariable and multivariable logistic regression analysis of risk factors for Brucella seropositivity

Risk factors	Category	No. Exam.	No. Positive (%)	Univariable		Multivariable	
				OR(95% CI)	P-value	OR (95% CI)	P-value
Districts	Lare	229	4(1.8)	1.0	-	1.0	-
	Jikawo	155	8(5.2)	3.1 (0.9,10.4)	0.072	2.8(0.8-9.9)	0.101
Herd size	Medium	111	1(0.9)	1.0	-	1.0	-
	Large	216	5(2.3)	2.6(0.30-22.6)	0.385	()	
	Small	57	6(10.5)	12.9 (1.5-110.3)	0.019	4.7(1.6-13.3)	0.004
Sex	Male	130	3(2.3)	1.0	-		
	Female	254	9(3.6)	1.6 (4.1-5.9)	0.513		
Age	Young	176	2(1.1)	1.0	-		
	Adult	208	10(4.8)	4.4(0.9-20.3)	0.058		
Presence of other species	No	175	2(1.1)	1.0	-	1.0	-
	Yes	209	10(4.7)	4.4(0.9-20.1)	0.060	4.91(1.0-23.8)	0.048

The univariable logistic regression analysis for the Brucella antibodies in mature female animals showed that both parity and abortion history were significantly associated ($p<0.05$) with seropositivity for brucellosis with animals having a history of abortion and giving at least one birth are more at risk than their counterpart. Multivariable logistic regression also showed that cows having more parity have higher odds of Brucella seropositivity (2.7) compared to those with small or no parity, which is marginally significant ($P=0.054$). Similarly, cattle with a history of abortion showed higher odds of brucellosis (44.6) compared to those with no history of abortion ($P<0.05$) (Table 3).

Table 3. Univariable and multivariable logistic regression analysis of risk factors for Brucella seropositivity in mature female cattle

Variable	Category	No. Exam.	No. Positive (%)	Univariable		Multivariable	
				OR (95% CI)	P value	OR (95% CI)	P value
Abortion history	No	144	7(4.8)	69.7 (5.6, 862.4)	0.001	44.6(3.4, 589.7)	0.004
	Yes	3	2(66.7)				
Parity	No parity	108	0 (0.0)				
	1-3 parity	71	4 (5.6)	3.0(1.2, 8.0)	0.024	2.7 (1.0, 7.6)	0.054
	>3 parity	75	5(6.7)				

Questionnaire Survey Analysis

Socio-economic characteristics of respondents

A total of 110 cattle owners were interviewed during the study period, of which 92 (83.6%) were male. The respondents' educational level showed that most of them (87.3% [96/110]) are illiterate. The income source of most respondents (52.7%) was based on animal sales, followed by animal and dairy product sales (32.7%) as shown in Table 4.

Table 4. Socio-economic characteristics of respondents in Lare and Jikawo districts

Variables	Categories	Frequency	Percent
Educational level	Illiterate	96	87.3
	Primary	13	11.8
	Secondary and above	1	0.9
Age	21-35	35	31.8
	36-49	41	37.3
	>49	34	30.9
Sex	Female	18	16.4
	Male	92	83.6
Family size	3-6	44	40
	7-10	42	38.2
	>10	24	21.8
Source of income	Crop sale	1	0.9
	Animal sale	58	52.7
	Dairy product sale	15	13.6
	Animal and dairy product sale	36	32.7

Analysis of knowledge, attitude, and practice of respondents

Most respondents66.4% (73/110) had heard about brucellosis. However, 92.7%(102/110) of the respondents did not know that brucellosis is a zoonotic disease, 77.2% (86/100) did not know that brucellosis causes abortion, and 89.1% (98/100) did not know that brucellosis can be transmitted to humans by handling aborted fetus and consumption of raw milk from infected cows. As part of the preventive measures for brucellosis adopted by cattle owners, most suggested using boiled milk, while others suggested testing and culling and improved sanitation. A few of them, 11.8% (13/110), never knew any control and preventive measures (Table 5).

Table 5. Respondents' knowledge of brucellosis in the study areas

Variables	Categories	Frequency	Percent
Have you heard about bovine brucellosis?	Yes	73	66.4
	No	37	33.6
Do you know brucellosis is a zoonotic disease?	Yes	8	7.3
	No	102	92.7
Do you know brucellosis causes abortion?	Yes	24	21.8
	No	86	77.2
Is brucellosis spread through the handling of aborted fetus and consumption of raw milk?	Yes	12	10.9
	No	98	89.1
Means of brucellosis transmission from animal to animal	Contact with infected domestic and wild animals	9	8.20
	Inhalation	20	18.18
	Contaminated feed	12	10.9
	Never know	69	62.72
Mode of transmission of brucellosis from animal to human	Eating raw meat	31	28.2
	Drinking raw milk	15	16.5
	Inhalation	5	4.5
	Sharing the same house with infected animals	8	7.3
	Contact with aborted material	1	0.9
	Never know	50	45.5
Methods of control of brucellosis	Test and culling	8	7.3
	Boiling	55	50
	Improving sanitary and hygienic standards	34	30.9
	Never know	13	11.8

Analysis of the attitude of respondents showed that only 17.3% (19/110) believed that some of their family members were at risk of contracting brucellosis if exposed to infected cattle. Moreover, most respondents do not think boiling milk before consumption, using gloves when handling infected cattle or aborted material and washing hands after close contact with infected or aborted material is necessary to prevent transmission of bovine brucellosis to humans (Table 6).

Table 6. Attitude of respondents toward brucellosis in study areas

Variable	Category	Frequency	Percent
Do you believe infected cattle can expose family members to *Brucella* infection?	Yes	19	17.3
	No	91	82.7
Do you think boiling milk is necessary before consumption to prevent brucellosis?	Yes	39	35.5
	No	71	64.5
Do you think it is necessary to use gloves when handling infected cattle or aborted material?	Yes	14	12.7
	No	96	87.3
Do you think washing your hands is necessary after close contact with animals or their abortus?	Yes	25	22.7
	No	85	77.3
Do you think the use of vaccination is necessary to prevent brucellosis?	Yes	109	99.1
	No	1	0.9

Most respondents used to practice risky activities such as not washing of hands before and after milking (80%), disposing of an aborted fetus with bare hands (90%), disposing of an aborted fetus in open fields (82.2%), handling animals with uncovered wounds (100%), and consumption of raw milk (85.5%) (Table 7).

Table 7. Practices of respondents regarding bovine brucellosis in the study areas

Variable	Category	Frequency	Percent
What type of Housing system do you use?	Open field	110	100.0
Do you practice hand washing before and after milking	Yes	22	20.0
	No	88	80.0
How do you dispose of aborted fetuses?	By protective materials	11	10.0
	By uncovered hand	99	90.0
Where do you dispose of aborted fetuses?	Incineration	4	3.6
	Deep burial	6	5.5
	Disposing to open field	91	82.2
	Throw it away for carnivores	9	8.2
Do you keep other animals in the herd?	Yes	56	49.1
	No	54	50.9
Do you use protective materials during assisting parturition?	Yes	17	15.5
	No	93	84.5
Do you cover wounds while handling animals?	Yes	10	9.1
	No	100	90.9
Form of milk Consumed?	Raw	94	85.5
	Boiled	9	8.9
	Processed	7	6.4
Do you assist dairy cows during parturition?	Yes	89	80.9
	No	21	19.1
Do you consume raw milk?	Yes	104	94.54
	No	6	5.46
How do you dispose of animals that died of suspected brucellosis?	Burn carcass	9	8.18
	Burring all carcass	7	6.36
	Cook and eat the meat	2	1.82
	Disposing to open field	92	83.64

Discussion

The present study revealed that the overall seroprevalence of bovine brucellosis was 3.1% in the Lare and Jikawo districts of the Gambella region at the individual animal level. This value was consistent with the 3.1% prevalence in Jimma zone Ibrahim et al., (2010) and 3.19% in the Tigray region by (Berhe *et al.*, 2007). However, the current prevalence was higher than the previous reports of Degefu *et al.*, (2011) 1.38% in Jijjiga Zone, Somalia, Kassahun *et al.*, (2010) 1.92% in Sidama Zone, Yohannes *et al.*, (2013) 1.97% in Guto-Gida district of East Wollega Zone, Bashitu *et al.*, (2015) 0.2% in Ambo and 0% in Debrebirhan town. In contrast to the current finding, a higher seroprevalence of 7.7% was reported by Haileselassie *et al.*, (2010) in the Tigray region, Ibrahim *et al.*, (2010) 15.0% in the Jimma zone of the Oromia region, Dinka and Chala, (2009) 11.2% in the East Shewa Zone of the Oromia region, and Berhe *et al.*, (2007) The present study showed no statistically significant difference in the seroprevalence of brucellosis between the two districts (Lare and Jikawo). This could be due to the similarity of traditional cattle management systems in both districts where pastoral livestock raising is predominant. In the current study, there was a higher seroprevalence of brucellosis in adult cattle than in young cattle. This finding agrees with the reports of Kassahun *et al.*, (2010) and Adugna *et al.*, (2013). It has also been well-documented that brucellosis is more associated with sexual maturity Radostits and Done, (2007), and a higher seroprevalence has been repeatedly reported in sexually matured animals.

The present study revealed that the presence of other livestock (sheep or goats) was the risk factor associated with the presence of seroreactor cattle. Although sheep and goats were not tested for brucellosis in this study, the finding corroborates reports of mixed farming importance in Brucella transmission dynamics in Egypt (Samaha *et al.*, 2008). On the other hand, *B. abortus* infection was isolated and reported from sheep and goats in Nigeria by Ocholi *et al.*, (2004), and *B. melitensis* was isolated from cattle in Egypt by (Samaha et al., 42.3% in the extensive cattle production system of the Tigray Region of Ethiopia. Also, a higher prevalence than the current study has been reported in different African countries, such as Zambia (18.7%) by Chimana *et al.*, (2010), and Algeria (9.7%) by (Aggad and Boukraa, 2006). The variation in prevalence reported from different regions of Ethiopia and other parts of Africa could be associated with the evolution of the disease, geographical origin, breeds, sample size, cattle rearing system, study frame, as well as the protocol adopted, such as the type and number of diagnostic tests used. The series of serology protocols used by researchers to screen and confirm the disease might be one test or more than one test, i.e., a screening test followed by confirmation of positive reactors by another test or in parallel interpretation (Chisi *et al.*, 2017).

2008). Accordingly, contact between cattle with sheep and goats was the most important risk factor identified in these studies. Thus, as the presence of other species in the bovine herd in the current study was also identified as one of the risk factors for seropositivity of bovine brucellisis, segregating sheep and goats from cattle might reduce the seroprevalence among cattle in mixed herds.

The prevalence of brucellosis was significant in cows with a history of abortion in the current study. Different authors also reported a different prevalence of brucellosis in cattle with a history of abortion (Adugna *et al.*, 2013; Berhe *et al.*, 2007; Ibrahim *et al.*, 2010; Tolosa *et al.*, 2008). The female animals were more positive reactors than the male animals in this study. It has been reported that males are usually more resistant than female cattle (Berhe *et al.*, 2007; Muma *et al.*, 2012; Tolosa *et al.*, 2008). Different factors are probably involved in the variation in sex susceptibility, including physiological and behavioral differences between males and females. Because of the preferential growth of *B. abortus* in the gravid uterus, it can enter the uterus as it disseminates from the main sites of carrier states (udder,

supra mammary lymph node) (Radostits and Done, 2007).

The existence of a previous history of abortion was statistically significantly associated with the prevalence of brucellosis ($p<0.05$) in the present study. This finding is in agreement with some studies, where significant associations between Brucella antibody seropositivity and history of abortion have been reported (Adugna *et al.*, 2013; Alemu *et al.*, 2014; Ibrahim *et al.*, 2010; Tolosa *et al.*, 2008). Similarly, studies in different African countries also show that individual animal brucellosis seroprevalence correlates with the presence of abortions (Muma *et al.*, 2012). This could be explained by the fact that abortion is a typical outcome of brucellosis (Alemu *et al.*, 2014; Minda *et al.*, 2016).

Based on parity, the difference observed in seroprevalence was statistically insignificant. Similar observations were recorded by Minda *et al.*, (2016) and Berhe *et al.*, (2007). Although there is an insignificant association between parity and brucellosis seropositivity, a higher seroprevalence was observed in cattle with greater than three parturitions (6.67%) than in cattle with one up to three parturitions (5.63%) in the study area. The higher seroprevalence of brucellosis in the multi-parturition cattle of this study was in line with the findings of Minda *et al.*, (2016) and Asmare *et al.*, (2013).

Improvement of knowledge, attitudes, and practices among cattle owners could have a significant impact on the reduction of many zoonotic infections, including brucellosis. The analysis of the KAP in the current study showed that most cattle owners in the studied area had heard about bovine brucellosis (66.4%), but most respondents did not know it was a zoonotic disease (92.7%). Similar results were reported in brucellosis KAP studies conducted in northern Uganda Nabirye *et al.*, (2017) and Kenya Obonyo and Gufu, (2015) where 63% and 79% of community participants had heard of brucellosis, respectively. Studies conducted in Egypt by Holt *et al.*, (2011), Nigeria by Buhari *et al.*, (2015), Uganda by Kansiime *et al.*, (2014) and Jordan Musallam *et al.*, (2015) showed that 83%, 93%, 99.3%, and 100% had heard of brucellosis, respectively. Contrasting results were found in a brucellosis KAP study in Tajikistan, where only 15% had heard of brucellosis (Lindahl *et al.*, 2015). Most of the respondents in the current study had heard about brucellosis from veterinarians working in veterinary clinics, indicating the importance of the role of government veterinary services in the current study. However, the primary sources of brucellosis information were stated as unspecified media in the Jordan study (Musallam *et al.*, 2015), community health workers in the Kenya study (Obonyo and Gufu, 2015), parents in the Nigeria study (Buhari *et al.*, 2015), and friends or family members in the Tajikistan study (Lindahl *et al.*, 2015). Poor hygienic practices and uncontrolled animal movements were practiced in extensive husbandry systems. This could pose a substantial risk of transmitting the disease within and in between the herds. The present study findings also agree with previous studies on the intensive farming system in Ethiopia (Minda *et al.*, 2016).

Cattle owners' knowledge, attitude, and practice regarding the disease are crucial steps in developing prevention and control measures (Prilutski, 2010). In the current study, most respondents have limited knowledge and attitudes about disease transmission and control. Moreover, they have been practicing risky activities such as assisting their animals during parturition, disposing of aborted fetuses and afterbirth in an open environment without protective gloves or masks, and consuming raw milk. These might have resulted in high risks of disease transmission within and between the herds and humans. The current findings agree with previous studies on extensive livestock production system (Adugna *et al.*, 2013; Megersa *et al.*, 2011). The occurrence of brucellosis in humans is associated with contacting aborted animals with bare hands and assisting animals during parturition (Kozukeev *et al.*, 2006).

Conclusion

The present study revealed a 3.13% and 12.5% overall seroprevalence of bovine brucellosis at individual animal and herd levels, respectively,

in the Gambella region, Ethiopia. The seroprevalence of the disease was associated with the presence of small ruminants and the size of the cattle herd. The present study also found that cattle owners' knowledge, attitude, and practice toward brucellosis in the study areas were low. This might contribute to the widespread of bovine brucellosis both in animals and humans. Therefore, creating awareness in the community on the mechanisms of transmission, zoonotic importance, prevention, control, and economic importance of the disease is recommended. Moreover, communication and cooperation between animal and human health professionals, the agricultural and education sectors, cattle owners, and other relevant stakeholders need to be strengthened to reduce disease transmission between animals and humans and improve control of brucellosis.

Ethics approval and consent to participate

This study was conducted following the Declaration of Helsinki. All study animal owners were informed about the study and informed consent was obtained from all cow owners and individuals who participated in this study. Participation in the study was voluntary. Confidentiality was assured by using code. We confirm that the animals were handled with the best practices of veterinary care. Ethical clearance was obtained from the Ambo University research and ethical review committee (Ref. No. አዩ/42/31/40/12).

Consent for publication

Not applicable.

Data Availability Statement

The data generated and analyzed during the current study are available in the (raw data compiled.xls) deposited in the Open Science Framework (OSF) repository as (**https://osf.io/g826x/files/osfstorage/63d31c938a2ec2010e635187**).

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Competing interests

The author reports no kind of financial, non-financial, professional or personal conflicts of interest in this work.

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