Bovine brucellosis: Seroprevalence and its potential risk factors in smallholder dairy farms in Hawassa Town, Southern Ethiopia

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Abstract

Bovine brucellosis is an infectious zoonotic disease causing significant economical loses in dairy industry. A cross-sectional study was carried out between October 2017 and July 2018 to estimate the seroprevalence and its associated risk factors in smallholder dairy farms in Hawassa town, Southern Ethiopia. A total of 370 blood samples were collected from cross-bred and local indigenous dairy cattle of above six months of age. One stage cluster sampling technique was used to get the sample of interest. Rose Bengal Plate Test (RBPT) was used as a screening, while serum samples testing positive to RBPT were subjected for complement fixation test (CFT) to confirm. Consequently, RBPT detected 18 of the 370 samples positive for brucellosis exposure. The positive sera when further retested using CFT, 10 out of the 18 RBPT positive sera were confirmed to be positive. The individual animal level prevalence of bovine brucellosis in the study area was 2.7% and the herd-level prevalence was 25.8%. Higher prevalence was observed in larger herd sizes than the small and medium herds (p<0.05). Likewise, parity number greater than six had more positive animals (p<0.05) than the corresponding group with lower parity number. Multivariable logistic regression anlysis revealed that herd sizes (OR: 9.13, 95% CI: 1.87-28.65, p<0.05), number of parity (OR: 11.6: 95% CI: 1.54-36.08, p<0.05), absence of separate parturition pen (OR: 7.9, 95% CI: 1.63-38.4, p<0.05) and stages of abortion (OR: 7.6, 95% CI: 1.89-31.36, P<0.05) were identified as the potential risk factors of bovine brucellosis. The results of this study showed that bovine brucellosis is not highly spread in dairy herds of Hawassa town. Therefore, in order to control spread of bovine brucellosis practicing better management is recommended.

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Introduction

Ethiopia is a resourceful country with estimated cattle population of 59.5 million (CSA, 2017). The livestock subsector has an enormous contribution to a national economy and livelihoods of many Ethiopians and still promising to rally round the economic development of the country. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 40% of the agricultural GDP excluding the values of draught power, manure and transport of people and products (Asresie and Zemedu, 2015). It also contributes 15% of export earnings and 30% of agricultural employment (Behnke, 2010).

However, trans-boundary and zoonotic animal diseases such as bovine brucellosis constrain the livestock sector of the country and affect livelihoods via their impact on animal health, animal food production, availability and quality. Bovine brucellosis has a great impact on both animal and human health as well as tremendous socio-economic impact in developing countries where rural income relies largely on livestock breeding and dairy products (Radostits *et al.*, 2007). Brucellosis is considered by Food and Agriculture Organization (FAO), World Health Organization (WHO) and World Organization for Animal health (OIE) as one of the most widespread zoonoses in the world (Schelling *et al.,* 2003). According to OIE, it is the third most important zoonotic disease in the world after rabies and anthrax. The disease affects cattle, swine, sheep, goats, camels and dogs. It may also infect other wild ruminants and marine mammals (Wadood *et al.*, 2009).

The disease is primarily caused by *B. abortus* and occasionally by *B. melitensis* where cattle are kept together with infected sheep or goats and characteristically associated with abortion at first gestation ("abortion storm" in naïve heifers) and is mainly caused by biovars (mainly biotype-1) of *B. abortus* (OIE, 2009; Godfroid *et al.*, 2010). Chronic infection of the mammary glands due to *B. suis* has also been reported (Lopes *et al.*, 2010). Clinically bovine brucellosis is characterized by impaired fertility specifically with abortion, metritis, orchitis and epididymitis (Radostits *et al.,* 2007). The mode of transmission of the bacteria varies with the epidemiological area, the animal reservoir and the occupational exposed groups (Radostits *et al.*, 2007). A precise diagnosis of

Brucella spp. infection is important for the control of the disease in animals and consequently in man.

In Ethiopia, the first cases of brucellosis reported in the 1970s. Since the first report of brucellosis the disease has been noted as one of the important livestock diseases in the country (Meyer, 1980; Tariku, 1994; Asfaw *et al.*, 1998; Bekele *et al.*, 2000; Alem and Solomon, 2002; Kebede *et al.*, 2008; Ibrahim *et al.*, 2010; Mekonnen *et al.*, 2010) demonstrating brucellosis is endemic.

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

Bovine Brucellosis was studied a decade ago in the areas of Sidama zone of Southern nation nationalities and peoples regional state by Asmare *et al.* (2007) on intensive and extensive management systems. In these ten years, there is expansion of the town, increasing in population pressure and increasing dairy farms but there was no documented information on status of bovine brucellosis in study area. There are many small and medium dairy farms mushrooming which supply raw milk and milk products for the communities in Hawassa town. The demand for consumption of milk and milk products in the areas also increasing this may leads to zoonotic diseases like brucellosis. So, this study achieved the gap and provided more information on seroprevalence of bovine brucellosis and its potential risk factors that predispose the animals in the study area. The objectives of the current study were to estimate seroprevalence of bovine *Brucella* antibody circulation in small holder dairy farms of the Hawassa town and to assess the possible risk factors associated with *Brucella* antibody in the study area.

Materials and methods

Description of the Study Area

The study was conducted in Hawassa town, Southern Ethiopia between October 2017 and August 2018. Hawassa is located in the Southern Nation's Nationalities and Peoples Region on the shores of Lake Hawassa in the Great

Rift Valley and located 275 Km away from Addis Ababa in southern direction. Geographically the City lays at 6⁰55'0" latitude N and 38⁰25'0" longitudes E. The annual mean rainfall is from 800-1000 mm and annual mean temperature is between 20.1-25°C (HCAFEDD, 2017). Hawassa town is the economic and cultural hub of the region, having a total area of about 50 km² being divided into eight sub-cities and 32 Kebeles. It is with estimated human population of 387,087 and the main livestock populations in the town are cattle, goats and sheep and estimated to be 125,284, 39,943 and 42,190, respectively. There are 4,401 equines used for draft service. Dairy production system in Hawassa town is mostly dominated by cross breed animals which the house holds use them for production of milk to sell for the urban communities. (HTLFRDD, 2018).
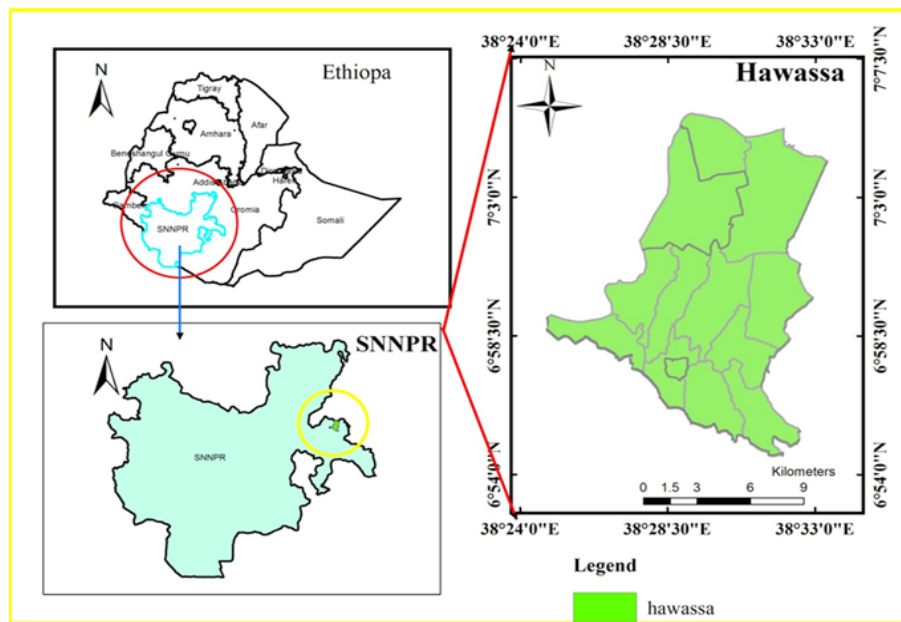

Figure 1. Map of the study area

Study animals and their management

The study consisted of dairy cattle that were managed under the intensive and semi-intensive production system. According to the criteria of Richard (1993), management systems were classified as semi-intensive husbandry system which includes all animals that both are kept indoor and outdoor while intensive system covers all animals which were kept in closed housing system and

feed concentrate as well as mixed feed. The cattle under study comprised of the cross breeds and local indigenous Zebu cattle. Animals of both sexes and different age group greater than six months were included in the study.

Dairy cows are kept under tethers feeding and some are natural grazing with supplementation of food industry byproducts. The cross-breed animals mainly the local with Holstein Frisian and Jersey are increasing in number.

Study design

A cross-sectional study design was carried out on using a pre-tested questionnaire and serological tests.

Sampling method and sample size determination

The study was included representative major dairy farms in Hawassa town. The sampling frame and sampling strategy were determined as follow:

A list of dairy farms was obtained from Hawassa town livestock and fishery resource offices and dairy owners. Farms were divided into small-scale (≤15 heads of cattle), medium-scale (≥16-29 heads of cattle) and large-scale (≥30 heads of cattle) farms depending on number of animals (Asgedom *et al.*, 2016). Those cattle that housed in the same barns were grouped together and considered as one herd (Tolosa *et al.*, 2008; Asgedom *et al.*, 2016). A one stage cluster sampling procedure was used. The clusters were randomly selected and all animals in each cluster were sampled. There are 82 herds in the town: 52 small, 25 medium and 5 large herds. Out of these, 19 small herds (152 head of animals), 10 medium herds (158 head of animals) and 2 large herds (60 head of animals) were sampled in the study area.

The sample size for this study was calculated using 14.14% of seroprevalence of bovine brucellosis as reported by Desalegn *et al.* (2011). Therefore, to determine the sample size of dairy cattle in this area, 14.14% was used as P_{exp} and 95% confidence interval and 5% required precision (Thrusfield, 2007)

$$n = \frac{(Z)^2 \, P_{exp} \, (1 - P_{exp})}{d^2}$$ Where,   n = required sample size,
Pexp = expected prevalence
d = desired absolute precision

Accordingly, the sample size was 186. The sample size was recalculated to get similar accuracy to that of simple random sampling. The intra-cluster correlation coefficient (ρ) of *Brucella abortus* was calculated from the results of cluster sample survey is 0.09 (Otte and Gumm, 1997). The new sample size formula (n′) was calculated by multiplying n by design effect i.e. n′= n x design effect, also Design effect=1+ρ (m-1), where m--average number of individuals sampled per cluster (m=12). Therefore, the required sample size for this study was 370 cattle above 6 months of age.

Data collection

Questionnaire survey

A total of 31 farm attendants/owners were interviewed using semi-structured questionnaire. A questionnaire survey with open and closed questions was used among the farm owners/ attendants whose farms were tested. The following data was collected on animal attributes: breed, sex, age and reproductive status, parity, stage of abortion (first trimester, second trimester and third trimester), history of abortion and retained fetal membrane and breeding systems. Based on its biological relevance, age was stratified into three categories (0.5-<3 years, ≥3-6 years and >6 years) (Asgedom *et al*., 2016). The reproductive status was also categorized (replacement heifers, pregnant cows, lactating cow, dry and bulls). Besides, information on farms such as: herd size was categorized into [small scale (≤15 heads of cattle), medium scale (≥16-29 heads of cattle) and large scale (≥30 heads of cattle)] and other managemental factors were collected. The presence of calving pens (No/Yes), waste disposal methods (placenta, aborted material and dead animal) was categorized into (burying, burning and open dump). Hygienic status of the farms was categorized as (clean and not clean) based on manure disposal, drainage and barn ventilation. Farmer's awareness about brucellosis (No/Yes) was assessed.

Blood sample collection and laboratory tests

Blood sample collection procedure

Animals were restrained by animal handlers and approximately 10 ml of blood sample was collected from the jugular vein of each animal using vacutainer tubes with 18-20-gauge hypodermic needles. Each sample from each animal

was labeled by using codes describing the specific animal and herd/farm. The samples were kept under the shade in a slant position for one hour and were centrifuged. If there was no light in the sample collection area, vacutainer tubes with serum were labeled and set tilted on a table overnight at room temperature to allow clotting. Next morning, the clotted blood in the tubes was centrifuged to obtain clear serum. The obtained serum was stored at -20^{0}C until they were tested by both Rose Bengal Plate Test and Complement Fixation Test. Corresponding to each sample, age, sex, breed of every animal, geo-reference information and other risk factors contributing to the occurrence of bovine brucellosis were collected and registered on a separate case book.

Serological tests

Rose Bengal plate test (RBPT)

It was employed as a screening test on the serum samples for the presence of *Brucella* agglutinins. The protocol of RBPT as recommended by OIE was used as screening test for the presence of *Brucella* antibody in the sampled sera. This test is generally considered to be as a sensitive test which reports to be 97.9% sensitive (Dohoo *et al.*, 1986). Before performing test, antigen and sera were brought to room temperature. 30 µL of serum was taken on a glass slide by micropipette and the antigen bottle was shaken well to ensure homogenous suspension and then one drop (30 µL) of Rose Bengal antigen was added. The antigen and serum were mixed thoroughly with the spreader and then the slide was rotated for 40 min. The result was read immediately after 4 min.

Complement fixation test (CFT)

All Sera that tested positive to RBPT were further tested using CFT at the National Veterinary Institute (NVI), Debre-Zeit, Ethiopia for confirmation using standard *B. abortus* antigen S99 (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health (OIE, 2009). Sera with 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 and above was classified as positive and lack of fixation/complete hemolysis was considered as negative.

Data storage and analysis

Data obtained from questionnaire survey and laboratory results were recorded and stored in Microsoft® Excel for Windows 2010 and transferred to Statistical Package for the Social Sciences (SPSS) version 20. Data were coded and analyzed using descriptive and analytical statistics as appropriate. The units of analysis were individual cattle and herd. Animal level seroprevalence was computed by the number of positive animals divided by the total number of animals tested and for herd level seroprevalence the number of positive herds was divided to the total number of herds tested. Associations between outcome (brucella sero-positivity) and explanatory variables (risk factors) for all units of analysis were investigated by using binary logistic regression model. The strength of the association between outcome (brucella sero-positivity) and explanatory variables was assessed using the adjusted odds ratios (OR). Multivariable logistic regression procedures were used to model the effects of potential risk factors on outcome variables. The backward elimination procedure was used to eliminate the factors that were not significant at p<0.05 in the overall model. Model fit was observed using the Hosmer-Lemeshow test. Subsequently, the predictive ability of the model was validated using the receiver operating characteristic (ROC) curve. In the analysis, a covariate was considered confounder and included in the model if its inclusion altered the OR of the estimated risk by more than 20% (Dohoo *et al*., 2009).

Results

Questionnaire survey

A total of 31 volunteer farm attendants and owners in the farms were interviewed to assess the awareness about brucellosis. Majority of cattle attendants and owners (54.8%) have no knowledge about brucellosis (Table 2). The vast majority of the interviewees (93.5%) were male. Forty-five percent of the respondents in the study area can write and read (Table 1). Fifty five percent of the participants have poor knowledge of the disease. However, the level of awareness was insignificantly lower (p>0.05) in intensive farms.

Table 1. Socio-demographic Characteristics of Respondents

Variables	Categories	Frequency	Percentage (%)
Sex of household	Male	29	93.5
	Female	2	6.5
Age of household	b/n 25-35	10	32.3
	b/n 36-46	12	38.7
	≥47	9	29
Educational status of households	Illiterate	10	32.3
	write and read	14	45.2
	Primary	5	16.1
	high school	2	6.5
	higher education	0	0.0
Total		31	100.00

Almost all of respondents practice intensive husbandry system 28 (90.3%) and only 3(9.7%) from the semi-intensive management systems. In intensive farms, artificial insemination (AI) is used for breeding in herds with cross breeds while most herds in semi-intensive farm use bull for breeding. Among intensive farms, 61.3% of farms were regarded as clean however, 38.7% of intensive farms and all of the semi-intensive farm management systems had poor hygienic practices including poor waste disposal, drainage and poor barn ventilation observed. Around 20% of respondents did not dispose waste products (aborted material or afterbirth) properly (Table 2). Generally, the frequency distribution of breed, parity, age group, reproductive status, retained fetal membrane and hygienic practice on farm were summarized in the following table (Table 2 and 3).

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Table 2. Respondents' response on management of dairy farm practices

Parameters	Categories	Frequency	Percentage (%)
Hygienic practice on farm	Not clean	12	38.70
	Clean	19	61.30
Level of awareness about brucellosis	No	17	54.80
	Yes	14	45.16
Animal introduction	No	20	64.50
	Yes	11	35.50
Separate parturition pen	No	14	45.16
	Yes	17	54.80
Management system	Intensive	28	90.30
	Semi-intensive	3	9.70
Breeding system	Natural mating	8	25.80
	AI	17	54.80
	Both	6	19.40
Disposal of aborted fetus/fetal membrane	Burning	14	45.16
	Burying	8	25.81
	Both	3	9.68
	open dump	6	19.35
Total		31	100.00

Out of 370 dairy animals, 105 (28.38%) were local breed whereas 265 (71.62%) were cross-breeds of indigenous zebu and Holstein Friesian. In addition, the herd characteristics of the studied animals were 107 (28.92%) milking cows, 21 (5.68%) pregnant cows, 126 (34.05%) non milking cows, 68 (18.38%) replacement heifers and 48 (12.97%) bulls. From the total studied animals, there was a history of retained fetal membrane in 29 (9.00%) and 25 (7.76%) were with a history of abortion (Table 3).

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Table 3. Frequency distribution of individual animal variables and percentage

Variables	Categories	Frequency	Percentage (%)
Breed	Local	105	28.38
	Cross	265	71.62
Reproductive status	Heifer	68	18.38
	Milking cow	107	28.92
	Non-milking cow	126	34.05
	Bull	48	12.97
	Pregnant cow	21	5.68
History of reproductive problems	No	268	72.43
	Abortion	25	7.76
	RFM	29	9.00
Sex	Male	48	12.97
	Female	322	87.03
Body condition score	Poor	160	43.24
	Medium	136	36.76
	Good	74	20.00
Age	0.5-<3	63	17.03
	≥3-6	129	34.86
	>6	178	48.11
Total		370	100.00

Overall seroprevalence

Individual animal level seroprevalence of bovine brucellosis

Out of 370 sera samples collected and screened by RBPT, 18 (4.86%) (95% CI: 2.67% -7.10%) were seropositive for brucella antibody. Among 18 brucella positive reactors, 10 (2.70%) (95% CI: 1.05% - 4.36%) of them were confirmed to be seropositive by CFT test. Animals with the age between 0.5-<3 years, replacement heifers, animals which abort at the stage of first trimester and semi-intensive management system tested were negative in both RBPT and CFT.

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Table 4. Animal level risk factors and brucella seropositivity in Hawassa town

Risk factors	Categories	No of tested	No of positive	Prevalence (%)	95% CI	P-value
Body condition score	Poor	160	6	3.75	0.81-6.69	0.38
	Medium	136	2	1.47	0.55-3.49	
	Good	74	2	2.70	0.99-6.40	
Sex	Male	48	1	2.10	0.15-16.15	0.17
	Female	322	9	2.80	0.30-0.92	
Parity	No	75	1	1.33	1.26-3.93	0.04
	1-3	129	1	0.78	0.74-2.29	
	4-6	67	2	2.99	1.09-7.06	
	>6	51	5	9.38	1.64-17.97	
Stage of abortion	Never	75	4	5.33	0.53-3.4	0.03
	1st trimester	129	0	0.00	0.00-2.89	
	2nd trimester	67	3	4.48	0.47-9.43	
	3rd trimester	51	3	5.88	0.58-12.34	
Breed	Local	105	4	3.8	0.15-7.47	0.92
	Cross	265	6	2.26	0.47-4.1	
Reproductive status	Heifer	68	0	0.00	0.00-0.06	0.48
	Milking cow	107	4	3.74	0.14-7.33	
	Non-milking cow	126	4	3.17	0.11-6.24	
	Bull	48	1	2.1	1.96-6.12	
	Pregnant cow	21	1	4.76	4.35-13.87	

CI: Confidence interval

Table 5. Herd level risk factors and *brucella* seropositivity in Hawassa town

Risk factors	Categories	No of tested	No of positive	Prevalence (%)	95% CI	P-value
Herd size	≤15	19	1	5.26	4.78-15.30	0.04
	16-29	10	4	40.00	9.64-70.36	
	≥30	2	2	100.00	100-100	
Hygienic practice on farm	Not clean	12	2	16.67	4.42-37.75	0.26
	Clean	19	6	31.6	10.68-52.5	
Animal introduction	No	20	6	30.0	9.92-50.1	0.83
	Yes	11	2	18.18	4.61-40.97	
Separate parturition pen	No	14	6	42.86	16.93-68.8	0.04
	Yes	17	2	11.76	3.6-27.1	
Management system	Intensive	28	8	28.57	11.84-45.3	0.82
	Semi-intensive	3	0	0.00	0.00-0.00	
Breeding system	Natural mating	8	4	50.0	15.35-84.7	0.54
	AI	17	3	17.7	0.47-35.77	
	Both	6	1	16.67	13.15-46.5	

CI: Confidence interval

Herd-level seroprevalence of bovine brucellosis

Out of 31 herds included in the study, 8 herds were sero-positive with at least one seropositive animal in the herd (8/31) (25.8%) (95% CI: 10.4%-41.2%). The range of herd level prevalence was within 5.56% up to 8.33% which at least one seropositive animal was observed based on CFT. In this study herds with larger herd size (greater 30 animals) has significantly higher prevalence than herds with small herd size ($p < 0.05$). The value of OR indicated that herds in the herd size of 16-29 animals were about 9.13 times more likely to be seropositive than herds in the ≤15 heads of animals. However, management systems ($p > 0.05$), Body condition score ($p > 0.05$ Sex ($p > 0.05$) and Breed ($p > 0.05$) were not associated with seropositivity of brucellosis (Table 4 and 5).

Table 6. Univariable logistic regression analysis of factors associated with *Brucella* seropositivity

Variables	Categories	No of tested animals	No of CFT positive (%)	OR (95% CI)	P-value
Sex	M (Ref)	48	1(2.1)		
	F	322	9(2.8)	4.1 (0.6-8.26)	0.17
Herd size	≤15 (Ref)	152	1(0.66)		
	≥16-29	158	5(3.16)	2.56(0.01-4.8)	0.05
	≥30	60	4(6.67)	3.4(0.2-4.08)	0.04
Parity	No (Ref)	75	1(1.33)		
	1-3	129	1(0.78)	0.87(0.01-2.96)	0.26
	4-6	67	2(2.99)	4.6(1.32-6.52)	0.09
	>6	51	6(9.38)	5.42(2.04-7.62)	0.04
Stage of abortion	Never	75	4(5.33)		
	1st trimester	129	0(0.00)	0.8 (0.03-0.98)	0.82
	2nd trimester	67	3(4.48)	7.4 (0.49-42.8)	0.03
	3rd trimester	51	3(5.9)	3.54(0.98-7.23)	0.05
Breed	Local	105	4(3.8)	0.122(0.01-1.67)	0.92
	Cross (Ref)	265	6(2.26)		
Management system	Intensive	334	10(2.99)	0.57(0.06-0.87)	0.82
	Semi-intensive (Ref)	36	0 (0.00)		
Observed abortion/RFM	No	268	2 (0.75)	0.4 (0.02-14.9)	0.29
	Yes (Ref)	54	7 (12.96)		
Separate parturition pen	No	167	6(3.6)	4.72(0.98-15.24)	0.04
	Yes (Ref)	203	4(1.97)		

OR: Odd ratio; CI: Confidence interval; Ref: Reference

Potential risk factors

Variables with a p<0.25 in the univariable analysis were included in the final multivariable logistic model. Two variables, age and parity that showed collinearity with each other, so age was removed from the model and parity stayed in the model. The rest variables; parity, herd size, separate parturition pen, stage of abortion was offered to the model. Further selection of variables in the final model was based on stepwise backward elimination procedure. A Hosmer-Lemeshow goodness-of–fit value (p=0.63), indicated that the model

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was fit the data. The area under the ROC curve was 0.54, indicating that the model had good predictive ability.

The sero-prevalence was significantly higher in the greater than six parity numbers of animals (9.38%) than the parity numbers between 4-6 ones (2.99%), consequently the seroprevalence increases as the parity of the animals increase. This study revealed that there is association between parity and seropositivity of bovine brucellosis with (p< 0.05).

Higher prevalence was observed in larger herd size (6.67%); old animals were affected more than young animal and no brucella reactors were observed in young animals in this finding. Seroprevalence of 12.0% was observed in animals with previous history of abortion.

The final multivariable logistic regression model showed that parity, absence of separate parturition pen, herd size and stage of abortion were independently associated with seroprevalence of bovine brucellosis. The multivariable logistic regression model revealed that herd size (OR: 9.13; 95% CI: 1.87-28.65, p<0.05), stage of abortion (OR: 7.6; 95% CI: 1.89-31.36, p<0.05), separate parturition pen (OR: 7.9; 95% CI: 1.63-38.4, p<0.05) and parity (OR: 11.6; 95% CI: 1.54-36.08, p<0.05) were potential risk factors for cattle seropositivity to circulating brucella antibodies (Table 7).

Table 7. Multivariable logistic regression analysis of potential risk factors with Brucella seropositivity

Variables	Categories	No of tested animals	No of CFT positive (%)	OR (95% CI)	P-value
Herd size	≤15 (Ref)	152	1(0.66)		
	16-29	158	5(3.1)	2.45(1.07-3.98)	0.034
	≥30	60	4(6.67)	9.13(1.87-28.65)	0.001
Separate parturition pen	No	167	6(3.6)	7.9(1.63-38.4)	0.021
	Yes (Ref)	203	4(1.97)		
Stage of abortion	Never (Ref)	75	4(5.33)		
	1st trimester	129	0(0.00)	1.8(1.2-2.14)	0.043
	2nd trimester	67	3(4.48)	5.5(1.07-18.2)	0.004
	3rd trimester	51	3(5.9)	7.6(1.89-31.36)	0.001
Parity	No (Ref)	75	1(1.33)		
	1-3	129	1(0.78)	2.45(0.35-4.03)	0.128
	4-6	67	2(2.99)	6.12(1.14-11.08)	0.041
	>6 (Ref)	51	6(9.38)	11.6(1.54-36.08)	0.001

OR: Odd ratio; CI: Confidence interval; Ref: Reference

Discussion

Awareness about brucellosis among farmers is crucial in controlling disease transmission. In this study, farm attendants/herd owners were interviewed to assess their awareness levels about brucellosis using semi-structured questionnaire. All farmers interviewed had no similar awareness about brucellosis and farmers which were aware about brucellosis were significantly lower in both farming systems. It also observed that poor hygienic practices and uncontrolled animal movements were practiced in semi-intensive husbandry systems. These could pose high risks of transmitting the disease within and in between the herds. This is in agreement with previous studies in intensive farming in Ethiopia by Tesfaye *et al*. (2011); Asgedom *et al*. (2016); Elemo and Geresu. (2018) and Waktole *et al*. (2018).

This low level of educational status may lead to reduced production of dairy farms because of low use of dairy innovations such as cultivation of improved forages, breeding techniques and use of modern dairy farming in the study area. This study revealed that the occurrence of abortion and retained fetal membrane was due to lack of knowledge on breeding methods and disease transmission, shortage of feed and lack of awareness on isolation of aborted animal from healthy animals could possibly be associated with the high prevalence rate of reproductive problems.

Similar prevalence to the current study of bovine brucellosis based on RBT and CFT has been reported from the highland areas of Ethiopia among cattle in intensive production systems (Asmare *et al*., 2004; Kebede *et al*., 2008; Jergefa *et al*., 2009). However; in certain parts of the Ethiopia some authors observed lower prevalence in indigenous cattle under intensive production systems by using the same diagnostic tests (Tolosa *et al*., 2010; Alemu *et al.,* 2014; Geresu *et al*., 2015; Asgedom *et al*., 2016). This variation could be due to differences in cattle management systems, husbandry practices.

The current finding was consistent with earlier findings of Asmare (2004) who reported 2.5% prevalence in Sidama zone dairy farms by using the same tests and management practices; Waktole *et al*. (2018) who reported a prevalence of 3% in selected dairy farms of Bishoftu town, Oromia region; Asmare *et al*. (2007) documented a seroprevalence of 2.46% in sidama zone and Tesfaye *et al*. (2017) who observed 2.08% seroprevalence in and around Kombolcha, Amhara regional state.

However, in Ethiopia a lower seroprevalence which contradict the current study were documented in previous findings of Bashitu *et al*. (2015) who observed a seroprevalence of 0.2% which conducted on 415 animals by using RBPT and CFT tests in Debrebirhan and Ambo towns and Sarba *et al*. (2016) who reported an overall seroprevalence of 0.49% on 816 animals using the same tests in selected towns of West Shewa, Ethiopia; Geresu *et al*. (2016) who observed 1.4% seropositivity of brucella on 570 animals using RBPT, CFT CT and i-ELISA tests in dairy cows in Asella and Bishoftu towns, Oromia regional state, Ethiopia and Tesfaye *et al*. (2011) who reported 1.5% prevalence in Addis Abeba dairy farms by using the same tests.

In the contrary, so far higher seroprevalence findings are reported by Elemo and Geresu (2018) who observed prevalence of 4.95% on 768 animals using RBPT and CFT tests in smallholder farms of Agarfa and Berbere districts of Bale Zone, South Eastern Ethiopia; Kebede *et al*. (2008) reported an overall seroprevalence of 11.0% on 1136 cattle using RBPT and CFT tests in Wuchale-Jida district; Alehegn *et al*. (2016) who reported 4.9% seroprevalence in and around Gondar Town, North West Gondar; Desalegn *et al*. (2011) who observed 14.14% of prevalence using the same tests in Assella Government Dairy Farm of Oromia Regional State, Ethiopia and Hailemelekot *et al*. (2007) who observed 4.6% of prevalence using the same tests in selected sites of Ethiopia.

The difference in seroprevalence might be due to the difference in management systems, age of the animals, sample sizes, parity of the animals, herd sizes and sex among dairy farms. It has been reported that susceptibility of cattle to *B. abortus* infection is influenced by age of an individual animal. Thus, sexually matured and pregnant cattle are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex. On the other hand, younger animals tend to be more resistant to infection and frequently clear infections, although latent infection may occur. This may be due to the fact that sex hormones and erythritol, which stimulates the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al*., 2007)

This study also estimated that there is association between parity and seropositivity of bovine brucellosis and hence, parity was one of the potential risk factors in the study area. This is probably due to increased contact with fetal materials and vaginal discharge from infected cows there by increasing the chance of being infected by *brucella* species. This finding was in agreement

with the findings of other authors (Desalegn *et al.*, 2011; Elemo and Geresu, 2018).

Even though age was not significantly associated with brucella seropositivity, a seroprevalence of 3.37 was found among age group of >6 years whereas no brucella seropositivity was observed in in the younger age group (6 months up to less than three years) of dairy cattle in the study area. Several earlier reports have indicated that the higher seroprevalence of brucellosis in adult age group of cattle which in contour with the findings of Magona *et al.* (2009) similar to the findings of this study. This report was in line with literatures which supports younger animals tend to be more resistant to infection and frequently clear infections. Sexually mature animals are more susceptible to Brucella infection than sexually immature animals, which are due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of Brucella organism, tend to increase in concentration with age and sexual maturity (Walker, 1999; Radostits *et al.*, 2007).

There was statistically significant association between (p<0.05) stage of abortion and seropositivity of brucella in the present study. This could be explained by the prevalence of higher seroprevalence in cows in the last trimester may due to the preferential localization of brucella in the uterus in which allantoic fluid factors such as erythritol could stimulate the growth of brucella and elevate in the placenta and fetal fluid from about the fifth months of gestation (Coetzer and Tustin, 2004; Radostits *et al.*, 2007).

In the present study, statistically significant variation has been observed in seroprevalence of brucellosis between different herd sizes; larger herd sizes were nine times more likely to be seropositive. Herd size has previously been reported as an important determinant for transmission of *Brucella* organism between susceptible and infected animals (Omer *et al.*, 2000) and thus; larger herds were more likely to have at least one positive animal than smaller herds (Al-Majali *et al.*, 2008). In larger herd sizes, the disease spreads by several modes of transfer, especially through contact with infected discharges from dam and its fetus (Radostits *et al.,* 2007). Thus, 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. However, in contrary to this Kebede *et al.* (2008), who reported that the risk of seropositivity was independent of herd size in Wuchale Jida district of East Wollega zone of Ethiopia. The observed variation of the reports among different region of

Ethiopia and other countries could be attributed to various factors including agro-ecology, management system. In the current finding the absence of separate parturition pen in the study area was significantly associated with brucella seropositivity. This is due to hygienic problems in dairy farms in which the sanitary systems of farm are not clean. This predisposes the animals to the disease.

In epidemiological studies, the use of two tests applied serially is recommended to maximize the accuracy of test results. A combination of rose Bengal and complement fixation tests is the most widely used serial testing scheme. Rose Bengal test is highly sensitive test and could easily apply in field conditions whereas, complement fixation test is highly specific usually used as a confirmatory test method (Samui *et al.*, 2007). The combination of these tests in this study could therefore maximize the accuracy of the findings. The false positive results in the RBT could be due to cross reactions with other bacteria such as *Yersinia enterocolitica*, *E. coli*, *Salmonella spp.* and *Pastuerella spp.*

Conclusion

In the current study, the seroprevalence recorded revealed that brucella antibody circulation is an established disease in dairy farms of Hawassa town. The current finding revealed that large herd sizes, absence of separate parturition pen, animals with the highest number of parity and animals which aborted at last trimester were identified as potential risk factors of brucella seropositivity. Even though age was not significantly associated with brucellosis, adult animals were highly predisposed than young animals and almost all of the positive reactors were female animals. From questionnaire survey, poor hygienic practices like improper disposal of aborted fetuses and fetal membranes were identified as potential risk factors which could create favorable condition for the entry and establishment of bovine brucellosis in the dairy farms. In conclusion, the prevailing *Brucella* seropositivity in the dairy farms indicates the disease has a major impact on human health, besides causing significant economic losses in dairy industry. Hence, using calving pens, improving hygiene, and awareness creation to farm attendants/owners are recommended to control further spread of the disease.

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