



Epidemiological investigation of brucellosis in breeding female cattle under the traditional production system of Jimma zone in Ethiopia

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ABSTRACT

Bovine brucellosis is a serious zoonotic infectious disease with widespread occurrence in developing countries like Ethiopia. A cross-sectional study was conducted from November 2017 to November 2018 to investigate the prevalence and associated risk factors of brucellosis in breeding female cattle under the traditional production system of the Jimma zone in Ethiopia. Blood samples were collected from a total of 423 breeding female cattle to test the presence of *Brucella* antibody. The presence of antibody against *Brucella* spp. was serially tested first by the Rose Bengal Plate test, then positive sera were confirmed using the complement fixation test. Potential risk factors for *Brucella* seropositivity were analyzed using multivariable logistic regression analysis. An overall 11.6% (95%CI: 6.25–16.94) and 4.3% (95%CI: 2.15–5.89) seroprevalence of brucellosis was recorded at herd and animal level, respectively in study areas. We identified age (OR=9.6, 95% CI: 2.08–44.07), breed (OR=4.5, 95%CI: 1.54–12.99), herd size (OR=10.4, 95%CI: 1.27–85.04) and species composition (OR=4.4, 95%CI: 1.31–14.89) as risk factors for *Brucella* infection. Herd level analysis of risk factor shows that herds kept with sheep and/goats was at higher risk (OR=3.7, 95% CI: 1.25–11.17) of acquired *Brucella* infection. This result showed that brucellosis was a widely spread disease in the study areas. Thus, important to carry out appropriate control methods and creating awareness on public zoonotic transmissions of brucellosis are recommended. Moreover, further investigation should be carried out to isolate and characterized brucellosis as a cause of reproduction problems in the study areas.

1. Introduction

Brucellosis is a widely prevalent disease that causes considerable economic losses and important human disease in many countries (Jergefa et al., 2009; Mai, Irons, Kabir, and Thompson, 2012; Matope, Bhebe, Muma, Lund, and Skjerve, 2010). This disease is the major breeding improvement problem in those countries by causing the reproductive inefficiency and abortion in breeding cattle (Asmare, Asfaw, Gelaye, and Ayelet, 2010; Ducrotoy et al., 2017; Franc, Krecek, Hasler, and Arenas-Gamboa, 2018; Ndazigaruye, Mushonga, Kandiwa, Samkange, and Segwagwe, 2018). *Brucella abortus* and *B. melitensis* are the foremost vital reason for brucellosis in bovine. Occasionally, bovine brucellosis has been related to *Brucella suis* (Szulowski, Iwaniak, Weiner, and Złotnicka, 2013). It is extremely characterized by inflicting abortion in late pregnancy, retained fetal membrane, and infertility in bovine (Radostits, Gay, Hinchcliff, and Constable, 2007). The common route of *Brucella* transmission is through direct contact with an aborting animal and aborted

fetus or by indirect contact with contaminated fomites (Ach and Szyfre, 2001). The epidemiology of brucellosis in cattle and cost-effective prevention methods is not well understood (McDermott & Arimi, 2002). Thus, brucellosis remains challenging widespread in cattle population and enormous economic and public health problems in developing countries (Ach and Szyfre, 2001; Memish & Mah, 2001). The epidemiology of bovine brucellosis is complex and influenced by several factors (Al-Majali, Talafha, Ababneh, and Ababneh, 2009). These can be classified into factors associated with the transmission of the disease between herds, and factors influencing the maintenance and spread of infection within herds (Megersa et al., 2011).

Ethiopia has a huge cattle population in Africa. Despite having a large cattle population, the country is unable to optimally use this potential resource as a result of different constraints affecting cattle production (CSA, 2017). Animal disease, poor genetic, management problems, nutrition deficiency, and lack of appropriate animal health service were the major constraints to cattle production in the country (Kebede, Melaku, and Kebede, 2014; Welay et al., 2018). Among the

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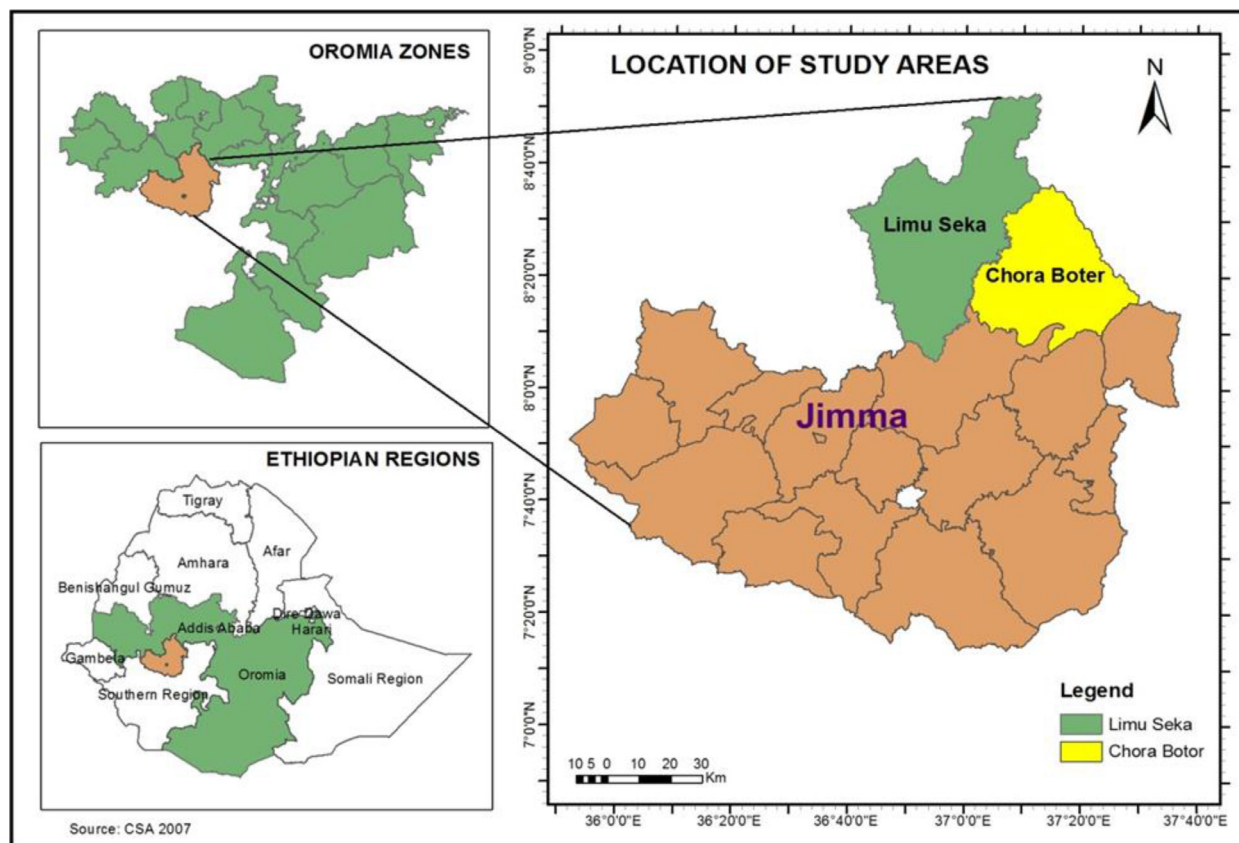


Fig. 1. Map of study areas.

infection of animal disease, brucellosis is among the serious problem in cattle and humans (Haileselassie, Kalayou, Kyule, Asfaha, and Belihu, 2011; Lakew, Hiko, Abraha, and Mengistu, 2019). Brucellosis causes heavy economic losses and public health concerns (Asfaw et al., 2016; FAO, 2010; OIE, 2009). The studies indicated that high seroprevalence of brucellosis in a place where people live very closely with livestock (Berhe, Belihu, and Asfaw, 2007). The evidence of *Brucella* infections in Ethiopian cattle has been serologically evaluated in different parts of the country by different authors (Adugna, Agga, and Zewde, 2013; Asmare et al., 2010; Haileselassie et al., 2011; Tolosa, Regassa, and Belihu, 2008). Seroprevalence of brucellosis is higher in the intensive farming systems than extensive cattle rearing systems (Degefa, Duressa, and Duguma, 2011; Deselegn and Gangwas, 2011).

Recent reports from different parts of the country by different authors (Asfaw et al., 2016; Pal, Lemu, Worku, and Desta, 2016; Tsegaye, Kyule, and Lobago, 2016) also indicated that brucellosis still widespread disease in the country, resulting in huge economic losses due to abortion and other reproduction problems. However, little information is available on risk factors that precipitated the occurrence and transmission of brucellosis in breeding female cattle under the traditional production system. Particularly information related to breeding female cattle brucellosis in the study area is unknown. For this reason, this study aimed to investigate the prevalence and associated risk factors of brucellosis in breeding female cattle under the traditional production system of Jimma zone, Ethiopia.

2. Materials and methods

2.1. Ethics consideration and clearance

All procedures were conducted according to the experiment practice and standards approved by the animals' welfare and research ethics committee at Jimma University School of Veterinary Medicine, College

of Agriculture, and Veterinary Medicine that is following the international guidelines for animal welfare with AgVmVM/16/1 reference number.

2.2. Description of study areas

The study was conducted in selected districts of Jimma zone. Limu Seka district is situated 109 km from Jimma town. The district has 19 peasant associations and 77 villages. The district is located at an altitude of 1400–2200 m above sea level, 09°29' North latitude and 37°26' East longitudes. The agroecology is characterized by 13% highland and 55% mid-highland and 32% lowland. The average temperature varies from a minimum 15.1 °C to a maximum 31 °C. There are two distinct seasons in Limu Seka: the rainy season (from late March to October), and the dry season (November to early March). The rainfall is often more than 1800 mm per annum. Limu Seka district has 295,627 cattle, 104,892 sheep, 89,079 goats and 134,370 human populations. Limu seka district has 324 herds. Local cattle breed are the most dominant one followed by some crosses of Holstein-Friesian. The management systems of the study area are extensive (crop-livestock production) systems and semi-intensive (urban production) systems. Chora Boter district is located 112 km from Jimma town. The district is divided in 16 peasant associations with 65 villages. The district is located at 9°–10°24' North latitude and 37°56'–40°35' East longitude with an altitude range of 1100–2200 m above sea level. The agroecology is characterized by 25% highland, 73.5% mid-highland, and 2.3% lowland. The annual average temperature ranges from 18.3 °C to 26.7 °C. Similar to Limu seka district, the district has two seasons. The rainfall is often more than 1800–2200 mm per annual. Chora Boter district has 228,846 cattle, 47,854 sheep, 68,037 goats and 215,348 human populations. About 228 herds of cattle in Chora Boter district. The herd is called *Ulle* in local language which characterized by the clustering of cattle share common grazing areas and watering points. *Abba Ulle* is an

important contact person in the village that facilitates cooperation among livestock owners. There are two management systems in the area are extensive (crop-livestock production) system and semi-intensive (urban production). Local cattle are dominated breed in the area and some crossbreed also in some place (Fig. 1).

2.3. Study population

All breeding female cattle in the Jimma zone were the target population. Source of the population was breeding female cattle in selected districts whereas the study population was the female cattle two years age and above in peasant associations that kept under the traditional production system. Study units were unvaccinated female cattle two years age and above were included in the sample.

2.4. Study design

The cross-sectional study design was carried out to study the prevalence and associated risk factors of brucellosis in breeding female cattle from November 2017 to November 2018 in Jimma zone. For this study breeding female cattle defined as female cattle with two years and above that used for the breeding purpose, since age at first calving cattle in tropical conditions were estimated to be 24–36 months (Haileselassie et al., 2011; Wathes, Brickell, Bourne, Swali, and Cheng, 2008).

2.5. Sampling methods

The multistage sampling strategy was carried out with zone as highest and herd as the lowest sampling stage, district, and village in between the two-stage. Jimma zone was selected purposively based on the dominant livestock production system while district, peasant association (PA), village, and herd were selected randomly. From ten districts of Jimma zone, two districts were selected by a lottery system, namely, Limu Seka and Chora Boter. Similarly, six and four peasant associations were selected from Limu Seka and Chora Boter districts, respectively based on cattle population. A total of twenty-seven villages were selected from those peasant associations by a simple random sampling method based on the number of the village in peasant associations. A total of 138 herds were selected randomly from those villages. The sampling unit was an individual animal two years age and above belonging to herds in the village. In each herd individual animal was chosen at random using the lottery method. The number of animals sampled from each herd could vary according to the number of cattle (while about 50% of the animals in large herds were to be sampled). The sampling frame of breeding female cattle was taken from respective peasant associations (Table 1).

2.6. Sample size determination

Since no previous study was done on breeding female cattle

Table 1
Number of peasant associations, villages, herds, and animals sampled from each selected districts of Jimma zone.

Peasant associations	Lemu Seka district			Peasant associations	Chora Boter district		
	No of villages	No of herds	No of animals		No of villages	No of herds	No of animals
Atinago	3	16	48	Mecha Dire	2	10	30
Dame	3	19	60	Chora Bage	3	13	39
Denebe	2	9	30	Soyoma	3	17	51
Cheka	2	8	24	Kobi	3	17	54
Doora	3	15	45				
Bontu	3	14	42				
Total	16	81	249		11	57	174

brucellosis in study areas. The sample size required for this study was determined using the formula described by Thrusfield (2005) based on the expected prevalence of brucellosis 50% and the desired absolute precision 5% with 95% confidence interval (CI). Hence, the number of breeding female cattle needed for this study was 384. The calculated sample size is for the desired precision or 95% CI as width assuming that there is no problem with non-response or missing value. Hence, it is wise to oversample by 10% to 20% of the computed number required (Naing, Winn, and Rusli, 2006). In this calculated the sample size was oversampled by 10% which is about 39 samples. Therefore, a total of 423 breeding female cattle with on history of vaccination against brucellosis were involved in the study.

2.7. Blood sample collection and serological tests

2.7.1. Blood sample collection

About 10 ml of blood samples were collected from the jugular vein of each cattle by using a sterile needle and plain vacutainer tube. Identification of each cattle was labeled on corresponding vacutainer tube and blood samples were allowed to stand overnight at room temperature to obtain the serum. The animals' codes were transferred to the cryovials to which the serum was decanted and serum samples were kept -20°C (OIE, 2009) in Jimma University microbiology laboratory until they transported to National Veterinary Institute, Debrezeitte using icebox for serological analysis.

2.7.2. Serological tests

The serum samples were screened for the presence of *Brucella* agglutinins by using Rose Bengal Plate Test (RBPT) (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT153, UK) following the recommended OIE (2004) techniques. The serum samples and antigens were taken from the refrigerator, and then it stays on room temperature for half an hour and processed following the recommended procedure. A total of 30 microliters of serum was dispensed onto the plate and 30 microliters of RBPT antigen were dropped on the slide with sera. The interpretation of both positive and negative control results was done according to the degree of agglutination and the reaction was read in good light source or a magnifying glass when micro agglutination was suspected. The RBPT results were interpreted 0, +, ++ and +++ as has been described by Dohoo, Martin, and Stryhn (2009) where 0 indicates no agglutination, + indicates barely visible agglutination (using magnifying glasses), ++ indicates fine agglutination and +++ indicates coarse clumping. Those serums identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were considered as positive.

All positive sera (RBPT) were confirmed using complement fixation test (CFT) by *B. abortus* antigen S99 and control sera (positive and negative) (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT153, UK). The antigen dilution was standardized at 1:10. Two-fold dilutions (1:5, 1:10, 1:20, and 1:40) of test sera were ready in standard 96-well U-bottom microliters plates before adding *Brucella* antigen, guinea pigs complement and 3% sensitized sheep red blood cells. The reagent was prepared and evaluated by titration according to protocols recommended by OIE (2009). The plates were incubated at 37°C for 30 min with agitations and results were read after the plates have been centrifuged at 2500 rpm for 5 min at 4°C . Sera with a strong reaction, more than 75% fixation of complement (3+) at 1:5 dilution or at least with 50% complement fixation (2+) at 1:10 dilution and above were considered as positive and lack of fixation/ complete hemolysis was considered as negative (OIE, 2004). A cattle was considered positive if test seropositive on both RBPT and CFT in serial interpretation. The combination of RBPT and CFT in serial most widely used is commonly recommended to maximize the specificity of the test result by ruling out false-positive serological cross-reactions (Dohoo et al., 2009).

2.8. Data collection and analysis

Data believed to be putative risk factors for breeding female cattle brucellosis were recorded in MS excel spreadsheet 2010 program. Individual data such as agro-ecology, age, body condition, breed, parity, pregnancy status, history of abortion, retained fetal membrane, abortion period, herd size, the introduction of new animals, management system, and species composition (mixed of cattle with sheep and/ goats) were recorded. Age of breeding cattle was categorized as <3, 3–6, and >6 years age groups and herd size group into small (<15 head of cattle), medium (15–30 head of cattle), and large (>30 head of cattle). Parity number breeding cattle were categorized as nulliparous, monoparous, and pluriparous.

All collected data were analyzed with SPSS version 20.0 (IBM SPSS 2011). The individual positive out-come was defined as any animal with RBPT+ and CFT+, while herd positive was any herd having at least one seropositive animal. Herd level seroprevalence was computed by dividing the number of RBPT and CFT positive herd with at least one infected animal to a total number of herd sampled. Apparent seroprevalence of brucellosis was calculated by dividing the number of RBPT and CFT seropositive samples to the total of animals tested. To estimate true prevalence in cattle the sensitivity and specificity estimate at each of RBPT (Se=0.981 and Sp=0.998) and CFT (Se=0.96 and Sp=0.998) in cattle predicted by EFSA (2006) were imputed in the Rogan and Gladen formula (Boukary et al., 2013): $TP = (AP + CSp_s - 1) / (CSe_s + CSp_s - 1)$ where TP = true prevalence; AP = apparent prevalence; CSe_s = combined sensitivity of the test series (SeRBPT × SeCFT), and CSp_s = combined specificity of the test series $(1 - (1 - spRBPT) \times (1 - spCFT))$. Animal level seroprevalence was calculated following adjustment for sample weighing. The Association between seroprevalence and risk factors of brucellosis was analyzed using the logistic regression model. A multivariable logistic regression model was established to identify risk factors associated with brucellosis with adjustment for clustering by the village and the strength of their associated was assessed using adjusted odds ratios (OR). In SPSS analysis the odds ratio is equal to the exponential function of the regression coefficient ($Exp(B) = OR$). Variable with a p-value less than or equal to 0.25 in univariable analysis were involved in the multivariable logistic model. The forward selection procedure was used for a further selection of variables. The variables were tested for interaction effect using cross-product terms and for multiple-collinearity using the collinearity matrix index before building the final model. The model validity and predictive ability were assessed using the Hosmer-Lemeshow test and ROC curve. Confidence level (CL) is at 95% and $P \leq 0.05$ were set for significance for all analysis.

3. Results

From a total of sampled breeding female cattle, 4.5% and 4.0% were positive for *Brucella* antibody by using RBPT and CFT, respectively. The prevalence of the *Brucella* antibody in each area was determined to be 4.8% in Limu Seka and 2.9% in Chora Boter districts of Jimma zone. An overall herd-level seroprevalence of 11.6% (95% CI: 6.25–16.94) was recorded assuming that antibody was detected at least from one herd based on CFT. The overall 4.3% (95% CI: 2.15–5.89) the true seroprevalence of the *Brucella* antibody was observed at the individual animal level (Table 2).

3.1. Herd level risk factors analysis

Species composition was significantly affected ($P < 0.05$) herd-level seroprevalence of brucellosis using both univariable and multivariable logistic regression. Mixed herd (sheep and/ goats) was about four times (OR = 3.7) more likely to have brucellosis than those having less or no contact. However, origin, agro-ecology, herd size, management system, and introducing of a new animal were not significantly associated

Table 2

Distribution of seroprevalence of brucellosis in breeding female cattle at individual and herd level across the study areas.

Study areas	Individual animal level		Herd level	
	Number of animals	Seroprevalence (%) (95% CI)	Number of herds	Seroprevalence (%) (95% CI)
Limu Seka	249	4.8 (2.16–7.48)	81	13.6 (6.12–21.04)
Chora Boter	174	2.9 (0.39–5.36)	57	8.8 (1.43–16.12)
Overall	423	4.3 (2.15–5.89)	138	11.6 (6.25–16.94)

CI: Confidence Interval.

($P > 0.05$) with seroprevalence of brucellosis at herd level (Table 3).

3.2. Individual animal level risk factors analysis

This result showed that age was significantly associated ($P < 0.05$) with the serostatus of the *Brucella* antibody. The odds of *Brucella* seroprevalence in older animals was 5.9 times higher (OR = 5.9) than their young counterparts. Moreover, there was a statistically significant difference ($P < 0.05$) observed between the two breeds. Local cattle breed had four times odds (OR = 4.0) of brucellosis as compared to cross-breed. There was statistically significant variation ($P < 0.05$) among pregnancy status of breeding female cattle, pregnant cattle had higher odds (OR = 3.0) of seropositivity to *Brucella* infection as compared to non-pregnant ones. Furthermore, there was a statistically significant variation ($P < 0.05$) in the seroprevalence of brucellosis between cows from different herd sizes. Cows from the large herd size category were eight times (OR = 8.3) more odds to be *Brucella* seropositive compared to those from small herd size groups. Similarly, a statistically significant difference in serostatus of brucellosis ($P < 0.05$) was observed in animals herded with sheep and/ goats; those having close contact with small ruminants had 4.4 times odds of seropositivity to *Brucella* infection as compared to those having less or no contact. However, body condition, parity, abortion history, abortion period, agro-ecology, management system, and retained fetal membrane were not able to explain seroprevalence of brucellosis ($P > 0.05$) (Table 4).

No significant interactions and multicollinearity between variables were detected. A Hosmer-Lemeshow goodness-of-fit value ($P = 0.94$), indicated that the model was fit the data. ROC curve (0.81) indicated that the model had a good predictive ability. The final multivariable logistic regression model showed that older breeding female cattle were more likely (OR = 9.6, $P < 0.05$) to be infected with brucellosis than younger cattle. Local breed breeding female cattle also more likely (OR = 4.5, $P < 0.05$) to be *Brucella* seropositive compared to crossbred cattle. Similarly, breeding female cattle contact with small ruminant was more likely (OR = 4.4, $P < 0.05$) to harbor *Brucella* organism and large herd size were also more likely (OR = 10.4, $P < 0.05$) to be exposed to *Brucella* infection than small herd size in the areas (Table 5).

4. Discussion

The overall of 4.3% true seroprevalence *Brucella* antibody was recorded at individual animal level in the present study. Similar level of prevalence was reported by Tibesso, Ibrahim, and Tolosa (2014) and Asmare (2014), who reported seroprevalence of 4.3% in Adami Tulu and 4.8% in southern Ethiopian breeding female cattle. Similarly, Hailemeleket et al. (2007) reported 4.6% from different parts of country; Ibrahim, Belihu, Lobago, and Bekana (2010) reported 3.1% in Jimma zone and Alehegn, Tesfaye, and Chane (2017) reported 4.9% in Gondar. A comparable prevalence finding was also reported in other African countries: 4.2% in Eritrea by Omer, Skjerve, Woldehiwet, and Holstad (2000) and 3.3% in Central Africa by Nakoune et al. (2004). However, prevalence report in this study is lower than some previous studies carried out in the country: 11.2% in East Shewa (Dinka and Chala, 2009); 6.1% in western Tigray (Mekonen, Kalayou, and Kyule,

Table 3
Univariable and multivariable logistic regression analysis of potential risk factors of brucellosis at herd level in breeding female cattle in study areas.

Variables	Category	Total herds examined	Total herds positive (%)	Univariable		Multivariable	
				OR (CI 95%)	p-value	OR (CI 95%)	p-value
Origin	Limu Seka	81	11(13.6)				
	Chora Boter	57	5(8.8)	1.6(0.54–4.99)	0.388		
Agro-ecology	Mid-altitude	115	15(13.04)	–	–		
	Lowland	23	1(4.35)	3.3(0.41–26.31)	0.260		
Species composition	Single	28	7(25.00)	–	–		
	Mixed	110	9(8.18)	3.7(1.25–11.17)	0.018	3.7(1.25–11.17)	0.018
Herd size	Small	57	9(15.79)	–	–		
	Medium	35	5(14.29)	1.1(0.34–3.68)	0.845		
	Large	46	2(4.35)	4.1(0.85–20.47)	0.080		
Management system	Semi-intensive	96	13(13.54)	–	–		
	Extensive	42	3(7.14)	2.0(0.55–7.56)	0.288		
Introduction of new animal	Yes	66	8(12.12)	–	–		
	No	72	8(11.11)	1.1(0.39–3.13)	0.853		

OR: Odds Ratio; CI: Confidence Interval.

2010); 14.1% in Assela (Deselegn and Gangwas, 2011); 10.6% in Borana (Megersa et al., 2011). Similarly, higher seroprevalence was also reported in other African countries, For instance 6.6% in Ghana (Kubuafor, Awumbila, and Akanmori, 2000), 41% in Togo (Domingo, 2000), 6.6% in Chad (Schelling et al., 2003) and 46.8% in Uganda (Kungu, Okwee, Ayebazibwe, Okech, and Erume, 2010). On the other hand, the seroprevalence reported in the current study was higher than the values 2.9% reported in central Oromia (Teshale, Kindahl, Bekana, Kelay, and Jergefa, 2009); 1.7% in Sidama zone (Asmare et al.,

2010); 1.5% in Addis Ababa dairy farms (Tsefaye, Speybroeck, Deken, and Thys, 2011) and 2.6% in Arsi zone (Tsegaye et al., 2016). The variation in seroprevalence in different study areas and countries may be related to differences in environmental factors, management system and animals breed used in each study.

The overall herd level seroprevalence (11.6%) in present study is similar with the finding of Tsegaye et al. (2016) 9.5% in Aris zone and Asmare et al. (2010) 13.7% in Sidama zone. however, this result is lower than the reports of Megersa et al. (2011) 26.1% in southern and

Table 4
Univariable logistic regression analysis of potential risk factors of brucellosis in breeding female cattle in the study areas.

Variable	Category	Total animals examined	Total animals positive (%)	Crude OR (CI 95%)	P-value
Origin	Lemu Seka	249	12 (4.8%)	–	–
	Chora Boter	174	5 (2.9)	1.7 (0.59–4.95)	0.321
Age	< 3 years	29	4 (13.8)	–	–
	3–6 years	204	8 (3.9)	3.9 (1.10–13.96)	0.035
	> 6 years	190	5 (2.6)	5.9 (1.49–23.52)	0.012
Breed	Cross	116	10 (8.6)	–	–
	Local	307	7 (2.3)	4.0 (1.50–10.89)	0.006
BCS	Good	54	4(7.4)	–	–
	Medium	264	11(4.2)	1.8 (0.56–6.01)	0.313
	Poor	105	2(1.9)	4.1 (0.73–23.23)	0.109
Parity	Nulliparous	184	11(6.0)	–	–
	Monoparous	112	3(2.7)	2.3 (0.63–8.47)	0.206
	Pluriparous	127	3(2.4)	2.6 (0.72–9.62)	0.144
Pregnancy status	Non-pregnant	165	11(6.7)	–	–
	Pregnant	258	6 (2.3)	3.0 (1.09–8.28)	0.034
Abortion history	No	282	8(2.8)	–	–
	Yes	141	9(6.4)	0.4 (0.16–1.14)	0.088
Abortion period	No history	282	8(2.8)	–	–
	Before 5th month	44	1(2.3)	1.3 (0.15–10.29)	0.832
	After 5th month	97	8(8.2)	0.3 (0.12–0.89)	0.029
Retained placenta	No	294	9(3.1)	–	–
	Yes	129	8(6.2)	0.5 (0.18–1.27)	0.138
Agro-ecology	Mid-altitude	351	16(4.6)	–	–
	Lowland	71	1(1.4)	3.4 (0.44–25.99)	0.240
Management system	Sem-intensive	222	10(5.0)	–	–
	Extensive	201	7 (3.2)	1.6 (0.60–4.31)	0.345
Introduction of new animal	No	134	8(6.0)	–	–
	Yes	289	9(3.1)	2.0 (0.75–5.24)	0.171
Herd size	Small	180	12(6.7)	–	–
	Medium	126	4(3.2)	2.2 (0.69–6.92)	0.187
	Large	117	1(0.9)	8.3 (1.06–64.60)	0.044
	Only cattle	40	5(12.5)	–	–
Species composition	Mixed with sheep and/ goat	383	12(3.1)	4.4 (1.47–13.26)	0.008

OR: Odds Ratio; CI: Confidence Interval, BCS: Body condition score.

Table 5
Multivariable logistic regression analysis of factors associated with *Brucella* seropositivity in study areas.

Variable	Category	Total animals examined	Total animals positive (%)	Adjusted OR (95% CI)	P-value
Age	<3 years	29	4 (13.4)	–	0.015
	3–6 years	204	8 (3.9)	4.3 (1.04–17.97)	0.045
	>6 years	190	5 (2.6)	9.6 (2.08–44.07)	0.004
Breed	Cross	116	10 (8.6)	–	–
	Local	307	7 (2.3)	4.5 (1.54–12.99)	0.006
Herd size	Small	180	12(6.7)	–	0.037
	Medium	126	4(3.2)	3.0 (0.87–10.22)	0.083
	Large	117	1(0.9)	10.4 (1.27- 85.04)	0.029
Species composition	Only cattle	40	5(12.5)	–	–
	Mixed with sheep and/ goat	383	12(3.1)	4.4 (1.31–14.89)	0.017

OR: Odds Ratio; CI: Confidence Interval.

eastern Ethiopia and Mekonen et al. (2010) 24.1% in western Tigray, Ethiopia. Similarly, higher herd level seroprevalence have been recorded by other authors; 62% from Zambia (Samui, Oloya, Munyeme, and Skjerve, 2007), 55.6% from Uganda (Faye, Castel, Lesnoff, Rutabinda, and Dhalwa, 2005) and 43.3% from Ethiopia (Berhe et al., 2007). This difference may be related to overall animal level prevalence status of the disease and numbers of animals pre studied herds (herd size). The species composition was the only factor effect on herd level seroprevalence and herd kept with sheep and/ goats had higher odd of seropositivity *Brucella* infection. This may be due to cross-species transmission brucellosis. Herding of sheep and/goat along with cattle has been reported risk factors for seropositivity of *Brucella* infection (Al-Majali et al., 2009; Kaou, Zaki, Shimaa, and Nasr, 2010; Megersa et al., 2011).

In this study an increase in age associated with increased risk of being seropositive; older animals (>6 years) were almost ten times (OR=9.6) more likely to acquire the infection compared to their younger counterparts. Similarly, several studies in Ethiopia (Asgedom, Damena, and Duguma, 2016; Berhe et al., 2007; Kebede, Ejeta, and Ameni, 2008; Megersa et al., 2011; Mussie, Tesfu, and Yilkal, 2007; Tolosa et al., 2008) and also elsewhere (Ali et al., 2017; Kushwaha, Rajora, Mohan, Upadhyay, and Kumar, 2016; Matope et al., 2010) reported age as one of the important risk factors influencing *Brucella* serostatus in cattle. This report is in line with standard veterinary literatures which supports younger animals tend to be more resistant to infection and frequently clear infections. Older animals are more susceptible to brucellosis than younger animals, which are due to sex hormones and erythritol that stimulate the growth and multiplication of bacterial is increased in concentration with age (Radostits et al., 2007).

This study revealed that breed shown statistically significant variations in *Brucella* serostatus with local breed was almost five times (OR=4.5) more likely to be acquired *Brucella* infection than cross breed. The better management in the cross herds, intensive feeding that minimize contacts between animals and may be responsible for this difference. This finding is consistent with some previous studies in Ethiopia (Alehegn et al., 2017; Asfaw et al., 2016; Jergafa et al., 2009) and elsewhere (Kong, Nsongka, Itoe, Hako, and Leinyuy, 2016; Kushwaha et al., 2016; Matope et al., 2010) stated that seropositive of *Brucella* antibody was statistically significantly associated with breed in cattle. However, Kebede et al. (2008), Tsegaye et al. (2016) and Shiferaw, Fikadu, and Waktole (2017), Yohannes, Mersha, Degefu, Tolosa, and Woyesa (2012) reported breed was not significant associated with *Brucella* seropositivity in cattle. Mai et al. (2012) and Anka et al. (2014), Muma, Samui, Oloya, Munyeme, and Skjerve (2007) also reported no significantly association between *Brucella* seropositivity and cattle breed in Zambia, Nigeria and Malaysia, respectively. This variation could be due to difference in breeds, environmental factors and management system.

In the present study, statistically significant variation has been observed in seroprevalence of *Brucella* antibody between different herd sizes; larger herd sizes were ten times (OR=10.4) more likely to be exposed to *Brucella* infection. 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 because of one positive animals was at least available in large herd cattle compared with small herd size (Al-Majali et al., 2009). Several authors also reported that large herd size enhances the exposure and maintenance potential following abortions through increased contact at common feeding and watering points promoting transmission of *Brucella* organisms (Asmare et al., 2010; Haileselassie et al., 2011; Matope et al., 2010; Terefe, Girma, Mekonnen, and Asrade, 2017; Tolosa et al., 2008). However, in contrary to the finding of Kebede et al. (2008), who reported that brucellosis was not associated with herd size. The observed variation of the reports among different region of Ethiopia and other countries could be attributed to various factors including agro-ecology, breed of animals and management system.

In this study, cows from households herding cattle together with goats and/ sheep were four times (OR=4.4) more likely to be *Brucella* seropositive than cattle those keeping only cattle. Herding of these animals together increases chance of cross-species transmission of *Brucella* organism. *Brucella* organism is not strictly host specific; *Brucella melitensis* has been isolated from cattle (Smits, 2013) and thus, herding together might have increased the spillover of the pathogen from small ruminants to cattle. Moreover, herding more animal species in one herd may increase density and contact among them, thus increase exposure to *Brucella* organism and increasing chance of acquiring the infection (Kaou et al., 2010). This result is in agreement with report of Megersa et al. (2011), who reported mixing of sheep and/ goats with cattle increased risk of *Brucella* seropositivity in cattle in Borana, Ethiopia. Moreover, reports from Eritrea (Omer et al., 2000), Jordan (Al-Majali et al., 2009) and Malaysia (Anka et al., 2014) also showed that mixing of sheep and/ goats with cattle was a risk for *Brucella* transmission among different animals species. This is inconsistent with the findings of Elabdin, Angara, Elfadil, Sanousi, and Ibrahim (2014), who reported that keeping sheep and/ goats with cattle not significantly associated with *Brucella* seropositivity in Sudan. This variation could be due to difference in environmental factors, animal breed and management system.

Potential limitation of brucellosis serology is that the tests used worldwide detected antibodies directed against epitopes associated to s-LPS, which is shared by the different *Brucella* spp. and other cross-reaction organism such as *Yersinia enterocolitica* O:9 (Corbel, 2006; Munoz et al., 2005). In epidemiological study, the use of two tests applied serially is recommended to maximize specificity (Godfroid, Nielsen, and Saegerman, 2010; OIE, 2009). When test specificities are conditionally independent of each other, the resulting

expected specificity of serial testing is said to be higher than the corresponding individual specificities of each test (Dohoo, Martin, and Stryhn, 2003). Application of series testing in diseased population maximizes specificity and positive predictive value, but may have the risk of missing true positive cases (Megersa et al., 2012). Given the serial nature of testing, it is not possible to exclude that RBPT-negative animals may be positive by CFT and/ c-ELISA. This combination is expected to reduce the occurrence of misclassification and increase the chance to detect antibodies against brucellosis when present for a given sera. On the other hand, serial testing using pairs of specificity-correlated serological testing (RBPT, CFT, c-ELISA) has been argued to have lower specificity than expected when applied to disease-free population (Mainar-Jaime et al., 2005). When such a test is applied to a low disease prevalence (<1%) or disease-free population, the positive predictive value of the test fall closer to zero and the increased proportion of non-infected animal are classified as seropositive (Dohoo et al., 2003; Mainar-Jaime et al., 2005). Test cut-offs have different diagnostic goals depending on their context, for example, a screening situation versus confirmatory diagnostic situation where a diagnostic cut-off is selected is always a trade-off between false negative and false positive, due to the overlap between normal and disease populations (Dohoo et al., 2003). In this study, the cut-off point used may increase the specificity of the test thereby ensuring that seropositive case are resulting from *Brucella* infection, but may have the short-coming of missing positive case.

5. Conclusion

This result showed that antibodies to *Brucella* organisms are prevalent in breeding female cattle. Although the existence of antibody does not necessarily mean cattle are infected, this result indicated presence of brucellosis in the study areas. This study also identified that age, breed, herd size and species compositions were risk factors for *Brucella* seropositivity in breeding female cattle. The presence of brucellosis in animal kept for milk production, certainly poses a threat to the public health of the communities. Thus, important to conduct appropriate control methods and increasing the public awareness on zoonotic transmission of brucellosis are suggested. Moreover, more investigation should be conducted to isolate and characterize brucellosis as causes of reproduction problems and the associated loss within the study areas.

Declaration of Competing Interest

The authors have not declared any conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2020.100117.

References

Acha, N. P., & Szyfres, B. (2001). *Brucellosis in zoonosis and communicable diseases common to humans and animals* (3rd Ed.). Washington, D.C.: Pan. Amer. Health, Organization40–62.

- Adugna, K. E., Agga, G. E., & Zewde, G. (2013). Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. *Review in Scientific and Technical of the Office International des Epizooties*, 32, 1–20.
- Alehegn, E., Tesfaye, S., & Chane, M. (2017). Seroprevalence of Bovine Brucellosis and its risk factors in cattle in and around Gondar Town, North West Gondar, Ethiopia. *Journal of Advanced Dairy Research*, 4, 166.
- Ali, S., Akhter, S., Neubauer, H., Melzer, F., Khan, I., & Nji, A. E. (2017). Seroprevalence and risk factors associated with bovine brucellosis in the Potohar Plateau, Pakistan. *BMC Research Notes*, 10, 1–11.
- Al-Majali, A. M., Talafha, A. Q., Ababneh, M. M., & Ababneh, M. M. (2009). Seroprevalence and risk factors for bovine brucellosis in Jordan. *Journal of Veterinary Science*, 10, 61–65.
- Anka, M. S., Hassan, L., Khairani-Bejo, S., Zainal, M. A., Mohamad, R. B., Salleh, A., et al. (2014). Case-Control Study of Risk Factors for Bovine Brucellosis Seropositivity in Peninsular Malaysia. *PLoS ONE*, 9, e108673.
- Asfaw, M., Ameni, G., Kassa, T., Tuli, G., Arenas, A., & Mamo, G. (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.
- Asgedom, H., Damena, D., & Duguma, R. (2016). Seroprevalence of bovine brucellosis and associated risk factors in and around Alage district, Ethiopia. *Springer Plus*, 5, 1–8.
- Asmare, K. (2014). Neospora caninum versus Brucella spp. exposure among dairy cattle in Ethiopia: A case control study. *Tropical Animal Health and Production*, 46, 961–966.
- Asmare, K., Asfaw, Y., Gelaye, E., & Ayelet, G. (2010). Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. *African Journal of Agricultural Research*, 5, 257–263.
- Berhe, G., Belihu, K., & Asfaw, Y. (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, 65–71.
- Boukary, A. R., Saegerman, C. A., batih, E., Fretin, D., Bada, R. A., Deken, R., et al. (2013). Seroprevalence and potential risk factors for *Brucella* spp. infection in traditional cattle, sheep and goats reared in urban, per urban and rural areas of Niger. *PLoS ONE*, 8, e83175. <https://doi.org/10.1371/journal.pone.0083175>.
- Corbel, M. J. (2006). In W. H. O. Press (Ed.). Geneva: World Health Organization.
- CSA. (2017). Livestock and livestock characteristics, agricultural sample survey. Addis Ababa, Ethiopia. *Statistical Bulletin*, 2, 9–13.
- Degefa, T., Duressa, A., & Duguma, R. (2011). Brucellosis and some reproductive problems of indigenous Arsi cattle in selected Arsi zones of Oromia Regional State, Ethiopia. *Global Veterinary*, 7, 45–53.
- Deselegn, T. B., & Gangwar, S. K. (2011). Seroprevalence study of bovine brucellosis in Assela government dairy farm of Oromia Regional State, Ethiopia. *International Journal of Science and Nature*, 2, 692–697.
- Dinka, H., & Chala, R. (2009). Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. *American-Eurasian Journal of Agricultural and Environmental Sciences*, 6, 508–512.
- Dohoo, I., Martin, S. W., & Stryhn, H. (2003). *Veterinary epidemiologic research*. Charlottetown, Prince Edward's Island: AVC Inc.34–56.
- Dohoo, I., Martin, W., & Stryhn, H. (2009). *Veterinary epidemiologic research* (2nd ed.). Charlottetown, Prince Edward Island: AVC239–249.
- Domingo, A. M. (2000). Current status of some zoonoses in Togo. *Acta Tropica*, 76, 65–67.
- Ducrottoy, M., Bertub, W. J., Matopec, G., Cadmus, S., Conde-Álvarez, R., Gusib, A. M., et al. (2017). Brucellosis in Sub-Saharan Africa: Current challenges for management, diagnosis and control. *Acta Tropica*, 165, 179–193.
- EFSA (European Food Safety Authority). (2006). Scientific opinion on performance of brucellosis diagnostic methods for bovines, sheep, and goats. *The EFSA Journal*, 432, 1–44.
- Elabdin, S. Z., Angara, E., Elfadil, A., Sanousi, E., & Ibrahim, A. (2014). Prevalence and risk factors of ruminants brucellosis in Jabel Aolia Locality, Sudan. *Sudan Journal of Science and Technology*, 15, 60–72.
- FAO. (2010). *Brucella melitensis* in Eurasia and the Middle East. *FAO Animal Production and Health Proceedings* No. 10. Rome.
- Faye, B., Castel, V., Lesnoff, M., Rutabinda, D., & Dhalwa, J. (2005). Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin in Uganda. *Preventive Veterinary Medicine*, 67, 267–281.
- Franc, K. A., Krecek, R. C., Hasler, B. N., & Arenas-Gamboa, A. M. (2018). Brucellosis remains a neglected disease in the developing world: A call for interdisciplinary action. *BMC Public Health*, 18, 125. <https://doi.org/10.1186/s12889-017-5016-y>.
- Godfroid, J., Nielsen, K., & Saegerman, C. (2010). Diagnosis of brucellosis in livestock and wildlife. *Croatian Medical Journal*, 51, 296–305.
- Haillemeleket, M., Kassa, T., Tefera, M., Belihu, K., Asfaw, Y., & Ali, A. (2007). Seroprevalence of brucellosis in cattle and occupationally related humans in selected sites of Ethiopia. *Ethiopian Veterinary Journal*, 11, 85–100.
- Hailleselassie, M., Kalayou, S., Kyule, M., Asfaha, M., & Belihu, K. (2011). Effect of Brucella infection on reproduction conditions of female breeding cattle and its public health significance in Western Tigray, Northern Ethiopia. *Veterinary Medicine International*, 201, 7.
- Ibrahim, N., Belihu, K., Lobago, F., & Bekana, M. (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, 35–40.
- Jergefa, T., Kelay, B., Bekana, M., Teshale, S., Gustafson, H., & Kindah, H. (2009). Epidemiological study of bovine brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. *Review in Scientific and Technical of the Office International des Epizooties*, 28, 933–943.
- Kaou, H., Zaki, M. M., Shimaa, A., & Nasr, A. (2010). Epidemiology of brucellosis among farm animals. *Nature and Science*, 8, 190–197.

- Kebede, H., Melaku, A., & Kebede, E. (2014). Constraints in animal health service delivery and sustainable improvement alternatives in North Gondar, Ethiopia. *Onderstepoort Journal of Veterinary Research*, *81*, 10. <https://doi.org/10.4102/ojvr.v81i1.713>.
- Kebede, T., Ejeta, G., & Ameni, G. (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). *Revue de Medecine Veterinaire*, *159*, 3–9.
- Kong, A. T., Nsongka, M. V., Itoe, S. M., Hako, A. T., & Leinyuy, I. (2016). Seroprevalence of Brucella abortus in the Bamenda Municipal Abattoir of the Western Highlands of Cameroon. *Greener Journal of Agricultural Sciences*, *6*, 245–251.
- Kubuafoa, D. K., Awumbila, B., & Akanmori, B. D. (2000). Seroprevalence of brucellosis in cattle and humans in the Akwapim-south district of Ghana: Public health implication. *Acta Tropical*, *76*, 45–48.
- Kungu, J. M., Okwee, A. J., Ayebazibwe, C., Okech, S. G., & Erume, J. (2010). Seroprevalence and risk factors for brucellosis in cattle in Gulu and Amuru districts, Northern Uganda. *Africa Journal of Animal and Biomedical Sciences*, *5*, 1819–4214.
- Kushwaha, N., Rajora, V. S., Mohan, A., Upadhyay, A. K., & Kumar, R. (2016). Comparison of serological tests for detection of Brucella antibodies in cattle of an organized dairy farm. *Indian Journal of Animal Research*, *50*, 69–74.
- Lakew, A., Hiko, A., Abraha, A., & Mengistu, H. S. (2019). Sero-prevalence and community awareness on the risks associated with Livestock and Human brucellosis in selected districts of Fafan Zone of Ethiopian-Somali National Regional State. *Veterinary and Animal Science*, *7*, 100047.
- Mai, H. M., Irons, P. C., Kabir, J., & Thompson, P. N. (2012). A large seroprevalence survey of brucellosis in cattle herds under diverse production systems in northern Nigeria. *BMC Veterinary Research*, *8*, 1–14.
- Mainar-Jaime, R. C., Munoz, P. M., de Miguel, M. J., Grillo, M. J., Marin, C. M., Moriyon, I., et al. (2005). Specificity dependence between serological tests for diagnosing bovine brucellosis in Brucella-free farms showing false positive serological reactions due to Yersinia enterocolitica O: 9. *The Canadian veterinary journal = La revue veterinaire canadienne*, *46*(10), 913–916.
- Matope, G., Bhebe, E., Muma, J. B., Lund, A., & Skjerve, E. (2010). Herd level factors for Brucella seropositivity in cattle reared in smallholder dairy farms in Zimbabwe. *Preventive Veterinary Medicine*, *94*, 213–221.
- Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., & Skjerve, E. (2012). Seroepidemiological study of livestock brucellosis in a pastoral region. *Epidemiol. Infect.* *140*, 887–896.
- McDermott, J. J. Arimi S.M. (2002). Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology*, *90*(1–4), 111–134. [https://doi.org/10.1016/S0378-1135\(02\)00249-3](https://doi.org/10.1016/S0378-1135(02)00249-3).
- Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., & Skjerve, E. (2011). Seroprevalence 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.
- Mekonen, H., Kalayou, S., & Kyule, M. (2010). Serological survey of bovine brucellosis in Barka and Orado breeds (*Bos indicus*) of western Tigray, Ethiopia. *Preventive Veterinary Medicine*, *94*, 28–35.
- Memish, Z. A. Mah M.W. (2001). Brucellosis in laboratory workers at a Saudi Arabian hospita. *American Journal of Infection Control*, *29*(1), 48–52. <https://doi.org/10.1067/mic.2001.111374>.
- Muma, J. B., Samui, K. L., Oloya, J., Munyeme, M., & Skjerve, E. (2007). Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, *80*, 306–317.
- Munoz, P. M., Marin, C. M., Monreal, D., Gonzalez, D., Garin-Bastuji, B., Diaz, R., & et al. (2005). Efficacy of several serological tests and antigens for diagnosis of Bovine Brucellosis in the presence of false-positive serological results due to Yersinia enterocolitica O:9. *Clinical and diagnostic laboratory immunology*, *12*(1), 141–151.
- Mussie, H., Tesfu, K., & Yilkal, A. (2007). Seroprevalence study of bovine brucellosis in Bahir Dar Milk shed, Northwestern Amhara Region. *Ethiopian Veterinary Journal*, *11*, 42–49.
- Naing, L., Winn, T., & Rusli, B. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences*, *1*, 9–14.
- Nakoune, E., Debaere, O., Koumand-Kotogne, F., Selenkon, B., Samory, F., & Talarmin, A. (2004). Serological surveillance of brucellosis and Q fever in cattle in the Central African Republic. *Acta Tropical*, *92*, 147–151.
- Ndazigaruye, G., Mushonga, B., Kandiwa, E., Samkange, A., & Segwagwe, B. E. (2018). Prevalence and risk factors for brucellosis seropositivity in cattle in Nyagatare District, Eastern Province, Rwanda. *Journal of the South African Veterinary Association*, *89*(0), <https://doi.org/10.4102/jsava.v89i0.1625> a1625.
- OIE. (2004). *Bovine brucellosis Manual of Diagnostic Tests and Vaccines for terrestrial Animals*. (5th Ed.). Paris: Office International des Epizooties 409–438.
- OIE. (2009). Manual of diagnostic tests and vaccines for terrestrial animals. *World Organization of Animal Health*, *2*, 5–35.
- Omer, M. K., Skjerve, E., Woldehiwet, Z., & Holstad, G. (2000). Risk factors for Brucella spp. infection in dairy cattle farms in Asmara, State of Eritrea. *Preventive Veterinary Medicine*, *46*, 257–265.
- Pal, M., Lemu, D., Worku, S., & Desta, G. (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.
- Radostits, O. M., Gay, C. C., Hinchcliff, K. W., & Constable, P. D. (2007). *Veterinary medicine. A text book of diseases of cattle, sheep, pigs, goats and horses* (10th Ed.). London: W.B., Saunders 963–985.
- Samui, K. L., Oloya, J., Munyeme, M., & Skjerve, E. (2007). Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. *Preventive Veterinary Medicine*, *80*, 306–317.
- Schelling, E., Dinguimbaye, C., Daoud, S., Nicoletti, J., Boertin, P., Tanner, M., et al. (2003). Brucellosis and Q-fever seroprevalence of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine*, *61*, 279–293.
- Shiferaw, J., Fikadu, A., & Waktole, H. (2017). Seroprevalence and associated risk factors for seropositivity of bovine Brucellosis at Wollega University Horro-Guduru animal breeding and research center. *Einstein International Journal Organization*, *1*, 45–56.
- Smits, H. L. (2013). Brucellosis in pastoral and confined livestock: prevention and vaccination. *Review in Scientific and Technical of the Office International des Epizooties*, *32*, 219–228.
- Szulowski, K., Iwaniak, W., Weiner, M., & Zlotnicka, J. (2013). Brucella suis biovar 2 isolations from cattle in Poland. *Annals of Agricultural and Environmental Medicine*, *20*, 672–675.
- Terefe, Y., Girma, S., Mekonnen, N., & Asrade, B. (2017). Brucellosis and associated risk factors in dairy cattle of eastern Ethiopia. *Tropical Animal Health and Production*, *49*, 599.
- Tesfaye, D., Speybroeck, N., Deken, R. D., & Thys, E. (2011). Economic burden of bovine trypanosomiasis in three villages of Metekel zone, Northwest Ethiopia. *Tropical Animal Health and Production*, *44*, 873–879.
- Teshale, S., Kindahl, H., Bekana, M., Kelay, B., & Jergefa, T. (2009). Epidemiological study of bovine brucellosis in three agro ecological areas of central Oromia Ethiopia. *Review in Scientific and Technical of the Office International des Epizooties*, *28*, 933–943.
- Thrusfield, M. (2005). *Veterinary Epidemiology* (3rd Edn.). England: Blackwell Publishing 345–543.
- Tibesso, G., Ibrahim, N., & Tolosa, T. (2014). Sero prevalence of bovine and human brucellosis in Adami Tulu, Central Ethiopia. *World Applied Sciences Journal*, *31*, 776–780.
- Tolosa, T., Regassa, F., & Belihu, K. (2008). Seroprevalence Study of Bovine Brucellosis in Extensive Management System in Selected Sites of Jimma Zone, Western Ethiopia. *Bulletin of Animal Health and Production in Africa*, *56*, 25–37.
- Tsegaye, Y., Kyule, M., & Lobago, F. (2016). Seroprevalence and Risk Factors of Bovine Brucellosis in Arsi Zone, Oromia Regional State, Ethiopia. *American Scientific Research Journal for Engineering, Technology and Sciences*, *24*, 16–25.
- Welay, G. M., Tedla, D. G., Teklu, G. G., Weldaeregay, S. K., Shibeshi, M. B., Kidane, H. H., et al. (2018). A preliminary survey of major diseases of ruminants and management practices in Western Tigray province, northern Ethiopia. *BMC Veterinary Research*, *14*, 293. <https://doi.org/10.1186/s12917-018-1621-y>.
- Wathes, D., Brickell, J., Bourne, N., Swali, A., & Cheng, Z. (2008). Factors influencing heifer survival and fertility. *Journal of Animal Science*, *2*(8), 1135–1143.
- Yohannes, M., Mersha, T., Degefu, H., Tolosa, T., & Woyesa, M. (2012). Bovine Brucellosis: serological Survey in Guto-Gida District, East Wollega Zone, Ethiopia. *Global Veterinary*, *8*, 139–143.