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Seroprevalence of Bovine Brucellosis and its Associated Risk Factors and Knowledge, Attitude and Practice of Cattle Owners Towards the Disease in Gambella and Itang Districts of Gambella Region, South-Western Ethiopia

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Abstract: A cross-sectional study was carried out from February 2019 to November 2019 to determine seroprevalence of Bovine Brucellosis, potential risk factors, knowledge-attitude and practice of cattle owners about brucellosis in Gambella and Itang districts Gambella regional state. A total of 400 blood samples were collected from local breed cattle of above six months of age. The RBPT screened 19 *Brucella* seropositive out of 400 (4.75%) (95% CI 1.04-8.05) and positive sera were further retested by using CFT and the combined result (RBPT and CFT tests) 8 (2%) (95% CI: 0.75-3.2) sera were confirmed seropositive. Out of 80 herds included in the study, 6(7.5%) (95% CI: 4.6-17.2) were seropositive using CFT with at least one seropositive animal in the herd. The overall seroprevalence of brucellosis was 2 and 7.5% at animal and herd level respectively. Besides, information was gathered on individual animal and herd to assess risk factors using a semi-structured questionnaire prepared for this purpose. The result of multivariable logistic regression analysis showed that herd size (OR: 9.481, 95%CI: 1.09-82.48, p=0.041), history of previous abortion (OR: 7.8, 95%CI: 5.75-12.38, P=0.003) and history of retain fetal membrane (OR: 32.18: 95%CI: 3.78-27.38, P=0.001) were found associated for *Brucella* seropositivity. The results of questionnaire survey showed that the majority (87.5%) of respondents did not have sufficient knowledge about brucellosis and its risk factors, about 93.75% of the have the habit of consumption of raw milk and 81.25% of respondents were assisting parturition without glove which put them at high risk of acquiring the infection. Hence, avoid raw milk consumption, increasing awareness creation, deep burring of aborted fetuses and fetal membrane measures should be implemented to reduce risk of infection and transmission of the disease in livestock and human in the study area.

Key words: Bovine · Brucellosis · Risk Factors · Seroprevalence · Gambella · Ethiopia

INTRODUCTION

Brucellosis is an infectious bacterial zoonotic disease caused by genus Brucella, characterized by their Gram-negative, facultative, intracellular coccobacillary organisms and comprised of species based upon biochemical features and their correlation with preferred host species [1, 2]. Bovine brucellosis is typically caused by *Brucella abortus*, less commonly by *B. melitensis* and rarely by *B. suis*, is characterized by late term abortion, infertility and reduced milk production [3]. Aborted foetuses and discharges containing large number of infectious organisms are implicated in transmission of the disease within and in between herds. Besides, chronically infected cattle can shed lower numbers of organisms through milk and reproductive tract discharges and vertically transmit infection to subsequently born calves and preserve disease transmission [4].

There are several factors that are understood to influence the epidemiology of cattle brucellosis as well as factors associated with disease transmission between herds, factors influencing the perpetuation and spread of infection within herds [5]. In order to design the proper strategy for the disease control and prevention measures

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perception of the epidemiology of brucellosis is crucial; yet, such information is inadequate in sub-Saharan Africa. Thus, appropriate preventive measures have not been undertaken in this part of the world [4].

The prevalence is highest in the Mediterranean countries, Central and South America, the Middle East and South Asia [6]. This could be due to endemicity of the disease in the area, large number of small ruminant population, subsistence of risk factors and relaxation of control measures in the areas. While the disease has been eradicated from most of the developed countries, it is yet a main public and animal health problem in many developing countries, where livestock are a main source of food and income [7]

In Africa, bovine brucellosis was first recorded in Zimbabwe (1906), Kenya (1914) and in Orange Free State of South Africa in the year 1915 [8]. However, the epidemiology of the disease in livestock and humans including appropriate preventive measures are not yet well understood and has left particularly of sub-Saharan African with inadequate information. In dairy farm production, the disease is a key impediment to the importation of high yielding breeds and signifies a major constraint to the improvement of milk production through cross breeding [9].

In Ethiopia, people living in rural area in which their livelihood is mainly dependent on livestock and their products and their relationship with animals is very close. Additionally, people often consume raw animal products [10]. Brucellosis is endemic in Ethiopia since 1970 [11]. Since then, studies have demonstrated the presence of antibodies against Brucella in animals and humans in different parts of the country [12-15].

Brucellosis has posed a significant impact on animal and human health including wide socio-economic impacts, particularly in countries in which rural income relies largely on livestock breeding and dairy products [16]. It causes losses due to breeding failure (Abortion) in the affected animal population, decreased milk production and posing reduced work capacity through sickness of the affected human [17].

The economy of Ethiopia is mainly dependent on agriculture that makes it mostly vulnerable to the effect of zonootic infectious diseases [18] and most of households have direct contact with domestic animals, favoring an occasion for infection and spread of disease. In the present study area all of the herds shared the communal grazing which allows unrestricted contact between animals that contributes the spread of brucellosis in extensive management system. The prevalence is linked to the practice of animal movement to communal watering points and other areas when searching for pasture and water [19].

Most of the studies on cattle brucellosis have been carried out in central and northern Ethiopia which were focused on dairy cattle's of urban and per-urban areas [15, 20]. However, the majority of livestock were found in rural areas where most households have direct contact with domestic animals and the habit of consuming raw milk, raw or undercooked meat is still a common practice, especially among rural communities [21, 22]. This could mainly be attributed to lack of knowledge of the zoonotic risks associated with the consumption of unpasteurized milk.

Several reports have indicated the occurrence of livestock and human brucellosis is increasing [20]. However, it is difficult to generate the general prevalence of animal and human brucellosis in the whole country due to lack of consistent studies in different parts of the country. Correspondingly, there were no studies undertaken on the seroprevalence, its associated risk factors and community awareness towards brucellosis. Consequently, the study was undertaken to determine the overall seroprevalence of bovine brucellosis, assess potential risk factors for infection of bovine brucellosis and knowledge, attitudes and practices of owners about brucellosis in the Gambella and Itang district, South-western Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in two purposively (logistic, accessibility) selected districts namely Gambella and Itang district of Gambella regional state from February 2019 to November 2019. According to the National Meteorology Agency, Gambella Branch (2005), Elevations in Gambella District ranges from 400–600 meters above sea level; annual rainfall is 800-1600mm and temperature of the area ranges from 19.6°C to 41.5°C. Around 20% of the Woreda is covered by dense forest [23]. Mixed crop- livestock, production system practiced in the area. Cattle are used as assets and the source of income [24].

There are about 95, 760 heads of cattle kept in both districts (20, 217 in Gambella and 75, 543 in Itang) the numbers of cattle found in each district are indicated in (Table 1) [24].

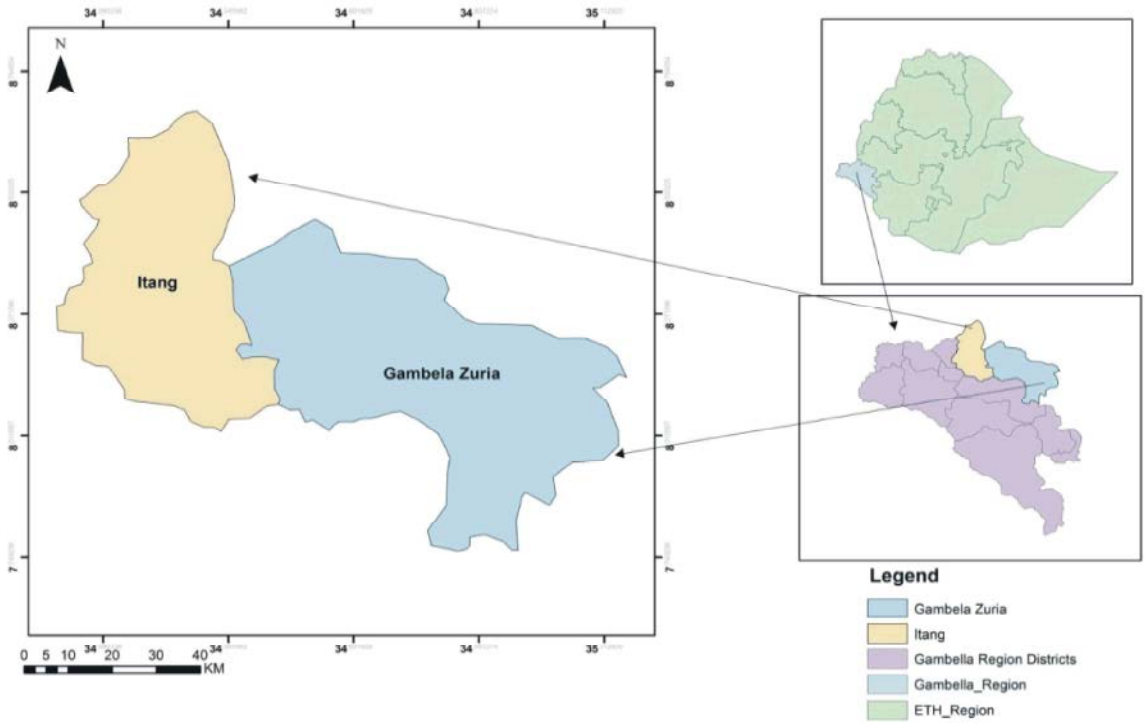

Fig. 1: Map of the study area

Study Animals: The local cattle breed with no history of vaccination against brucellosis in Gambella and Itang districts were considered as study animals. Unrestricted animal movement, communal grazing and watering, poor shelter, under feeding, etc., are livestock management problems, which might have their own part effect as factor for various animal diseases. Both sexes and different age group greater than six month were included in the study, while the cattle less than 6 months of age due to maternal antibody may interfere with test result.

Study Design: A cross-sectional epidemiological study was carried out to determine seroprevalence of brucellosis (at animal and herd level) and its association with different risk factors using two serological tests, Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) and questionnaire survey were used for Knowledge, Attitude and Practice (KAP).

Sampling Procedure and Sample Size Determination: The study districts were selected purposively on the basis of prior information on the problem, logistics and accessibility. The selection of Peasant associations (PA's) was done based on the proportions of PA's found in each districts. Accordingly, five PAs from Gambella district (Abol, Opagna, Bonga, Pinkwo and Ileyi) and eight PAs from Itang special district (Achewa, Baziel, Drong, War, Watgach, Mekod, Ibago and Eliya) were selected randomly. It was followed by made decision on the number of sampling herds (households) from each districts. Accordingly 20 and 60 herds were selected by systematic random sampling from Gambella and Itang special district respectively. The number of herds taken from each PAs was based on the number of herds in the PAs. Therefore, (6, 5, 3, 3, 3) herds from (Bonga, Ileyi, Abol, Pinkwo, Opagna) PAs of Gambella district and of 60 herds of Itang district about (10, 10, 10, 9, 8, 6, 4, 3) herds from (Mekod, Watgach, Baziel, Achewa, Drong, War, Eliya, Ibago). PAs respectively were sampled randomly. The numbers of animals sampled from each PAs were also determined by the proportion of the cattle population existing in each PAs. Accordingly, (30, 20, 14, 10 and 10) cattle from (Bonga, Ileyi, Abol, Pinkwo and Opagna) PAs and (60, 55, 45, 45, 40, 36, 20 and 15) cattle from (Mekod, Watgach, Baziel, Achewa, Drong, War, Eliya and Ibago). PAs respectively found in both districts were sampled by simple random sampling technique. Generally about 80 herds and 400 heads of cattle were sampled, of this about 77.5% (n=310) of the study animal were female and 24% (n=96) of them were young.

The selection of PAs, herds and sampled animals were based on data obtained from the districts agricultural office. Those cattle that housed in the same barns or under individual households were considered as one herd [14, 25].

According to data obtained from the district agricultural office [24], the number of households in each PA's varies from 80 to 150. Averages of 7 herds (households) were selected by systematic random sampling method from each PA. Animals above six months of age within the herds were selected using simple random sampling method. The Herd sizes were divided into three categories; small (≤15 heads of cattle), medium (≥15-30 heads of cattle) and large (≥30 heads of cattle) depending on number of animals [26]. The number of animals existing in each herd ranges from 15-200 heads of cattle were found respectively.

To determine the desired sample size, there were no previous reports of bovine brucellosis prevalence in the present study area. Therefore, the average expected prevalence was assumed to be 50% for the area within 95% confidence interval (CI) at 5% desired precision as stated by Thrusfield [27]. Hence, using the formula, calculated sample for the current study becomes 384 heads of cattle; however, a total of 400 serum samples of both sexes were sampled in the study areas to increase the precision of the result.

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

where,

n = required sample size
P_{exp} = expected prevalence
d = desired absolute precision
Z = confidence statistics

Blood Sample Collection: Approximately 10 ml of blood was collected from the jugular vein of each selected animal using plain vacutainer tubes and needle. During the sampling, animals were restrained and the area was first disinfected by using 70% alcohol before puncturing. Identification of each animal was labeled on corresponding vacutainer tubes and centrifuged at 2500/rpm for 5 minutes then after the sera were collected in to the sterile cryovial tube (2ml), to which animal's identification was coincided. Sera were kept at -20°C in National Animal Health Diagnostic and Investigation Center (NAHDIC) until serological tests were conducted. All serum samples were screened by Rose Bengal Plate Test (RBPT) at NAHDIC. The sera that tested positive to the RBPT were further subjected to the Complement Fixation Test (CFT) for confirmation at NAHDIC, Sebeta.

Questionnaire: A questionnaire was designed to collect information on factors that were believed to influence the spread and prevalence of Brucella infection. These include herd size (small <15 cattle; Medium 15-30 cattle; and large >30 cattle) and composition (bovine, caprine, ovine, canine), management system (extensive purchase source and replacement dairy cattle (own farm or outside source), handling of animal products (milk, meat) and handling of calving/abortion (parturition pen, burring, burning, thrown to environment). The following data were collected on animal attributes: sex, age of the animal (cattle: >0.6-3 years=young); 3-5years= adult; >5 years= old) and reproductive status, parity, history of abortion and retained fatal membrane and breeding (natural, AI).Questionnaire surveys with open and closed questions were used among the owners or attendants whose animals were tested. The data collected were ethical respected with confidential consideration involvement and the farmers interviewed from selected kebeles /districts were proportionally selected from each site by randomly sampling techniques.

Serological Tests: All serum samples collected were screened for Brucella antibodies using the Rose Bengal Plate Test (RBPT) at NAHDIC and the RBPT antigens were obtained from NAHDIC Sebeta, Ethiopia. Testing was done according to the procedures stipulated by OIE [3]. Before performing test, antigen and sera are brought to room temperature. Then 30 µl of each serum sample was placed on a clean white tile and mixed with an equal volume of antigen. Subsequently, an equal volume of antigen was placed near each serum spot. The serum and antigen were mixed thoroughly using a clean tooth pick to produce a circle approximately 2 cm in diameter and the mixture was agitated gently for 4 min. at ambient temperature and the result was noted based on the presence or the absence of agglutination.

The interpretation was performed as follows: 0 = no agglutination, + = barely perceptible, ++ = fine agglutination, some clearing, +++ = coarse clumping, definite clearing. Those samples identified with no agglutination were recorded as negative and those with +, ++, +++ were recorded as positive.

Complement fixation test (CFT) was used to all sera tested positive by Rose Bengal Plate Test (RBPT) for further confirmation. *B. abortus* antigen for CFT was used to detect the presence of anti-Brucella antibody in the sera like RBPT. Test was done according to the protocol of recommended by OIE [3] 2004 at NAHDIC, Sebeta. 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 hemolysis) with clear water supernatant, result was recorded as ++++, nearly complete fixation (75% clearing) as +++, partial hemolysis (50%) as ++ and some fixation (25% clearing) as +. Complete lack of fixation (complete hemolysis) was recorded as 0. For positive reactions final titrations was registered [3]. 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 at a dilution of 1:10 and at dilution of 1:20 were classified as positive [3].

Data Analysis: All the data collected was entered in to Microsoft excel spread sheet and coded appropriately. Descriptive statistic was utilized to summarize data after coded and transferred to Statistical Package for the Social Science (SPSS) version 20. Two epidemiological parameters were generated namely individual animal and herd level seroprevalence. An animal was considered positive if it tested seropositive on both RBPT and CFT test. Individual animal seroprevalence was calculated by the number of positive animals divided by the total number of animals tested. Similarly, herd level prevalence was calculated by the number of positive herds with at least one seropositive animal in the herd divided by the total number of herds screened.

Univariable logistic regression analysis was used to select the individual explanatory variable that may predict the outcome variable in the model. The explanatory variables ($P \leq 0.25$) were further checked for multicollinearity using the variance inflation factor (VIF) and tolerance factor (TF) before multivariable logistic regression analysis. Variance inflation factor values of greater than 3 or tolerance less than 0.1 were considered the cut-off points for the collinearity diagnostics. The strength of association between outcome (*Brucella* seropositivity) and risk factors was assed using the odd ratio (OR). Multivariable logistic regression analysis was conducted to calculate the probability of disease happening as a function of several independent variables. The backward elimination procedure was used to eliminate the factors that were not significant at $P<0.05$ in overall model. Factors that were significant ($P \leq 0.05$) were retained in the final model and model fit was observed using the Hosmer-Lemeshow test.

RESULTS

Seroprevalence of Bovine Brucellosis: From the total of 400 Animals, 90(22.5%) male and 310(77.5%) female animals above 6 month of age were sampled and tested for Brucella antibodies. Of which 19 (4.75%) (95% CI 1.04-8.05) were positive to RBPT and positive sera were further retested by using CFT and the combined result (RBPT and CFT tests) 8 (2%) (95% CI: 0.75-3.2) sera were confirmed seropositive which giving over all seroprevalence of 2% (Table 1). Out of 80 herds included in the study, 2 herds from Gambella and 4 herds from Itang or 6 (7.5%) were found seropositive using RBPT+CFT with at least one seropositive animal in the herd. The individual animal seroprevalence of bovine brucellosis in the two district of Gambella region ranged from 1.89 to 2.38% (Table 1). Comparatively, higher seroprevalence of brucellosis was recorded in Gambella District (2.38%) than Itang District (1.89%).

Animal Level Risk Factors Analysis: The result of Univariable analysis had shown the association of predictor variable and *Brucella* seropositivity (Table 2). Accordingly, seroprevalence of bovine brucellosis was not significantly related with study districts ($P>0.05$). Even though there were no significant difference among study districts and *Brucella* seropositivity, slightly higher proportion of seropositivity was observed in Gambella district (2.38%) when compared to Itang district (1.89%). Sex had no a significant association with brucellosis seropositivity ($P>0.05$) despite females having a slightly higher proportion of infection 2.25% (n=310) compared to males 1.1% (n=90). Seroprevalence of bovine brucellosis was significantly related with cows had history of RFM ($P<0.05$) and aborting cow ($P<0.05$). Age was also found a significant factor for brucellosis infection ($P< 0.05$) with old age having a higher proportion of infection. Of 310 female animals tested 42 (13.5%) showed history of abortion and was significantly associated with seropositivity ($P< 0.05$), 43 (13.9%) with history of retained placenta, 84(27.1%) were pregnant, 60(19.3%) were lactating and 81(26.1%) dry, heifer and calves).

Table 1: Overall individual animal and herd level brucellosis seroprevalence

Individual animal level prevalence				Herd level prevalence		
District	NA	RBPT +	RBPT+CFT	NH	RBPT+	RBT+CFT
Gambella	84	6(7.14%)	2(2.38%)	20	4(20%)	2(10%)
Itang	316	13(4.11%)	6(1.89%)	60	15(25%)	4(6.7%)
Total	400	19(4.75%)	8(2%)	80	19(23.75%)	6(7.5%)

NA = number of tested animals, NH = number of tested herds

Table 2: Univariable logistic regression analysis of common risk factors associated with Brucella seropositivity at individual animal level

Factor	N. tested	CFT+ (%)	OR	95%CI	P-value
Districts					
Gambella	84	2(2.3%)			
Itang	316	6(1.9%)	0.794	(0.157-4.005)	0.779
Sex					
male	90	1(1.1%)			
Female	310	7(2.25%)	2.056	(0.250-16.935)	0.503
Age					
Young	96				
Adult	143	2(1.4%)	4.25	(2.75- 26.35)	0.051
Old	161	6(3.7%)	7.861	(1.098-53.726)	0.040
History of abortion					
No	268	4(1.5%)			
Yes	42	3(7.1%)	69.22	(8.25-78.51)	0.001
History of RFM					
No	267	2(0.7%)			
Yes	43	5(11.6%)	28.784	(5.60-147.75)	0.000
RP-status					
Lactating	60				
Dry/heifer	166	2(1.2%)	35.989	(0.317-56.69)	0.896
Pregnant	84	5(5.9%)	0.208	(0.039-1.098)	0.064

N = number of tested animal, OR = Odds Ratio, CI = Confidence Interval, RP = retain placenta

Table 3: Univariable logistic regression analysis of common risk factors associated with Brucella seropositivity at herd level

Factors	Categories	NH	CFT +ve	OR	95%CI	P-value
District	Gambella	20	2(10%)			
	Itang	60	4(6.7%)	0.999	(0.964-1.036)	0.971
Herd size	Small*	27				
	Medium	25	1(4%)	0.037	(0.011-0.993)	0.042
	Large	28	5(17.8%)	0.072	(0.013-0.881)	0.038
New. animal	No*	57	2(3.50%)			
	Yes	23	4(17.4%)	4.636	(0.79-27.25)	0.089
Maternity pen	No*	67	4(5.9%)			
	Yes	13	2(15.4%)	1.017	(0.109-9.497)	0.981
Disposal after birth	No*	71	5(7.04%)			
	Yes	9	1(11.1%)	1.028	0.194-5.431	0.974

NH = number of herds, * = reference, OR = Odds Ratio, CI = Confidence Interval

Herd Level Risk Factors Analysis: The herd level Univariable logistic regression analysis revealed that herd sizes were found to be strongly associated with seropositivity to *Brucella* infection ($P < 0.05$). There was no significant difference of *Brucella* seropositivity according to district difference ($P>0.05$). However relatively higher proportion of seropositivity was observed in Gambella District (10%) when compared to Itang District (6.7%). The study also fails to detect a significant variation in *Brucella* seropositivity among other risk factors at herd level (Table 3).

The result of multivariable logistic regression analysis showed important risk factors for *Brucella* seropositivity (Table 4). Risk factors with p-value ≤0.25

Table 4: Multivariable logistic regression analyses identifying the association of potential risk factors to Brucella seropositivity in cattle

Factors	Categories	OR	95% CI	P-value
Herd size	Small (<15 heads of cattle)ref*			
	Medium (>15-30 heads of cattle)	0.257	(0.049-1.353)	0.052
	Large (>30 heads of cattle)	9.481	(1.092-82.483)	0.040
HRM	No*			
	Yes	32.182	(3.781-273.8)	0.001
HMA	No*			
	Yes	7.8	(5.759-12.389)	0.003

OR= Odds ratio, CI = confidence interval, * = reference category, HMA = history maternal abortion, RFM = history of retain fetal membrane

Table 5: Socio-demographic characteristics of respondents in relation to herd seropositivity according to District

Variables	Categories	NR	NPH(CFT)
District	Gambella	20(25%)	2(33.3%)
	Itang	60(75%)	4(66.7%)
Educational Status	Illiterate	61 (76.25%)	5(83.3%)
	Write and read	15 (18.75%)	1(16.7%)
	6-8 grade	4(5%)	
Sex of respondents	Male	67 (83.75%)	4(66.7%)
	Female	13 (16.25%)	2(33.3%)

NR = number of respondents, NPH = number of positive herds

in the univariate logistic regression model were included in the separate multivariable logistic regression model fitted. Accordingly, Age, Herd size, history of maternal abortion, introduction of new animal, reproductive status (pregnancy) and history of retain fetal membrane were significantly associated with *Brucella* seropositivity were included in the final logistic regression model. Of all of this, in the final analysis though animal's seropositivity was significantly influenced more by herd size, maternal abortion and prior history of retain fetal membrane, while introduction of new animal was not included in the multivariable regression because of its multicollinearity with herd size. Age and reproductive status (pregnancy) were found not significantly associated with Brucella infection and the rest of the variables were not included in the final model. Thus multivariable logistic regression analysis showed that animals involved in the large herd are 9.4 times more likely to be at higher risk for *Brucella* infection than animals in small herd with (95% CI: 1.092-82.483, OR=9.4 P<0.05). Similarly, the multivariable regression analysis revealed that the seroprevalence of brucellosis was significantly associated with animal which had prior history of retain fetal membrane and those animal with RFM were found to be 32 times more likely to be at higher risk for *Brucella* infection compared with no history of RFM with (95% CI: 3.781-273.8, OR=32.1, P<0.05). Seroprevalence of brucellosis was also significantly associated with female animals those had prier history of abortion (95% CI: 5.759-12.389, OR=7.8, P=0.003). This might be explained by the fact that abortion is typical outcomes of brucellosis.

Socio-Demographic Characteristics of Respondents: From the total of 80 respondents selected systematical, about 20(25%) and 60(75%) of them were from Gambella and Itang district respectively and 2 and 4 totally (6) of their herds were found seropositive to Brucella infection respectively. Of the total households interviewed, 76.25% of them were illiterate, while 18.75 % of them were able to write and read, only 5% of them were attended 6-8 grade education and none of them were proceeded this level. Majority of the respondents (83.75%) were male and 16.25% female and found with 4 and 2 of their herds were positive respectively (Table 5).

Herd Management and Husbandry Systems of Respondents: From the total households interviewed, 88.75% of the respondents were gained the skill from their parents and found with 5 positive herds, only 11.25% of them were acquired skill from extension/agricultural training and found with 1 seropositive herd. Regarding the housing type, 90% of the herds were housed in corral and about 10% were housed in barn/open field and holds 4 and 2 positive herds respectively. Only 16.25% farmers were had separating maternity pen and found with 1 seropositive herd, most of the respondents (83.75%) had no maternity pen and 5 seropositive herds were with them (Table 6).

Knowledge-Attitudes and Practices of Farm Owners about Brucellosis: The majority of herd owners or respondents (87.5%) was not aware of bovine brucellosis and holds all positive herds. Respondents were also interviewed to describe the occurrence of some

Table 6: Response of respondents on herd management and husbandry system

Variables	Categories	NR	NPH
Source of skill	Agri, training/Extension	9(11.25%)	1(16.7%)
	Parent	71(88.75%)	5(83.3%)
Housing type	Barn/Open field	8(10%)	2(33.3%)
	Corral	72(90%)	4(66.7%)
Separation of maternity pen	Yes	13(16.25%)	1(16.7%)
	No	67(83.75%)	5(83.3%)

NR = number of respondents, NPH = number of positive herds

Table 7: Knowledge-attitudes and practices of farm owners about brucellosis

Variables	Categories	NR	NPH
Awareness about brucellosis	Yes	10 (12.5%)	
	No	70(87.5%)	6(100%)
Awareness about Abortion	Yes	11(13.75%)	1(16.7%)
	No	69(86.25%)	5(83.3%)
Separation of aborted cow	Yes	4(5%)	
	No	76(95%)	6(100%)
Proper disposal of after birth	Burial/burning	3(3.75%)	
	Thrown	77(96.25%)	6(100%)
Raw milk consumption	Yes	75(93.75%)	5(83.3)
	No	5(6.25%)	1(16.6%)
Assisting cow during parturition with out glove	Yes	65(81.25%)	4(66.7%)
	No	15(18.75%)	2(33.3%)

NR = number of respondent, NPH = number of positive herds

reproductive problems that causes abortion and Most of the respondents (86.25%) had no knowledge on causes of abortion and as brucellosis cause abortion in cattle and found with most of (5) positive herd. The practices of disposing after birth were done mostly (96.25%) in the way thrown to the environment, with shared 100% of positive herds. About 95% of respondent were not separating aborted animal and found with all positive herds. The majority of the respondents consume raw milk (93.75%) and about 5 of their herds were positive. Similarly, most of the farmers (81.755%) have habit of assisting cows during parturition, without using of protective glove; they shared 4 positive herds of all positive herds (Table 7).

DISCUSSION

During the present study an overall seroprevalence of *Brucella* antibodies of 2% (95% CI: 0.75-3.2) was resulted. This finding is slightly in agreement with other studies conducted by different authors on cattle under similar production systems in different parts of Ethiopia; 1.7% from Arsi Zone [28], 1.97% from East Wollega [29], 2% from Sudan [30] abroad the country. However, higher prevalence was observed by various other authors than the present study in other parts of the country [25, 31, 32, 33], 4.63 11.1, 7.7, 14.14 and 3.3% seroprevalence was recorded respectively.

On the other hand the lower prevalence than the present study was reported by different authors; Tefera [34] with prevalence of 1.13% in intensive and extensive farms of Addis Ababa and Sululta town [35], who found an overall prevalence of 1.49% in extensive and semi-intensive farms of Tigray Region Degefu *et al*. [13] who found an overall prevalence of 1.38% from Agro pastoral cattle's of Jijjiga, Somali region [36] withprevalence of 1.3 in Humbo districts of Wolaita zone, Roba [37] with prevalence of 1.1% in Dida Tuyura Ranch and pastoral herds of Borena zone.

The differences in prevalence observed between the reports from different parts of Ethiopia and the present study may be due to sample size, differences in herd size, management conditions, agro ecological and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds. With regard to districts (Gambella and Itang), there was non-significant difference in seroprevalence of brucellosis. This could be due to similarity in management system and agro ecological.

In the present study, the seroprevalence of bovine brucellosis was not statistically significant between the sexes; though the result showed that infection was higher in female (2.25%) than male (1.1%). This finding is in agreement with the findings of, Berhe, *et al.* [35] in Tigray region, Deselegn and Gangwar [33], in Asella dairy farm Asgedom *et al.* [25] in and around Alage districts

who reported higher prevalence in female than male. The lower prevalence of male reactors in this study could be due to smaller number of males tested as compared to female and it has also been reported that the organism favor gravid uterus for growth and multiplication relative to testicle and epididymis [15]. Though no controlled study has been conducted on the relative susceptibility of female and male cattle to brucellosis, based on reactor rates it is probable that bulls are more resistant than sexually mature heifers and cows, however, are less resistant than sexually immature heifers [38]. The lower prevalence of male reactors in this study could be due to smaller number of males tested as compared to female.

This study revealed that, all infected animals were adult though there was not statistically significant difference (P>0.05) in seroprevalence of *Brucella* among different age groups. This finding was in agreement with Lidia [39] in central highland of Ethiopia and Ibrahim *et al.* [14], in selected site of Jimma zone, who reported only older age category reactors [15, 25, 28]. According to some authors [36, 37, 40, 50] susceptibility to brucellosis is reported to increase as the animals approach to the breeding age. Thus, sexually mature cattle are more susceptible to infection with *Brucella* organism than sexually immature animal of either sex [41]. In this study there was no seropositive reactor in animals less than 3 years of age. This finding is in agreement with the prevalence report of 0.0% in nullparous animals by Ibrahim *et al*. [14], Kebede *et al*. [32] and Berhe *et al*. [35]. This shows that brucellosis is highly related with age and sexual maturity of animals.

In this study herd size remained significantly associated with Seropositivity to brucellosis. This finding is in agreement with the reports [10, 15, 31, 36, 42]. An increase in herd size is usually accompanied by increase in stocking density, as well as an increase in risk of exposure to infection. Stocking density is an important determinant of the potential for transmission between susceptible and infected animals [43]. There is also undeniable fact that the spread of the disease from one herd to another herd and from one area to another is almost frequently due to the movement of an infected animal from an infected herd to a non-infected susceptible herd [44]. Therefore, brucellosis should never be viewed as the disease of individual animals, but should be considered in the context of herd and also the animal population in the region.

The cow with history of retain fetal membrane was significantly associated with seropositivity in the present study (p=0.001). Seropositivity to Brucellosis was higher in animals with history of retain fetal membrane (11.6%) compared to with no history of RFM (0.75%) animals. Association between brucellosis seroprevalence and occurrence of RFM also reported [14, 28 35, 36, 42].

Even though pregnancy was not significantly associated with seropositivity, pregnant cattle were showed more susceptible (5.9%) than nonpregnant (2.4%) to *Brucella* organism. This finding is in agreement with the reports of Yohannes [36], Adugna *et al.* [42], Omer *et al.* [43] in their study found that pregnancy status of cattle has no significant effect on the seroprevalence of brucellosis.

This study revealed that, the history of previous abortion was found significantly associated with Seropositivity to brucellosis with (P=0.001). Among the cows that had history of previous abortion was exhibited more than 7% (3/42) Brucella antibody in their serum than those cows which had no previous history of abortion 1.5% (4/268). This is in agreement with other authors [14, 28 35, 36, 42].

The information gathered with questionnaire survey has provided about the socio-demographic characteristics of the respondents, herd management and husbandry practice, knowledge- attitude and practices of cattle owners about brucellosis in selected districts of Gambella region. The educational status attained by majority of the respondents was low which falls between illiterate and lower grades. This low level of knowledge may lead to be at higher risk of acquiring and transmission of the disease, reduced production gained from animals because of the effects of the disease. Knowledge of diseases is a crucial step in the development of prevention and control measures [45]. Irrespective to enormous efforts of the government institutions to improve animal production in the areas, most farmers were not familiarized with new technologies. In addition to this, proper disposal of aborted materials, unprotected contacted with infected tissues (feotus, retain placenta), the habit of raw milk consumption, use of a separate parturition pen and assisting parturition by using protective gloves were not under consideration. Generally, the awareness of the respondents was very low. These could have effect on the transmission of the disease within and between the herds and human. This finding is in agreement with previous studies in extensive livestock production system [15, 42, 46]. The occurrence of brucellosis in humans is associated with contact with domestic animals, exposure to aborted animals and assisting animal parturition [47, 48, 49]. In this study, the majority of the respondents

have the habit of drinking raw milk and assisting parturition without using protective glove. This concludes that the lack of awareness about the impacts of the disease and this in turn, contributes to the spread and transmission of the infection to human in the area. Thus, there is a need to design and implement control measures aiming at preventing further spread of the disease in the Region through the use of better management practices [49, 51].

CONCLUSIONS

The present study showed that the seroprevalence of bovine brucellosis was found to be low in Gambella and Itang special districts of Gambella region. The finding of positive serological reactors indicates the presence of foci of infection that could serve as sources of infection for the spread of the disease into unaffected animals and herds. The study revealed that herd size, abortion and retain fetal membrane were found to be significantly associated with *Brucella* seropositivity. The study also clearly showed that cattle owners had less knowledge of the disease and at higher risk of acquiring the infection that was realized by consuming raw milk, assisting parturition and handling of aborted materials without using protective gloves. Hence, avoid raw milk consumption, increasing awareness creation, deep burring of aborted fetuses and fetal membrane measures should be implemented to reduce risk of infection and transmission of the disease in livestock and human in the study area.

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