Global Veterinaria 24 (2): 68-78, 2022
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2022.68.78

Sero-Prevalence of Bovine Brucellosis in Selected Dairy Farms of Debreberhan Milkshed, Central Highlands of Ethiopia

[1]Admasu Lakew, [2]Gebeyehu Goshu, [2]Gezahegne Mamo,
[2]Ashenafi Mengistuand and [2]Tilaye Demissie

[1]Department of Animal Science, College of Agriculture and Natural Resource Sciences, Debre Berhan University, Debre Berhan, Ethiopia
[2]Addis Ababa Universities, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

Abstract: A Cross-sectional study was conducted in Debre Berhan milk sheds central highlands of Ethiopia with the objectives to assess the prevalence of brucellosis in dairy cattle and its zoonotic significance in the study area. A total of 744 blood samples were collected from non-vaccinated, crossbred and local breeds of dairy cattle. Of which 11 (1.5%) animals tested positive by RBPT and 9 animals were confirmed positive by CFT, giving seroprevalence of 1.21 % with a combined test of result of RBPT and CFT. The risk of having brucellosis in those herds experiencing abortion was 22.97 times higher (OR =22.97, CI = 2.72-193.73) than those without abortion. Herds with the previous history of abortion and placenta retention showed 23 and 11times more likely to be seropositivity to brucellosis than those herds with no history of abortion and placenta retention ($p \leq 0.01$) and ($p \leq 0.01$) respectively. Dairy farmers addressed in this study were used to throw retained fetal membrane, aborted fetus and different discharges to the environment. They also consume raw milk that may be associated with high risk of public health problem. In conclusion, isolation of aborted and new entry animals, movement control between different herds and creation of public awareness by educating people are forwarded to reduce disease transmission within animals and from animals to humans.

Key words: Abortion · Bovine Brucellosis · Debreberhan · Ethiopia · Prevalence · Zoonosis

INTRODUCTION

Brucellosis is one of the infectious diseases that are highly characterized primarily by causing an abortion in late pregnancy, retained placenta, endometritis and infertility in subsequent pregnancies in cattle. It has also a significant economic, social and public health impact in many parts of the world [1]. As cited by Schelling *et al.* [2], nine *Brucella* species are currently recognized; seven of them that affect terrestrial animals are *Brucella abortus*, *B. melitensis, B. suis, B. ovis, B. canis, B. neotomae* and *B. microti* and the two other species that affect marine mammals are B. ceti and B. pinnipedialis. Seleem *et al.* [3] reported that brucellosis is considered as the most important and widespread zoonotic disease with economic and public health impacts, particularly for human and animal populations. Hika *et al*. [4] reported that brucellosis is a public health problem with adverse health implications both for animals and human beings as well as economic implications for individuals and communities. Currently, there is an increasing trend of establishment of a number of small and medium dairy farms that supply raw milk and milk products for the communities as well as the large milk processing plants to the capital city of Addis Ababa. Due to these reason epidemiological investigations are very vital to identify the major risk factors that predominantly influence the disease occurrence and thus contribute to designing appropriate and feasible national controlling strategies [5]. Previous studies were also limited in their scope as they were concentrated only on a few parts of the country reported by Yohaness [6], Abeje [7] and Edao *et al*. [8], which hardly represent the whole country in general and DebreBerhan milk shed in particular. Therefore the objective of this study was to determine the seroprevalence, potential risk factors and their association with Brucella seropositivity in Debre Berhan milk shed, central highlands of Ethiopia.

Corresponding Author: Admasu Lakew, Department of Animal Science, College of Agriculture and Natural Resource Sciences, DebreBerhan University, Debre Berhan, Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The cross-sectional study was conducted in selected districts ofDebreBerhan town, BasonaWorana and Angolelanatara, North Shewa administrative Zone of the Amhara National Regional State, central highlands of Ethiopia (Figure 1). Debre Berhan town, the administrative and commercial center of North Shewa Zone which is 130 km away to the Northeast of Addis Ababa, on the paved highway of Addis Ababa to Dessie. The topography of the study area is predominantly characterized by undulating hilly landscape with gentle slopes. The study area is located between 9° 30' and 9° 50' latitudes and 39° 20' and 39° 44' longitudes. The elevation ranges from 2840 to 2943 masl [9]. The mean annual minimum and maximum temperatures averaged between 6.7°C and 19.9°C, respectively. The mean annual rainfall is 1026 mm with potential evapotranspiration of 1396 mm. Rainfall distribution is bimodal, usually, the long rains (*meher*) last from June to the beginning of September and the period of the short rains (*belg*) falls between February and May. About 86% of the annual rain falls between June and September which is the main cropping season. Most of the study area is covered by moderately and poorly drained soils, predominantly black Vertisol [9]. Crop-livestock farming is the predominant farming systems in the areas. The livestock species reared include cattle, sheep, goats, donkeys, horses, mules and poultry. Cattle production with indigenous and cross-bred animals predominates the livestock production followed by sheep production [10].

Study Population: The study animals were indigenous Jersey and crossbred cattle to determine the seroprevalence of brucellosis and their association with different risk factors using two serological tests Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). A structured questionnaire survey was performed to assess the associated risk factors. Relevant individual animal and farm level information was collected using a semi-structured questionnaire.

Sample Size Determination: In order to determine the desired sample size, there were no previous reports of the prevalence of brucellosis in the present study area. Therefore, the average expected prevalence rate was assumed to be 50% for the area within a 95% confidence interval (CI) at 5% desired precision as stated by Thrusfield [11]. Hence, using the formula, calculated sample for the current study becomes 384 heads of cattle;

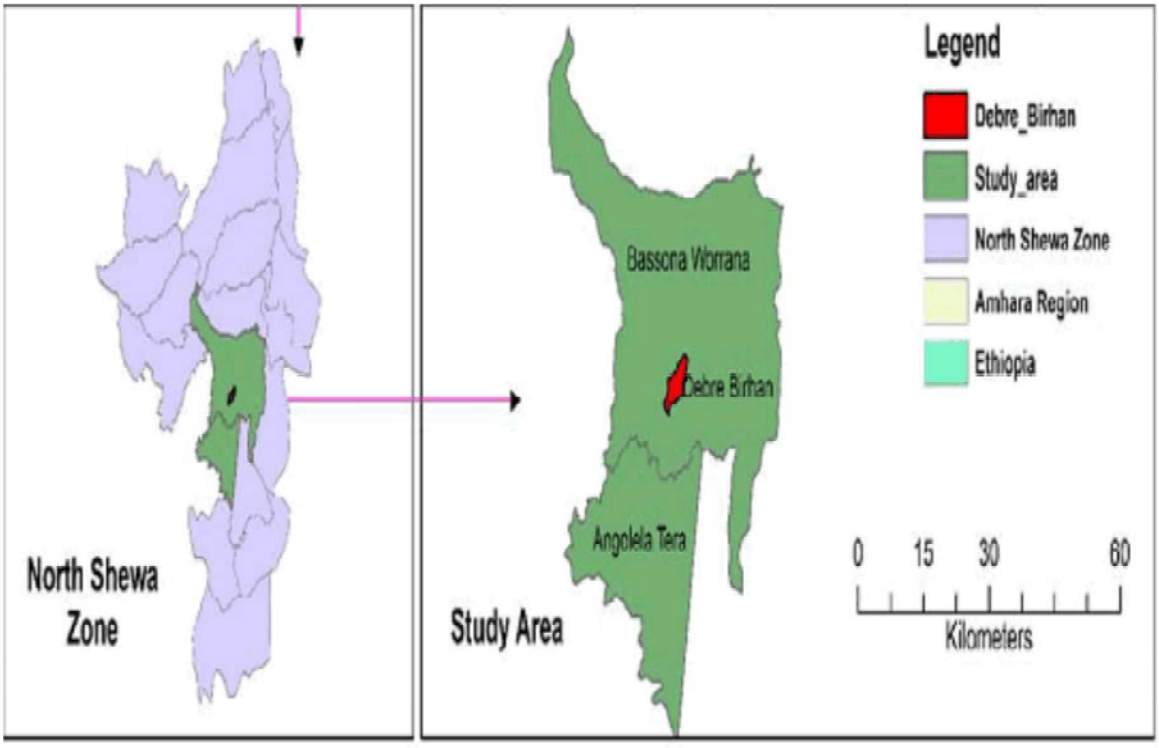

Fig. 1: Map of the study areas

however, a total of 744 serum samples (194 from Debere Berhan town, 237 from Basona Worana and 313 from AngolelanaTeraWereda) of both sexes having different ages were sampled to increase the precision of the result based on the willingness of the owners but individual animals within the herds were selected using simple random sampling method except for those cows with a history of abortion or active case of abortion which were included purposively.

$N = 1.96^2(P)(1-P)/d^2$

where,

N = Total calculated sample size
P = Expected prevalence
d = Absolute precision

Out the total 744 animals sampled, 93.4% were female and 6.6% male cattle at least 6 months of age and female's age 2 years and above, none milking, replacement heifers and bulls were included. A total of 117 small farms (34 DebreBerhan, 39 BasonaWorana, 44 Angolelana Tera Wereda) (with an average herd size of 5-10), 20 medium farms (5 DebreBerhan, 14 BasonaWorana, 1 Angolelana Tera Wereda) (with an average herd size of 11-20) and 8 large farms (3 DebreBerhan, 1 BasonaWorana, 4 Angolelana Tera Wereda (with an average herd size greater than 20) were randomly sampled. Finally, a total of 145 farms (67 in urban and 78 in peri-urban farms) were selected using random sampling method and individual dairy cattle were also selected randomly based on the willingness of the owners.

Questionnaire for the Assessment of Risk Factors for Brucellosis: The potential risk factors for the prevalence of bovine brucellosis at herd and individual animal level such as parity, breed, age, farm type, herd size, farm location, breeding system and history of reproductive problem were extracted by face-to-face interviewing from farmers by using a semi-structured questionnaire. Furthermore, the clinical indicators such as history of abortion, testicular swelling and other reproductive problems were interviewed and recorded. The type of management system was categorized into intensive, semi-intensive and extensive. Herd size was categorized into 5-10 animals (small); 11 - 20 animals (medium) and > 20 animals (large) as reported by Alehegn *et al.* [12]. Presence or absence of separate calving pen, retained fetal membrane and aborted fetus disposal methods, use of personal protective equipment during handling aborted cows, retained fetal membrane and awareness of farmers on diseases causing abortion in late pregnancy, breeding system, source of replacement stock were the risk factors considered at the herd level. While types of sex, age, breed and occurrence of previous abortion; retained placentas of the relevant animal were the risk and clinical factors considered at animal level.

Blood Sample Collection: Approximately 7-10 ml of blood was collected from the jugular vein of each selected animal using plain vacutainer tubes. Before puncturing, animals were restrained and the area was disinfected by using 70% alcohol. The identification of each animal was labeled on corresponding vacutainer tubes and kept overnight at room temperature to allow clotting. The next morning, sera were collected into the sterile cryovial tube (2ml), labeled and kept at - 20°C in DebreBerhan University Biology laboratory until serological tests were conducted. All serum samples were screened by Rose Bengal Plate Test (RBPT).All RBPT positive sera were further tested using the Complement Fixation Test (CFT) for confirmation at the National Veterinary Institute (NVI) at Bishoftu.

Serological Laboratory Techniques

Rose Bengal Plate Test (RBPT): RBPT was conducted following the procedure of OIE [13]. About 30µl sera sample was dispensed onto the plate and 30µl of RBPT antigen was dropped alongside the sera. The plate was shaken by hand for 4 minutes and the test was read by comparing it with the positive and negative control sera by examining for agglutination in natural light. Results of RBPT were interpreted as 0, +, ++ and +++ as described by OIE [13]. 0 = no agglutination; + = barely visible agglutination (seen by using magnifying glass); ++ = fine agglutination and +++ = coarse agglutination. Samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive.

Complement Fixation Test (CFT): The CFT procedure was undertaken on those samples which were found to be positive on RBPT as described in OIE protocol [13]. The preparation of the reagents was performed according to OIE protocols. A titration of hemolysin and antigen was performed before the test. The minimum hemolytic dose was also estimated for each run. As for the interpretation of test results, positive reactions were indicated by sedimentation of Sheep Red Blood Cells (SRBC) and the absence of hemolysis. Negative reactions were revealed by the hemolysis of SRBC. According to OIE [13], sera with a strong reaction, more than 75%

fixation of complement at a dilution of 1:10 and at least with 50% fixation of complement at a working dilution (1:5) was classified as positive.

Data Analysis: Data was collected and stored in Microsoft (MS) Excel Spread Sheet program and Categorical variables were summarized as frequency and percentages. Descriptive statistical analysis of various risk factors and dependent variables were done using (STATA software version 13). The Fishe's exact test was used to test Brucella-seroprevalence association with incriminated categorical risk factors. The total prevalence was calculated by dividing the number of RBPT- and CFT positive animals by the total number of animals tested. Herd prevalence was calculated by dividing the total number of herds with at least one reactor in RBPT and CFT by the number of all herds tested. In these study a herd, defined as the total number of cattle belonging to the same household. Univariate logistic regression was used to test the significance of the effect of different risk factors on sero-prevalence of brucellosis. Odds ratio (OR) was utilized to measure the degree of association between risk factors and Brucella-seropositivity. All risk factors that had non-collinear effect and p-value < 0.25 in the univariable logistic regression analysis were subjected to multivariable logistic regression analysis.

RESULTS

A total of 744 animals, 49(6.6%) male and 695(93.4%) female animals above 6 months and 2years of age were sampled and tested for Brucella antibodies. Of the total 744 animals tested with RBPT, 11 were positive for brucellosis with seroprevalence of 1.5%. Of which 11 animals tested positive by RBPT, 9 animals were confirmed positive by CFT, giving seroprevalence of 1.21 % with a combined test of result of RBPT and CFT.

There were no significant differences between urban and peri-urban and farm types (P>0.05). However, a relatively higher proportion of seropositivity was observed in peri-urban and intensive farm types (0.8%) and (0.81%) respectively when compared to urban, extensive and semi-intensive systems. The occurrence of brucellosis was significantly higher in animals (1.1%) having age greater than 6years (P<0.01), whereas, no animal less than 3 years old was found to be sero reactive. Higher Brucella-seropositivity was observed in crossbreeds than local breeds and Jersey (P<0.05) respectively. Regarding parity, the prevalence of brucellosis was higher in those cattle with multiparous.

The prevalence of bovine brucellosis was statistically significant higher between herd sizes of the studied animals (P<0.05), herd size greater than 10 more likely to be infected with Brucella organisms than those with herd size less than <10 animals. All seropositivity animals were females and were either pregnant or lactating. Among 695 female animals, 52(0.7%) showed a history of abortion and 34(0.7%) with a history of retained fetal membrane. The seroprevalence of brucellosis were significantly associated with cows with a history of abortion and cows with a history of retained fetal membrane (Table 1).

Univariable Logistic Regression Analysis of the Variables Associated with Animal Level Sero-Positivity: The results pertaining that the association of interpreter variable and Brucella-seropositivity by univariable analysis are presented in Table 2. The results indicate that the highest seroprevalence was observed for animals in herd size greater than >20 of (0.54%)than animals found in herd size less than <10 and between 11-20 animals respectively. There was a significant association (p<0.05) between herd size with the odds of seropositivity being at least 6 times more likely to be infected with Brucella organisms. The results indicate that the odds for Jersey breeds were 9.69 times at high risk of infection compared to cross breeds and local breeds respectively.

Age showed statistically significant associations (P<0.05) with seropositivity of brucellosis. The odds of ages >6years were 8.08 at high risk of an infection with seropositivity of brucellosis. Similarly, animals with history of abortion and retention of placental were found to be significantly associated with seropositivity. The prevalence of brucellosis was significantly ($p \leq 0.001$) higher (0.7%) in dairy animals with a history of abortion with 17.00 times more likely to be seropositive than dairy animals with no history of abortion. The prevalence of Brucella seropositivity significantly higher 5(0.7%) in animals having a history of retention of placenta with 28.32 times more likely to be seropositive than dairy animals with no history of placenta retention. The details of the association with seropositivity were indicated in Table 2.

Brucella Seropositivity at Herd-level Risk Factors Analysis: The results pertaining that out of 145 herds studied, 9 (6.2%) were positive using combined RBPT and CFT result. The prevalence of Brucella seropositivity analysis revealed that herds with a history of retention of placenta and abortion were found to be strongly associated with seropositivity to Brucellosis ($p < 0.05$).

Table 1: Association of risk factors with brucellosis seropositivity at the individual animal level

Risk factors	No. Tested	RBPT positive No. (%)	RBPT+CFT positive No. (%)	Fisher's exact test p-value
Areas				0.741
Urban	314	3(0.4)	3(0.4)	
Peri-urban	430	8(1.1)	6(0.8)	
Farm types				0.696
Intensive	413	6(0.81)	6(0.81)	
Semi-intensive	256	3(0.4)	2(0.3)	
Extensive	75	2(0.3)	1(0.13)	
Age				0.009
<3 years	100	0(0)	0(0)	
3-6 years	307	1(0.13)	1(0.13)	
>6years	337	10(1.34)	8(1.1)	
Sex				1.00
Female	695	11(1.5)	9(1.21)	
Male	49	0(0)	0(0)	
Breed				0.044
Cross	685	8(1.1)	7(0.94)	
Local	48	2(0.3)	1(0.13)	
Jersey	11	1(0.13)	1(0.13)	
Herd size				0.034
Small	449	3(0.4)	2(0.30)	
Medium	137	4(0.54)	3(0.4)	
Large	158	4(0.54)	4(0.54)	
Mating system				0.118
Non serviced	41	0(0)	0(0)	
Natural	418	8(1.2)	6(0.9)	
AI	202	1(0.14)	1(0.14)	
Both	34	2(0.3)	2(0.3)	
Parity				0.138
Null parous	73	0(0)	0(0)	
Mono parous	178	0(0)	0(0)	
Multiparous	444	11(1.6)	9(1.3)	
Abortion				$p \leq 0.001$
Absent	643	6(0.9)	4(0.6)	
Present	52	5(0.7)	5(0.7)	
RFM				$p \leq 0.001$
Absent	661	4(0.6)	4(0.6)	
Present	34	7(1.0)	5(0.7)	

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test

Herds with the previous history of abortion and placenta retention showed 23 and 11times more likely to be seropositivity to Brucellosis than those herds with no history of abortion and placenta retention ($p \leq 0.01$) and ($p \leq 0.01$) respectively. The odds of large herd sizes were 45.6 times at higher risk ($p \leq 0.001$) than small and medium herd sizes. There was no significant association ($P > 0.05$) between urban and peri-urban production systems (Table 3).

Analyses of Multivariable Logistic Regression for Brucella Seropositivity: The results from Table 4 indicated that the risk factors with p-value ≤ 0.25 in the univariate logistic regression model were included in the distinct multivariable logistic regression model. Age, breeds, herd size and history of abortion were included in the final logistic regression model. However, breed, herd size and history of abortion were significantly associated with Brucella seropositivity. In this case, retention of fetal membrane was not included in the final multivariable regression because of their multicollinearity with abortion. As a result the final multivariable logistic regression analysis shows that animals with a history of abortion are 10 times more likely to be seropositive to Brucellosis (OR= 10 [2.53-42.42).

Table 2: Univariable logistic regression analysis based on the individual animal level seroprevalence of brucellosis and associated risk factors

Risk factors	No. Tested	RBPT+CFT positive (%)	95%CI	OR	P-value
Areas					
Urban	314	3(0.4)			
Peri-urban	430	6(0.81)	0.36-5.91	1.47	0.59
Farm types					
Intensive	413	6(0.81)			
Semi-intensive	256	2(0.3)	0.107- 2.67	0.53	0.445
Extensive	75	1(0.13)	0.109- 7.72	0.92	0.936
Age					
<3 years	100	0(0)			
3-6 years	307	1(0.13)			
>6years	337	8(1.1)	1.09-59.81	8.08	0.041
Breed					
Cross	685	7(0.94)			
Local	48	1(0.13)	0.25- 17.10	2.06	0.503
Jersey	11	1(0.13)	1.09-86.23	9.69	0.042
Herd size					
Small	449	2(0.30)			
Medium	137	3(0.4)	0.82-30.26	5.0	0.079
Large	158	4(0.54)	1.08-32.01	5.95	0.043
Abortion (n=695)					
Absent	643	4(0.6)			
Present	52	5(0.7)	4.42-65.41	16.995	p≤0.001
RFM (n=695)					
Absent	661	4(0.6)			
Present	34	5(0.7)	7.22-111.04	28.32	p≤0.001

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test; OR = odds ratio

Table 3: Univariable logistic regression analysis based on herd-level seroprevalence of brucellosis and associated risk factors

Risk factors	No. Tested	RBPT+CFT positive (%)	95%CI	OR	P-value
Farm location					
Urban	67	3(2.1%)			
Peri-urban	78	6(4.1%)	0.34-6.36	1.46	0.613
Herd size					
Small	117	2(1.4%)			
Medium	20	3(2.1%)	1.48-60.84	9.50	0.017
Large	8	4(2.8)	6.69-310.77	45.6	p=0.001
Abortion					
Absent	106	2(1.4%)			
Present	39	7(4.8%)	2.72-193.73	22.97	0.004
RFM					
Absent	122	4(2.8%)			
Present	23	5(3.4%)	2.42-50.12	11.02	0.002

RBPT = Rose Bengal Plate Test; CFT = Complement Fixation Test; OR = odds ratio

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

Variable	Level	Odds Ratio	[95% Conf. Interval]	P-value
Age	<3 years			
	3-6 years			
	>6years	8.07	0.98-66.57	0.05
Breed	Cross	1		
	Local	3.04	0.27-34.74	0.37
	Jersey	27.26	1.75-424.28	0.018
Herd size	Small	1		
	Medium	5.88	0.81-42.73	0.08
	Large	10.01	1.30-77.50	0.027
Abortion history	Absent	1		
	Present	10.36	2.53-42.42	p≤0.001

OR = oddsratio, 1 = referring

Table 5: Knowledge-attitudes and practices of farm workers about Brucella infection in the study area

Variables	Urban (n=67)	Peri-urban (n=78)	Overall mean (N=145)
Isolation of aborted animal from others			
Yes	7.5	1.3	4.1
No	92.5	98.7	95.9
Perception of the cause of abortion			
Yes	7.5	6.4	6.9
No	92.5	93.6	93.1
Awareness about brucellosis			
Yes	9.0	6.4	7.6
No	91.0	93.6	92.4
Raw milk consumption			
Yes	32.8	6.4	18.6
No	67.2	93.6	81.4
Raw meat consumption			
Yes	82.1	92.3	87.6
No	17.9	7.7	12.4
RFM and Dead fetus/After birth disposal			
Water canal	23.9	46.2	35.9
Burning/Burying	1.5	7.7	4.8
Thrown on field or Open dump	68.7	41.0	53.8
Give to dog	6.0	5.1	5.5
Assisted parturition			
Yes	71.6	48.7	59.3
No	28.4	51.3	40.7
Using protective glove during assisting			
Yes	10.4	3.8	6.9
No	89.6	96.2	93.1

Table 6: Characteristics of dairy farms in the study area (%)

Variables	Urban (n=67)	Peri-urban (n=78)	Overall mean (N=145)
Service type (%)			
Natural	28.4	42.3	35.9
AI	28.4	23.1	25.5
Both	43.3	34.6	38.6
Replacement stock (%)			
Raised own	59.7	74.4	67.7
Purchased	3.0	1.3	2.1
Both	37.3	24.4	30.3
Parturition pen (%)			
Yes	0.0	3.8	2.1
No	100	96.2	97.9
Cleaning of calving pen (%)			
Flushing with water	98.5	15.4	53.8
Disinfect with detergent	1.5	5.1	3.4
No sanitation service	0.0	79.5	42.8
Culling criteria (%)			
Reproductive problem	21.7	36.0	26.8
Feed shortage	6.5	12.0	8.5
Decrease milk production	71.7	52.0	64.8
Feed and water supply (%)			
Own	64.2	34.6	48.3
Communal	22.4	9.0	15.2
Both	13.4	56.4	36.6

Occupational Risk and Awareness among Dairy Cattle Farmers about Brucellosis: Most of the respondents (93.1%) had no knowledge of the cause of abortion in dairy cattle and 95.9% of them had not isolated the aborted animals from others (Table 5). The result indicated that the majority (92.4%) of respondents in the study areas were not aware of bovine brucellosis. Half of the respondents disposed of retention of fetal membrane and

dead fetus/after birth to open dump or thrown on the field in the environment followed by water canal or rivers, burning/burying and few respondents fed aborted materials to dogs respectively. Most of the respondents (71.6%) in urban areas and 48.7% of respondents in peri-urban areas practiced assisted parturition, of which only a few (6.9%) of them use a protective glove. Few numbers of the respondents in the study area consume raw milk. More than 87.6% percent of respondents in urban and peri-urban areas consume raw meat.

The results of Table 6 indicate that both Natural and artificial insemination (AI) breeding system were common practices of breeding in the study area. The study further indicates that 59.7% of the respondents in urban and 74.4% of the respondents in peri-urban areas were raised their own replacement animals. The practices of the provision of separate pens for parturition were 100% in urban and 96.2 % in peri-urban dairy producers and majority of the respondents in the study areas were used flushing with water to clean their animals' pens after parturition. Decrease in milk production due to age and reproductive problems were prominent culling criteria in all farms. The majority (64.2%) of the respondents in the urban and 48.3% in peri-urban areas were used separate feed and water supply for each animal.

DISCUSSION

In the present study, bovine brucellosis at the individual animal level was 1.21% (9/744) using CFT (95%, CI=1.08-4.85). This finding is similar to the report of (1.2%) by Haileselassie *et al.* [14] in Western Tigray. It is also comparable with the earlier reports of 1.0% by Meles and Kibeb [15] in Chencha district of Gomogofa zone, Geresu *et al*. [16] who reported (1.4%) of in Asela and Bishoftutowns, Asmare *et al*. [17] who reported (1.66%) of prevalence from Sidama zone, Moti *et al*.[18] who reported (1.97%) of from East Wollega and Tsegaye *et al*. [19] who reported (1.7%) from Arsi Zone. Similarly, reported by Senein and Abdelgadir [20] and Scacchia *et al*. [21] who reported 2% and 2.77% from Sudan and Eritrea. Lower prevalence of brucellosis also has been reported in intensive farms [22-23]. On the other hand, the present study is lower than the previous report of Kebede *et al.* [24]11%; Berhe *et al*. [25] 3.2%; Hunduma and Regassa [26] 11.2%; Ibrahim *et al*. [27] 3.1%; Hailesillasie *et al.* [14] 4.9%; Megersa *et al*. [5] 8.0%; Tibesso *et al*. [29] 4.3% and Alehegn *et al*. [12] 4.9% from the highland areas of Ethiopia based on the same diagnostic tests. This variation in the seroprevalence findings might be seen due to relatively better farm management and location.

In the present study the seroprevalence of brucellosis was significantly associated with a cow with a history of abortion and placental retention. Therefore, history of abortion and retention of placenta were found to be 17 and 28 times more likely to be seropositive when compared to no history of abortion and retention of placenta respectively. These risk factors as explained by the respondents are were reported by other researchers [6, 7, 19, 27, 30]. The results further illustrated that in most case, the occurrence of abortion leads to placenta retention. The results as obtained in the current observation are in agreement with the previous reports by Schelling *et al.* [2].

Cow with a history of retained fetal membrane was significantly affected herd seropositivity. The herd seroprevalence of brucellosis was higher in herds that had a history of RFM (3.4%), compared with no history of RFM (2.8%).This could be explained by the fact that retained placenta is typical outcomes of brucellosis. Similarly, the presence of a cow with a history of abortion had significantly affect herd seropositivity. Seroprevalence of brucellosis was higher in herds that had a history of abortion (4.8%) compared with non-aborted (1.4%). This finding is in agreement with the previous reports by Yohannes [6], Abeje [7], Kebede *et al*. [24], Adugna *et al*. [30].

The final multivariable logistic regression model showed that animals kept in large (OR = 10, 95% CI = 1.30-77.50) and medium (OR = 5.88, 95% CI =0.81-42.73) herd sizes were more likely to be exposed to Brucella infection than those maintained in small herds. This finding was in line with that of Berhe *et al.* [25], Mekonnen *et al*. [31], Geresu *et al*. [16], Yohaness [6] and Abeje [7] indicated that herds of larger and medium sizes had higher seroprevalence of bovine brucellosis as compared to smaller herds. This can be explained by the fact that an increase in herd size is usually accompanied by an increase in stocking density, one of the determinants for exposure to Brucella infection especially following abortion calving [32]. On the other hand, reports by Kebede *et al*. [24], Jergefa *et al*. [33] and Asmare *et al*. [28] reported that the risks of seropositivity were independent of herd size. Similarly, among the potential risk factors considered in the present study, the breed of cattle was shown to have a significant effect on the serological prevalence rate of bovine brucellosis [(OR=27.26, 95% CI=1.75-424.28)]. In this study, breed

prevalence of 0.94% (7/744) of the seropositive animals were cross-breeds. This finding was in line with that of Abeje [7], the seropositive associate with cross-breeds animals. On the other hand, the present finding was contradicting with higher prevalence rates reported in indigenous cattle in Nigeria (32.2%), by Junaidu *et al.* [34]. On the other hand, reports by Yohannes [6], Geresu *et al*. [16], Kebede *et al*. [24], Asmare *et al.* [28] and Amenu *et al*. [35] indicated that the seropositivity of Brucella infection was independent of the breed. Radostits *et al.* [36], confirmed that there is no association between the breed of cattle and the seroprevalence of bovine brucellosis. The present result indicated that a previous history of abortion was significantly associated ($P<0.001$) with animal level brucellosis sero positivity. This finding was in line with that of Yohaness [6], Abeje [7] and Adugna *et al.* [30]. In contrary to this finding, Brucella infection is not associated with abortion history [24, 28].

The present findings revealed that the occurrence of major reproductive problems was mainly repeat breeding, anoestrus, abortion, retention of placenta, dystocia and stillbirth and uterine prolapsed. Similar findings were reported by a study conducted in the central zone of Tigray region, northern Ethiopia [37]. In the present study, majority of the interviewed farmers or farm workers were not aware of bovine brucellosis (92.4%). Animal attendants with a primary and lower level of education are hence likely at a higher risk of exposure to the disease. Similar findings were reported by a study conducted in Addis Ababa dairy cattle [8]. The majority (83.4%) of farmworkers were male. This could be due to the fact that farm works in urban and peri-urban dairy production are labor demanding, as a result of which most farm owners prefer to employ male farmworkers. Practices affectation a high risk of Brucella transmission is very common, because of most participants reported assisting in animal parturition, disposing of aborted fetuses/after birth in thrown on the field or open dump without protective gloves or masks and consumption of unpasteurized milk. The reason could be poor knowledge of the disease and risks of transmission but also lack of resources used for personal protection such as gloves, aprons and antiseptics. The creation of awareness of the farm owners and attendants is important to control brucellosis.

CONCLUSION AND RECOMMENDATIONS

Dairy cattle were essential part of livestock keeping in the study areas and they were mainly kept for source of income generation, mainly from milk sell and sometimes sell of animals. Dairy products also significantly contributed to family nutrition. However, repeat breeder, RFM and abortion were the most important reproductive problems. The present study revealed that the overall seroprevalence of bovine brucellosis at the animal level was 1.2%. In this study, it was found a very low-level seroprevalence at the individual animal level. History of abortion and retained placenta were found to be important risk factors associated with the occurrence of bovine brucellosis. Lack of calving pen and poor awareness of dairy owners on the zoonotic importance of brucellosis and consumption of unpasteurized milk, assisting parturition and handling of aborted materials were to be risk factors for human brucellosis and induce the high risk of to animals and public health. Based on this, control measures such as isolation or culling of aborted animals, proper disposal of the aborted fetuses, pasteurization or boiling of milk before consumption should be carried out to reduce the risk of infection and transmission of the disease in animal and human in the study area. Further research is needed on the major reproductive health disorders and further isolation and identification based on biochemical and molecular techniques should be carried out to isolate and identify Brucella species in order to design evidence-based disease control measures.

ACKNOWLEDGMENTS

The authors like to acknowledge Addis Ababa University, Research and Technology Transfer and DebreBerhan University for financial support. The authors like to thank the staff of the Debre Berhan, Bosona Worana and Angolelaena Tera district agriculture and rural development offices, development agents who assisted in data collection and livestock owners for their cooperation.

REFERENCES

1. Addis, S.A. and A.Y. Desalegn, 2018. “Comparative Seroepidemiological Study of Brucellosis in Sheep under Smallholder Farming and Governmental Breeding Ranches of Central and North East Ethiopia, Journal of Veterinary Medicine, 1-12.
2. Schelling, E., C. Diguimbaye, S. Daoud, J. Nicolet, P. Boerlin, M. Tanner and J. Zinsstag, 2003. Brucellosis and Q-fever sero prevalence of nomadic pastoralists and their livestock in Chad. Preventive Veterinary Medicine, 61: 279-93.

3. Seleem, M.N., S.M. Boyle and N. Sriranganathan, 2010. Brucellosis: a re-emerging zoonosis. Veterinary Microbiology, 140: 392-398.
4. Hika, W., A. Adane, A. Fufa, M. Gezahegn and A. Hagos, 2020. Sero-Prevalence of Bovine Brucellosis and its Associated Risk Factors in Three Districts of Arsi Zone Oromia Regional State, Ethiopia. Advances in Biological Research, 14(4): 207-214.
5. Megersa, B., D. Biffa, F. Abunna, A. Regassa, J. Godfroid and E. Skjerve, 2011. Sero prevalence of brucellosis and its contribution to abortion in cattle, camel and goat kept under pastoral management in Borana, Ethiopia. Tropical Animal Health and Production, 43: 651-656.
6. Yohannes, H.M., 2017. Seroprevalence of Bovine Brucellosis under Extensive Production System in Wolaita Zone, Southern Ethiopia. MVSc. Thesis, Addis Ababa University, Bishoftu, Ethiopia.
7. Abeje, A., 2018. Seroepidemiogical Investigation of Bovine Brucellosis and Its Zoonotic Implication in Selected Towns of Oromia Region, Central Ethiopia. MVSc. Thesis, Addis Ababa University, Bishoftu, Ethiopia.
8. Edao, B.M., G. Hailegebreal, S. Berg, A. Zewude, Y. Zeleke, T. Sori, G. Almaw, A.M. Whatmore, G. Ameni and J.L.N. Wood, 2018. Brucellosis in the Addis Ababa dairy cattle: the myths and the realities. BMC Vet. Res., 14: 1-9. https://doi.org/10.1186/s12917-018-1709-4.
9. Molla, A., 2013. Farmers' knowledge helps develop site specific fertilizer rate recommendations, central highlands of Ethiopia. World Applied Sciences Journal, 22: 555-563.
10. Hassen, A., A. Ebro, M. Kurtu and A.C. Treydte, 2010. Livestock feed resources utilization and management as infuenced by altitude in the Central Highlands of Ethiopia. Livest Res. Rural Dev., 22: 1-19.
11. Thrusfield, M., 2007. Sampling in Veterinary Epidemiology. 3rd ed. Black Well Science Ltd, London, pp: 46-65: 228-242.
12. Alehegn, E., S. Tesfaye and M. Chane, 2016. Seroprevalence of Bovine Brucellosis and its Risk Factors in Cattle in and around Gondar Town, North West Gondar, Ethiopia. Journal Advanced Dairy Research, 4: 4.
13. World Organization for Animal Health (OIE), 2012. Bovine brucellosis. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.
14. Haileselassie, M., S. Kalayou and M. Kyule, 2010. Serological survey of bovine brucellosis in barkaandaradobreeds (Bosindicus) of Western Tigray, Ethiopia. Preventive Veterinary Medicine, 94(1-2): 28-35.
15. Meles, Y. and L. Kibeb, 2018. Sero-prevalence of bovine brucellosis in and around Chencha district, Gomogofa zone, south eastern Ethiopia. World Journal of Pharmaceutical Research, 7: 1448-1460.
16. Geresu, M.A., G. Ameni, T. Kassa, G. Tuli, A. Arenas and G.M. Kassa, 2016. Seropositivity and risk factors for Brucella in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. African Journal of Microbiology Research, 10: 203-213.
17. Asmare, K., Y. Asfaw, E. Gelaye and G. Ayelet, 2010. Brucellosis in extensive management system of zebu cattle in Sidama zone, southern Ethiopia. African Journal of Agricultural Research, 5(3): 257-263.
18. Moti, Y., T. Mersha, H. Degefu, T. Tolosa and M. Woyesa, 2012. Bovine brucellosis: serological survey in Guto-Gida District, East Wollega Zone, Ethiopia. Global Veternaria, 8(2): 139-143.
19. Tsegaye, Y., M. Kyuleb and F. Lobagob, 2016. Seroprevalence and Risk Factors of Bovine brucellosis in Arsi Zone, Oromia Regional State, Ethiopia. American Sci. Res. J. Engin., Technol. Sci., 24: 16-25.
20. Senein, M. and A. Abdelkadir, 2012. Serological survey of cattle brucellosis in Eldein, eastern Darfur, Sudan. Acad. J., 6(31): 6086-6090.
21. Scacchia, M., A. Di provvido, C. Ippoliti, U. Kefle, T. Sebhatu, A.D. Angelo and De F. Massis, 2013. Prevalence of brucellosis in dairy cattle from the main dairy farming regions of Eritrea. J. Vet. Res., 80(1): 448.
22. Bashitu, L., B. Afera, G. Tuli and F. Aklilu, 2015. Sero-Prevalence Study of Bovine Brucellosis and its Associated Risk Factors in Debrebirhan and Ambo Towns. Journal of Advanced Dairy Research, 3: 131.
23. Pal, M., D. Lemu, S. Worku and G. Desta, 2016. Sero-prevalence Study of Bovine Brucellosis and Reproductive Problems in Small-scale Dairy Farms of North Shewa, Ethiopia. International Journal of Livestock Research, 6: 1-10.
24. Kebede, T., G. Ejeta and G. Ameni, 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue MedicineVeterinaire, 159: 3-9.

25. Berhe, G., K. Belihu and Y. Asfaw, 2007. Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. International Journal of Applied Research in Veterinary Medicine, 5: 65.
26. Hunduma, D. and C. Regassa, 2009. Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa zone, Oromia Regional State, Ethiopia. America, Eurasian Journal. Agriculture Environ. Science, 6: 508-512.
27. Ibrahim, N., K. Belihu, F. Lobago and M. Bekana, 2010. Sero prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. Tropical Animal Health and Production, 42: 34-40.
28. Asmare, K., B. Sibhat, W. Molla, G. Ayelet, J. Shiferaw, A.D. Martin, E. Skjerve and J. Godfroid, 2013. The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. ActaTropica, 126: 186-192.
29. Tibesso, G., N. Ibrahim and T. Tolosa, 2014. Seroprevalence of bovine and human brucellosis in Adami Tulu, Central Ethiopia. World Applied Science Journal, 31: 776-780.
30. Adugna, K.E., G.E. Agga and G. Zewde, 2013. Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. Reveiew Science Technology, 32: 765-773.
31. Mekonnen, H., S. Kalayou and M. Kyule, 2010. Serological survey of bovine brucellosis in Barka and Arado breeds (Bosindicus) of Western Tigray, Ethiopia. Prev. Vet. Med., 94: 28-35.
32. Crawford, R.P., J.D. Huber and B.S. Adams, 1990. Epidemiology and Surveillance. In animal brucellosis. CRC Press Inc., Florida, pp: 131-148.
33. Jergefa, T., B. Kelay, M. Bekana, S. Teshale, H. Gustafson and H. Kindahl, 2009. Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromia, Ethiopia. Rev. Sci. Tech. Off. Int. Epiz., 28: 933-943.
34. Junaidu, A.U., S.I. Oboegbulem and M.D. Salihu, 2008. Seroprevalence of brucellosis in prison farm in Sokoto, Nigeria. Asian Journal of Epidemiology, 1: 24-28.
35. Amenu, K., E. Thys, A. Regassa and T. Marcotty, 2010. Brucellosis and Tuberculosis in Arsi-Negele District, Ethiopia: Prevalence in Ruminants and People?s Behavior towards Zoonoses: Tropicultura, 28: 205-210.
36. Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Mastitis. In: Veterinary Medicine, 9th ed., W.B. Saunders Company Ltd., London, pp: 603-687.
37. Bahlibi, W., 2015. Assessment of Major Reproductive Problems of Dairy Cattle In Selected Sites of Central Zone of Tigrai Region, Northern Ethopia.Msc thesis, Mekelle University, Ethiopia.