academicJournals

Vol. 10(7), pp. 203-213, 21 February, 2016
DOI: 10.5897/AJMR2015.7707
Article Number: E303AFC57233
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Seropositivity and risk factors for *Brucella* in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia

Minda Asfaw Geresu[1]*, Gobena Ameni[2], Tesfu kassa[2], Getachew Tuli[3], Angella Arenas[4] and Gezahegne Mamo Kassa[5]

[1]School of Agriculture, Animal and Range Sciences Course Team, Madda Walabu University, Bale-Robe, Ethiopia.
[2]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia.
[3]National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia.
[4]College of Medicine, Texas A and M University, College Station, TX 77845, U.S.A.
[5]College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia.

Received 7 August, 2015; Accepted 1 September, 2015

A cross-sectional study was conducted in Asella and Bishoftu towns of Oromia Regional State of Ethiopia to determine seropositivity and associated risk factors exposing dairy cattle to brucellosis from December, 2013 to March, 2014. A total of 570 dairy cattle from 35 herds were purposely selected for inclusion in the study based on abortion history. From 35 farms studied, 80, 55.56 and 100% of the farm owners in small, medium and large herd sizes responded as they were aware of brucellosis, respectively. It was also found out that all farm owners of the study area were dependent on culling of the known *Brucella* infected animals, while most of the farm owners dispose the after birth to open dump in small and medium herd size farms. All sera sample collected were tested and confirmed serologically using the card test (CT), rose Bengal plate test (RBPT), indirect enzyme linked immuno sorbent assay (i-ELISA) and complement fixation test (CFT). Out of 570 samples tested in the present study, an overall sero prevalence was estimated 1.4% (95% CI: 0.241, 3.461) by complement fixation test (CFT). Among the tested samples, 13 (2.28%), 15 (2.63%) and 16 (2.81%) were found positive by the aforementioned tests, respectively. The higher seroprevalence, 3.23% (95% CI: 3.0, 7.4) was observed in Asella compared to Bishoftu (0.52%) town. A Chi-square computed statistical analysis indicated that origin ($\chi 2=6.63$; $P<0.05$), breed type ($\chi 2= 8.49$; $P<0.05$), abortion history ($\chi 2=92.43$; $P<0.001$) and abortion period ($\chi 2=192.97$; $P<0.001$) were the major risk factors for *Brucella* infection in the study areas. Multivariable logistic regression statistical analysis revealed that origin and breed type were significantly associated with *Brucella* seropositivity ($P<0.05$). Consequently, origin was statistically identified to be the major risk factor for brucellosis to occur in relation to other factors (OR=7.56). In conclusion, the prevailing *Brucella* seropositivity in most of the dairy farms of the study areas signifies the economic importance of brucellosis in the dairy cattle industry and the potential public health implication for human population. Therefore, more proactive measures should be taken to protect the cattle populations from *Brucella* infection to reduce its economic impact to the dairy industry and the risk of zoonotic infection in exposed human population in the study areas.

Key words: Asella, Bishoftu, brucellosis, dairy cattle, seropositivity, risk factors.

INTRODUCTION

Brucellosis is endemic in many developing countries and is caused by *Brucella* species that affect man, domestic and some wild animals, and marine mammals (Seleem et al., 2010). It causes abortion and sterility in livestock leading to serious economic losses and has even more serious medical impact in humans, leading to more than 500,000 infections per year worldwide (Godfroid et al., 2005).

Bovine brucellosis is an infectious and contagious disease known for its impact on reproductive performance of cattle in Africa and is predominantly a disease of sexually mature animals (Rahman et al., 2012; Asmare et al., 2013). The disease is primarily caused by *Brucella abortus* and occasionally by *Brucella 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, 2009a; Godfroid et al., 2010). Chronic infection of the mammary glands due to *Brucella suis* has also been reported (Lopes et al., 2010). Clinically bovine brucellosis is characterized by impaired fertility specifically with abortion, metritis, orchitis and epididymitis (Seleem et al., 2010).

The mode of transmission of the bacteria varies with the epidemiological area, the animal reservoir and the occupational exposed groups (Seleem et al., 2010). Sources of infection for the transmission of the bovine brucellosis are aborted fetuses, the fetal membranes after birth, and vaginal discharges and milk from infected animals (Radostits et al., 2000; Tolosa et al., 2010). The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitute a very important source of infection. The bacteria can be transmitted to humans through direct contact with infected tissue via breaks in skin, ingestion of contaminated tissues or milk products, and inhalation or mucosal exposure to aerosolized bacteria (Seifert, 1996; Radostits et al., 2007).

The prevalence of brucellosis is influenced by a number of risk factors related to production systems, biology of the individual host and environmental factors. These include age, herd size and composition, hygienic status of the farm, rate of contact between infected and susceptible animals, farm biosecurity and climate (Radostits et al., 2007; McDermott and Arimi, 2002).

A precise diagnosis of *Brucella* spp. infection is important for the control of the disease in animals and consequently in man. Clinical diagnosis is based usually on the history of reproductive failures in livestock, but it is a presumptive diagnosis (Poester et al., 2010) that must be confirmed by laboratory methods (Nicoletti, 2002; Poester et al., 2010). Although blood and tissue cultures remain the 'gold standard' for diagnosis, they show low sensitivity, are time consuming, and represent a risk for laboratory personnel (Bricker et al., 2002; Navarro et al., 2004).

Serology is a standard method for the epidemiological surveillance of brucellosis (Köppel et al., 2007; Leuenberger et al., 2007). However, cross-reactions between *Brucella* species and other Gram- negative bacteria, such as *Yersinia enterocolitica* O:9, *Francisella tularensis*, *Escherichia coli* O:157, *Salmonella urbana group* N, *Vibrio cholerae* and *Stenotrophomonas maltophilia*, are a major problem of the serological assays (Muñoz et al., 2005; Al Dahouk et al., 2006). The source of antigenic cross reactions is the O-chain of the smooth lipopolysaccharide (S-LPS) present on the surface of the bacterial cell, which shows great similarity in smooth *Brucella* spp. and the above mentioned bacteria (Hinić et al., 2009). False positive serological results are due only to *Y. enterocolitica* O: 9 affect up to 15% of the cattle herds in regions free from brucellosis, generating considerable additional costs for surveillance programs (Muñoz et al., 2005). False negative results have also been observed in serological diagnosis of brucellosis (Godfroid et al., 2002; Tessaro and Forbes, 2004). They occur mostly due to the fact that the antibody response is dependent upon the stage of infection during sample collection (Hinić et al., 2009).

Since the first report of brucellosis in the 1970s in Ethiopia, the disease has been noted as one of the important livestock diseases in the country (Asfaw, 1998; Eshetu et al., 2005; Kebede et al., 2008; Ibrahim et al., 2010). Prevalence of bovine brucellosis varies widely across Ethiopia, with reported seroprevalences ranging from 0.2% in south-western Ethiopia (Tolosa, 2004) to 38% in western Ethiopia (Rashid, 1993).

The dairy industry has been growing to meet an ever increasing demand for milk and milk products in the country. Crossbreeding indigenous cattle with high yielding exotic cattle is the main policy established by the Ethiopian government to bridge the gap between supply and demand for dairy products. Owners of dairy cattle and institutions promoting the dairy industry require current, reliable scientific data on such important diseases as brucellosis. Therefore, it is of paramount importance to know the magnitude of brucellosis, major

*Corresponding author. E-mail: minda.asfaw@gmail.com. Tel: +251910431505.

potential risk factors exposing to the disease and use of different serological tests which in turn assist in controlling and eradicating the disease and devising baseline information to develop national wide brucellosis information.

The main aims of this study were: i) to determine seropositivity of brucellosis in dairy cattle and major potential associated risk factors, ii) to assess knowledge-attitude and practices (KAP) of the farm owner's regarding this disease, and iii) comparison of four different serological test agreement in diagnosing brucellosis.

MATERIALS AND METHODS

Description of the study area

The study was conducted in two purposely selected sites in central Ethiopia, Bishoftu, East Shewa Zone and Assela, East Arsi Zone. These study areas were selected based on the abundance of dairy farms that constituted the known milk sheds (Land O'Lakes, 2010).

Bishoftu is located at 47 km southeast of Addis Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 meters above sea level in the central high land of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26 and 14°C respectively, with mean relative humidity of 61.3% (ADARDO, 2007). Farmers in the vicinity of Bishoftu town use a mixed crop and livestock farming system. Moreover, Bishoftu and its surrounding area have variable and yet representative agro-ecologies of the country. These agro-climatic zones are inhabited with different plant and animal species (Conway and McKenzie, 2007).

The second study area was Asella, which is located at 175 km southeast of Addis Ababa, and the altitude and annual rainfall of the area ranges from 502-4130 meters above sea level and 200-400 mm with mean annual temperature of 22.5°C, respectively. It is one of the highly populated areas in Ethiopia with estimated human population of 2,521,349 and livestock population of cattle-82,190; sheep-51,292; goat-8, 11,479; poultry- 5, 62,915; equine- 22,055 (Deselegn and Gangwar, 2011).

Definitions

In Ethiopia dairy cattle production systems are classified into rural smallholder (mixed crop-livestock) production, pastoral and agro-pastoral production, urban and peri-urban smallholder dairy production, and commercial dairy production systems (Asmare et al., 2013; Land O'Lakes, 2010). This study focuses on the latter two production systems. Urban and peri-urban dairy is one of the four dairy production systems in Ethiopia producing milk either as a full-time or a part-time business. These smallholder dairy farms predominantly keep a small number (≤10 animals) of cross-bred cows in a zero grazing system to produce milk for both home use and sale. Commercial dairy farms are also farms located in urban and peri-urban areas mainly in and around the major cities and produce milk exclusively for sale.

The farms were classified according to herd size and level of production into smallholder farms (<10 animals), medium farms (10 to 50 animals) and large farms with more than 50 animals (Megersa et al., 2011).

The disease is primarily caused by *B. abortus* in cattle. However, occasionally there were reports as the disease is caused by *B. melitensis* where cattle are kept together with infected sheep or goats (Godfroid et al., 2010) and chronic infection of mammary glands due to *B. suis* has also been reported when keeping cattle with pigs (Lopes et al., 2010). Though the above reports revealed that as the cattle were infected by different species of *Brucella* upon mixing with sheep, goats or pigs, in our study area, there were no sheep/goat/pig mixing practices with the dairy cows by the owner of the farms.

Risk factors assessment

In this study covariates (hypothesized explanatory variables) were assessed at both individual and farm level. Information was extracted from herd records where possible, and if this information was not available owners were interviewed using semi-structured questionnaires.

The presence of abortion history in the farm, separate parturition/maternity pen, separation of cows during parturition, awareness about brucellosis (knowledge), brucellosis test in the farm, frequent contact between animals with other herds were categorized as yes or no variables while breed of dairy cattle were categorized as Holestein-Friesian, cross or local. Breeding was characterized by service types (artificial insemination (AI), bull or both). The method of after birth disposal (placenta, aborted material and dead fetus) was also categorized into burying/burning, or thrown to open dump.

The culling criterion of animals from the farm was categorized as reproductive problems, non-reproductive problems or both variables. The method of cleaning of calving pen after parturition was categorized as flushing with water, disinfecting with detergents or both variables. The replacement stock of each farm was defined as buy in, raise own replacement or both. Culling, test and slaughter or both were considered as the measures taken against the known *Brucella* infected animals. Individual animals were categorized as young (≤ 36 months) and adult (>36 months), origin of each individual animal was defined as either Asella or Bishoftu while the location of the farm was classified as urban or peri-urban. Parity of the animals in the farm were categorized as primiparous, pluriparous or not applicable variables whereas the abortion stage was classified as first trimester, second trimester or third trimester.

Study design and sample size determination

A cross-sectional study design was conducted to determine the seropositivity of *Brucella* infection in dairy cattle in the two selected towns and to identify the potential risk factors associated with the seropositivity. Dairy cattle above six months of age were selected for this study. Relevant individual animal biodata and farm level information were collected using a semi-structured questionnaire.

The sampling was performed using a two level approach, selecting first individual farms with abortion history and then randomly selecting individual animals systematically inside each farm. About 57% of the sampled cattle were from smallholder farm (small herd size) while the remaining 43% were from medium and large herd size around urban and peri-urban including the commercial dairy farms. A list of dairy farms was prepared for each of the two study areas in collaboration with the respective district livestock health departments.

The sample size for cattle in Asella was calculated using a 14.14% seroprevalence of bovine brucellosis (Deselegn and Gangwar 2011), 95% confidence interval (CI) and 5% required precision (Thrusfield, 2007). In Bishoftu, 50% expected prevalence, 95% confidence interval and 5% required precision were used, resulting in a sample size of 384 cattle for this study. Hence, a total of 570 dairy cattle (186 from Asella and 384 from Bishoftu) were

considered for this study from 35 farms in the study areas.

Study methodology

Serological blood sample collection

Blood samples (10 ml) were collected from the jugular vein of each animal, using sterile needles and plain vacutainer tubes. The blood samples were allowed to stand overnight at room temperature and centrifuged at 1500 × g for 10 min to obtain the serum. Sera were decanted into cryovials, identified and transported to the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia in ice packs and stored at -20°C until screened for antibodies against natural *Brucella* exposure using serological analysis.

Serological laboratory techniques

Rose Bengal plate test (RBPT)

All sera samples collected were initially screened by RBPT using RBPT antigen (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom) according to OIE (2004) and Alton et al. (1975) procedures. Briefly, sera and antigen were taken from refrigerator and left at room temperature for half an hour before the test to maintain to room temperature and processed following the recommended procedures.

Indirect enzyme linked immunosorbent assay (i-ELISA)

For further laboratory analysis, i-ELISA was performed using a commercial i-ELISA kit (BRUCELISA(160+400), (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey KT15 3NB, United Kingdom) to detect circulating antibodies of *Brucella* in cattle serum sample, and the protocol provided by the developers was followed precisely. The test sera were analyzed at a final dilution of 1/200. The positive and negative controls were used at a dilution of 1/40 as has been indicated by the manufacturer. Following the addition of the conjugate and substrate-chromogen mixture at a recommended strength, the plate was incubated and examined for the intensity of reaction with an automated ELISA reader at 405 nm. Color development within a well indicates that the tested serum has antibodies to *Brucella.* A positive/ negative cut-off was calculated as 10% of the mean of the optical density (OD) of the eight positive control wells. Any test serum with an OD value equal to or above this value was considered positive.

Card test (CT)

The brucellosis card test is a macroscopic agglutination procedure utilizing disposable materials, a stained buffered whole cell antigen suspension of *B. abortus* strain119-3 and contained in compact kits of minimal size. The card test for brucellosis is a rapid, sensitive and reliable procedure for detecting serologic evidence of *Brucella* infection. This test was performed according to instructions of the manufacturer (United States Department of Agriculture (USDA), APHIS, Veterinary Services, USA). A positive serum showed characteristic agglutination, moderate to large clumps where as a negative one showed a pattern of dispersed particles without characteristic clumps and those showed no clumping.

Complement fixation test (CFT)

Sera that tested positive to the card test, i-ELISA and RBPT were further tested using CFT 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, 2009b). Sera with a strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.

Case definition

Animals were considered as seropositive on the complement tests result, i.e., an animal was considered positive if tested seropositive on CT/ RBT/ i-ELISA and CFT in serial interpretation. The test was regarded as valid if the negative control serum showed complete haemolysis and the positive control shows inhibition of haemolysis. Due to its high accuracy, complement fixation is used as confirmatory test for *B. abortus*, *B. melitensis*, and *Brucella ovis* infections and it is the reference test recommended by the OIE for international transit of animals (OIE, 2009a, b).

Data analysis

Data generated from the questionnaire survey and laboratory investigations were recorded and coded using a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA). The seroprevalence was calculated as the number of seropositive samples divided by the total number of samples tested. To identify association of *Brucella* seropositivity with the risk factors (origin, age, management system, breed type, herd size, separate parturition, abortion history, abortion period and parity) were computed by Pearson's Chi-square test. After the association of exposure variables with *Brucella* seropositivity was analyzed at individual animal level by the Chi-square test, those variables significantly associated with *Brucella* seropositivity (origin, breed type, abortion history and abortion period) were further analyzed by multivariable logistic regression. A multivariable logistic regression model was used to identify the potential risk factors associated with *Brucella* infection in animal and variables with a p-value lower than or equal to 0.05 (in Chi-square analysis) were included in the multivariable logistic regression model. Further selection of variables was based on backward elimination procedure using a LR-test at 0.05 as cut point. Prior to building a final model, variables were tested for interaction effects using cross-product terms and for multiple-collinearity using the collinearity matrix index. The validity of the model to the observed data was assessed by computing the Hosmer-Lemeshow goodness-of-fit test. Finally, deviant covariate patterns and their influences on parameter estimates of the model were identified.

The agreement between CT, RBPT, i-ELISA with CFT, considering as gold standard test, were done using kappa test and interpreted according to the recommendations of (Dohoo et al., 2003) who states Kappa values as: <0.2: slight agreement, 02–0.4: fair agreement, 0.4–0.6: moderate agreement, 0.6–0.8: substantial agreement and >0.8: almost perfect agreement. The 95% confidence interval and a significance level of $\alpha = 0.05$ were used.

RESULTS

Knowledge, attitudes and practices (KAP) of the farm owner about brucellosis

From 35 farms studied, 80, 55.56 and 100% of the farm

Table 1. Knowledge, attitudes and practices (KAP) of farm owner's about *Brucella* infection in small, medium and large herd size in the study areas.

Variables	Proportion of respondents (n)		
	Herd size		
	Small(n=20) n (%)	Medium(n=9) n (%)	Large(n=6) n (%)
Awareness about brucellosis			
No	4(20)	0)	0(0)
Yes	16(80)	5(55.56)	6(100)
Brucella infected animal			
Test and slaughter	0(0)	0(0)	0(0)
Culling	20(100)	9(100)	6(100)
Both	0(0)	0(0)	0(0)
After birth disposal			
Burrying/ Burning	1(5)	1(11.1)	5(83.33)
Open dump	19(95)	8(88.89)	1(16.67)

n= number.

owners in small, medium and large herd sizes responded as they were aware of brucellosis, respectively. It was also found out that all farm owners of the study area were dependent on culling of the known *Brucella* infected animals while most of farm owners dispose after birth to open dump in small and medium herd size farms (Table 1).

Farm characteristics

Of the 35 farms assessed by a questionnaire survey, it was found that all of the large herd size farms had bulls on their farms, whereas only 5 (25%) of small farms have bulls. The study revealed that all farms in the study area had no frequent contact with other herds. The majority (95%) of small farms and all of the large farms were using AI for breeding purposes. The practices of provision of separate parturition pens were 83.3% in large farms whereas they were only 5% for small farms (Table 2).

Seroprevalence of brucellosis

In the present study, an overall seroprevalence was estimated 1.4% (95% CI: 0.241, 3.461) by CFT. Among 570 tested samples, 13 (2.28%), 15 (2.63%) and 16 (2.81%) were found positive by RBPT, iELISA, and card test, respectively. The higher seroprevalence, 3.23% (95% CI: 3.0, 7.4) was observed in and around Asella town compared to Bishoftu (0.52%) (Table 3).

Comparison of serological test agreement

The kappa statistics showed that there was substantial agreement between the card test and RBT with CFT as gold-standard test, while almost perfect agreement was observed between i-ELISA and CFT (Table 4).

Chi-square analysis of association of the putative risk factors with *Brucella* seropositivity

A Chi-square analysis revealed that origin, breed, abortion history and abortion period were significantly associated (P<0.05) with seropositivity of bovine brucellosis than among other factors considered during the study (Table 5).

Multivariable logistic regression analysis of risk factors associated with *Brucella* sero positivity

The logistic regression analysis of the putative risk factors indicated that cattle those originated from Asella were more likely to be infected (OR= 6.4, 95% CI: 1.27 - 31.85) with *Brucella* than cattle from Bishoftu (Table 6).

DISCUSSION

In the present study, based on the questionnaire survey, most of the respondents were well aware about brucellosis and practice culling of the known *Brucella* infected animals in their farms. Among the prevention of brucellosis transmission, culling is the most known measures against animal brucellosis (Radostits et al., 2000). In addition, most of the respondents in this study with the small herd size (95%) did not bury afterbirth (aborted fetus, still birth and retained foetal membrane), rather they left them on open dump. Moreover, all the

Table 2. Summary of the proportion of variables in the three herd (farm) size.

Variables category	Herd size		
	Small (n=20) Frequency (%)	Medium (n=9) Frequency (%)	Large (n=6) Frequency (%)
Bull			
No	15 (75)	2 (22.2)	0
Yes	5 (25)	7 (77.8)	6 (100)
Frequent contact with other herd			
No	20 (100)	9 (100)	6 (100)
Yes	0 (0)	0 (0)	0 (0)
Service type			
AI	19 (95)	7 (77.8)	6 (100)
Bull	0 (0)	0 (0)	0 (0)
Both	1 (5)	2 (22.2)	0 (0)
Parturition pen			
No	19 (95)	5 (55.6)	1 (16.7)
Yes	1 (5)	4 (44.4)	5 (83.3)
Cleaning of calving pen			
Flushing with water	15 (75)	6 (66.7)	1 (16.7)
Both*	4 (20)	2 (22.2)	5(83.3)
Replacement stock			
Buy in	0 (0)	0 (0)	0 (0)
Raise own stock	19 (95)	7 (77.8)	5 (83.3)
Both	1 (5)	2 (22.2)	1 (16.7)

Both* = Flushing with water and disinfection with detergent.

Table 3. Results of CT, RBT, iELISA and CFT of brucellosis by origin.

Origin	N	Card test	RBT	i-ELISA	CFT
		Number positive (%)	Number positive (%)	Number positive (%)	Number positive (%)
Bishoftu	384	4(1.04)	3(0.78)	3(0.78)	2(0.52)
Asella	186	12(6.45)	10(5.38)	12(6.45)	6(3.23)
Total	570	16(2.81)	13(2.28)	15(2.63)	8(1.40)

N=number of animal tested.

respondents did not use protective gloves while handling calving or aborting animals. These factors combined with the poor cleaning practice by the owners could pose a great risk of the spread of the disease to unaffected animals (Tolosa, 2004).

The present study revealed that the overall seroprevalence of bovine brucellosis was 1.40%. This finding is consistent with the earlier reports of Degefu et al. (2011) (1.38%) in agro pastoral areas of Jijjiga zone of Somali Regional State. Comparable to this finding, Asmare et al. (2007) reported (1.92%) in Sidama zone, southern Ethiopia, Tolosa et al. (2012) in Jimma area(1.97%) and Tesfaye et al. (2011) (1.5%) in Addis Ababa.

On the other hand, there were reports with a relatively higher seroprevalence of bovine brucellosis in other parts of the country (Hunduma and Regassa, 2009) (11.2%); (Megersa et al., 2012) (8.0%). However, most of the reports were from the extensively managed herds, where cattle from several owners mingle at grazing or watering

Table 4. Kappa test for agreement between CT, RBPT, i-ELISA and CFT.

Variables	CFT		Kappa Value	Kappa value interpretation	P-value
	-	+			
Card Test			0.660		0.001
Negative	554	0		Substantial agreement	
Positive	8	8			
Rose Bengal Test			0.758		0.001
Negative	557	0		Substantial agreement	
Positive	5	8			
i-ELISA			0.839		0.001
Negative	559	0		Almost perfect agreement	
Positive	3	8			

*Common interpretation of kappa: <0.2 = slight agreement, 0.2 to 0.4 = fair agreement, 0.4 to 0.6 = moderate agreement, 0.6 to 0.8 = substantial agreement, >0.8 = almost perfect agreement.

Table 5. Association of risk factors with *Brucella* seropositivity.

Variables	Level	Number tested	Number positive	χ^2 (*P*-value)
Origin	Bishoftu	384	2(0.52%)	6.63(0.010*)
	Asella	186	6(3.23%)	
Age	Young	131	0(0)	2.42(0.120)
	Adult	439	8(1.82%)	
Management	Extensive	8	0(0)	1.51(0.469)
	Semi-intensive	178	1(0.56%)	
	Intensive	384	7(1.82%)	
Herd size(farms)	Medium	163	0(0)	3.73(0.155)
	Small	85	1(1.18%)	
	Large	322	7(2.17%)	
Breed type	HF	94	0(0)	8.49(0.014*)
	Cross	468	7(1.50%)	
	Local	8	1(12.5%)	
Abortion history	No	524	0(0)	92.43(0.001**)
	Yes	46	8(17.39%)	
Separate parturition	No	160	0(0)	3.17 (0.075)
	Yes	410	8(1.95%)	
Abortion period	First trimester	19	0(0)	192.97(0.001**)
	Second trimester	5	0(0)	
	Not applicable	523	0(0)	
	Third trimester	23	8(34.78%)	
Location	Urban	100	0(0)	1.73(0.189)
	Peri-urban	470	8(1.70%)	
Parity	Not applicable	160	0(0)	4.66(0.097)
	Primiparous	119	1(0.84%)	
	Pluriparous	291	7(2.41%)	

*, statistically significant;**, statistically highly significant.

Table 6. Multivariable logistic regression analysis of putative risk factors with *Brucella* sero positivity.

Variables	Level	No. of animal tested	Prevalence (%)	Crude OR (95%CI)	Adjusted OR (95% CI)
Origin					
	Bishoftu	384	0.52	1	1
	Asella	186	3.23	6.37(1.27,31.85)	7.56(1.48,38.61)
Breed type					
	HF	94	0	1	1
	Cross	468	1.50	-	-
	Local	8	12.50	0.11(1.02,86.99)	0.19(0.53,52.45)

OR, Odds ratio; CI, Confidence interval; 1, Reference.

points. Hence, the low seroprevalence observed in this study could possibly be explained by the developed awareness and instituted informal culling practice, as well as proper disposal of afterbirths as it has been also suggested by Tesfaye et al. (2011) and/or the prevailing management differences between the intensive, semi-intensive and extensive production systems (McDermott and Arimi, 2002; Matope et al., 2011).

The present study revealed that origin of dairy cattle was significantly associated with brucellosis in dairy cattle (P<0.05) and the results showed higher individual animal seroprevalence in Asella (3.23%) when compared to Bishoftu (0.52%). The reasons for the variations in brucellosis seroprevalence among the study areas might be related to the difference in management practice performed in the two study sites. At the onset of the dairy schemes in Asella, farm owners purchased *Bos taurus* cattle from commercial farms, but the screening of these for brucellosis was not done due to limited availability of veterinary services, while the practice of screening for brucellosis was developed before purchasing cattle in most of Bishoftu dairy farms. According to the report of different studies, purchasing of cattle from commercial farms without screening for brucellosis increases the chances of contact with infected herds (Muma et al., 2007).

In addition, different studies revealed that the seroprevalence of brucellosis is lower in low land agro-climate which is unsuitable for survival of *Brucella* organisms than highland (Radostits et al., 1994). Therefore, the practice of purchasing cattle from commercial farms without screening for brucellosis together with other agro-ecological factors could partly explain the observed higher seroprevalence of dairy cattle brucellosis in Asella as compared to Bishoftu.

In the present study, the higher seroprevalence of dairy cattle brucellosis was observed in large herd size in the study sites. This study finding was in line with that of Asfaw (1998) in which he found significant association between *Brucella* seropositivity and large herd size. However, in contrary to this, Kebede et al. (2008) reported that the risk of *Brucella* seropositivity was independent of herd size in small holder farms from Wuchale Jida district of East Wollega zone of Ethiopia. Higher seropositivity in large herd size 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 or calving (Crawford et al., 1990).

Even though age was not significantly associated with *Brucella* seropositivity (P> 0.05), a sero prevalence of 1.82% was found among the adult age group whereas no *Brucella* seorpositivity was observed in the young age group of dairy cattle in the study sites. Several previous reports have indicated that higher seroprevalence of brucellosis in adult age group of cattle (Magona et al., 2009) similar to the findings of this study. This could be explained by sexual maturity and pregnancy due to the influence of sex hormones and placenta erythritol on the pathogenesis of brucellosis (Radostits et al., 2007).

The finding of this study revealed that higher seroprevalence of brucellosis was observed in intensive production systems. Similar to this finding, previous reports have indicated that higher seroprevalence of *Brucella* was found among dairy cattle in intensive production systems in highland areas of Ethiopia (Jergefa et al., 2009; Asmare et al., 2010). The higher seroprevalence of brucellosis in intensive production systems particularly in Asella could be explained by the fact that there is a greater chance of contact between infected and healthy animals in these systems, or between healthy animals and infectious materials, since most of farm owners' do not follow hygienic practices which was in agreement with the report of Jergefa et al. (2009).

The present study revealed that a history of previous abortions was significantly associated (P<0.001) with *Brucella* seropositivity. A seroprevalence of 17.39% was recorded for the occurrence of previous abortion in these study areas based on questionnaire survey. This finding was consistent with Tolosa (2004) who reported 17.6% in selected sites of Jimma Zones. This could be explained by the fact that abortions or stillbirths and retained

placentas are typical outcomes of brucellosis (Radostits et al., 1994; Swell and Brocklesby, 1990). In addition, in highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is a cardinal feature of the disease (Radostits et al., 2000). In contrary to this finding, a relatively lower seroprevalence (6.1%) was reported by Tesfaye (1996) in Mekele dairy cattle and 6.7% by Yayeh (2003) in North Gondar, Ethiopia.

There is still a controversy among different researchers on the issue of breed susceptibility to brucellosis. This study revealed that significant difference between breed type and *Brucella* sero positivity in dairy cattle. This might be due to the origin of the animal from the previously infected or exposed herds (Deselegn and Gangwar, 2011). In spite of the small sample size of local breed in the present study as potential limitation, a higher seroprevalence of 12.50% was found in local bred cattle in the study sites. In contrast to our finding, Yohannes et al. (2012) reported a seroprevalence of 1.7% in local bred cattle in Asella, Oromia Regional State, Ethiopia. In contrary to the present study, Jergefa et al. (2009) found that breed of cattle has significant effect on the serological prevalence of brucellosis and he reported higher seroprevalence of brucellosis in cross-bred than in indigenous (local) ones.

There was statistically significant association ($P<0.05$) between abortion period and sero positivity of brucellosis in the present study. This could be explained by the presence of higher seropositivity in cows in the last trimester which may be 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 5th month of gestation (Radostits et al., 2007; Coetzer and Tustin, 2004).

On the basis of parity, the difference observed in seroprevalence was statistically insignificant. Similar observations were made by Berehe et al. (2007). Though there is insignificant association between parity and brucellosis seropositivity, the higher seroprevalence was observed in pluriparous (2.41%) than primiparous cattle (0.84%) in the study areas. The higher seroprevalence of brucellosis in the pluriarous cattle of this study was in line with Asmare et al. (2013) who reported 2.5% in pluriparous dairy cattle and breeding farms with special emphasis on cross and exotic bred. With regard to serological test comparison, almost perfect agreement with significant association was observed between i-ELISA and CFT (K=0.839) whereas substantial agreement was found between CT (K=0.66) and RBPT (K=0.76) with CFT. This finding is inconsistent with Asfaw (1998) who reported a moderate agreement (K=0.44) between RBT and CFT. On the other hand, almost perfect agreement (K=0.98) was reported between RBPT and CFT by Abay (1999).

In conclusion, serological findings indicated that bovine brucellosis is an established disease in Asella and Bishoftu dairy farms. Higher seropositivity of *Brucella* was observed in Asella dairy farms compared to Bishoftu. Origin, local breeds, cattle with history of abortion, and the third trimester abortion period were the risk factors significantly associated with *Brucella* seropositivity in the study areas. Moreover, origin was statistically identified as the major potential risk factor for brucellosis to occur in relation to other factors. Therefore, more proactive measures should be taken to protect the cattle populations from *Brucella* infection and to reduce its economic impact to the dairy industry and the risk of zoonotic infection in exposed human population in the study areas.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

We acknowledge Dr. Fekadu Regassa, Dr. Ashenafi Feyissa and Dr. Endris Hansar for their encouragement, material support and information provision while collecting the sample by following abortion cases in dairy cattle farm. We also express our deepest gratitude and appreciation to National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia for providing us valuable support and assistance during sample processing in the laboratory. We are grateful to the staff members of the center, especially to Dr. Fasil Aklilu for his devoted cooperation in serology laboratory while conducting three different serological tests.

Abbreviations: AI, Artificial Insemination; **CFT,** Complement Fixation Test; **CI,** Confidence Interval; **CT,** Card Test; **HF,** Holestein Fresian; **iELISA,** Indirect Enzyme Linked Immuno Sorbent Assay Test; **KAP,** Knowledge, Attitude and Practices; **NAHDIC,** National Animal Health Diagnostic and Investigation Center; **OD,** Optical Density; **OR,** Odds Ratio; **RBPT,** Rose Bengal Plate Agglutination Test; **S-LPS,** Smooth Lipopolysaccharide; **USDA,** United States Department of Agriculture.

REFERENCES

Abay B (1999). Bovine brucellosis*:* A sero epidemiological study in selected farms and ranches in south eastern Ethiopia. DVM thesis*.* Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia*.*

ADARDO (2007). Ada'a District Agricultural and Rural Development Office.

Al Dahouk S, Nöckler K, Scholz HC, Tomaso H, Bogumil R, Neubauer H (2006). Immunoproteomic characterization of *Brucella abortus* 1119-3 preparations used for the serodiagnosis of *Brucella* infections. J. Immunol. Methods 309:34-47.

Alton G, Jones LM, Pietz DE (1975). Laboratory techniques in brucellosis. 2nd edition. WHO ,Geneva. pp. 23-124.

Asfaw Y (1998). The epidemiological study of bovine brucellosis in intra and peri-urban dairy production systems in and around Addis

Ababa, Ethiopia. Trop. Anim. Health Prod. 46:217-224.
Asmare K, Asfaw Y, Gelaye E, Ayelet G (2010). Brucellosis in extensive management system of zebu cattle in Sidama zone, Southern Ethiopia. Afr. J. Agric. Res. 5:257-263.
Asmare K, Prasad S, Asfaw Y, Gelaye E, Ayelet G, Zeleke A (2007). Seroprevalence of brucellosis in cattle and in high risk animal health professionals in Sidama Zone, Southern Ethiopia. Ethiop. Vet. J. 11:69-83.
Asmare K, Sibhat B, Molla W, Ayelet G, Shiferaw J, Martin AD, Skjerve E, Godfroid J (2013). The status of bovine brucellosis in Ethiopia with special emphasis on exotic and cross bred cattle in dairy and breeding farms. Acta Trop. 126:186-192.
Berehe G, Belihu K, Asfaw Y (2007).Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. Int. J. Appl. Res. Vet. Med. 5:65-71.
Bricker BJ (2002). PCR as a diagnostic tool for brucellosis. Vet. Microbiol. 90:435-446.
Coetzer JW, Tustin RC (2004). Infectious diseases of livestock, 3rd edition. South Africa: Oxford University press. pp. 34-39.
Conway P, McKenzie E (2007). Poultry coccidiosis and effect of coccidiosis diagnostic and testing procedures. 3rd edition. Blackwell Publishing, Ames, Iowa, P 5.
Crawford RP, Huber JD, Adams LG (1990). Epidemiology and surveillance. In: Animal brucellosis. Edited by Nielsen K, Duncan B. Orlando: CRC Press. pp. 131-151.
Degefu H, Mohamud M, Hailemelekot M, Yohannes M (2011). Seroprevalence of bovine brucellosis in agro-pastoral areas of Jijiga zone of Somali National Regional State, Eastern Ethiopia. Ethiop. Vet. J. 15:37-47.
Deselegn TB, Gangwar SK (2011). Seroprevalence study of bovine brucellosis in Assela government dairy farm of Oromia Regional State, Ethiopia. Int. J. Sci. Nat. 2:692-697.
Dohoo I, Martin SW, Stryhn H (2003). Veterinary epidemiologic research. Price Edward's AVC Inc. Charlotte town; Island, Canada; P 360.
Eshetu Y, Kassahun J, Abebe P, Beyene M, Zewdie B, Bekele A(2005). Seroprevalence study of brucellosis on dairy cattle in Addis Ababa, Ethiopia. Bull. Anim. Health Prod. Afr. 53:211-214.
Godfroid J, Nielsen K, Saegerman C (2010). Diagnosis of brucellosis in livestock and wildlife. Croat. Med. J. 51:296-305.
Godfroid J, Saegerman C, Wellemans V, Walravens K, Letesson JJ, Tibor A, Mc MA, Spencer S, Sanna M, Bakker D (2002). How to substantiate eradication of bovine brucellosis when a specific serological reaction occurs in the course of brucellosis testing. Vet. Microbiol. 90:461-477.
Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, Fretin D, Walravens K, Garin-Bastuji B, Letesson JJ(2005).From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet. Res. 36:313-326.
Hinić V, Brodard I, Thomann A, Holub M, Miserez R, Abril C (2009). IS*711*-based real-time PCR assay as a tool for detection of *Brucella* spp. in wild boars and comparison with bacterial isolation and serology. BMC Vet. Res. 5:22.
Hunduma D, Regassa C (2009). Seroprevalence study of bovine brucellos is in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. Am. Eurasian J. Agric. Environ. Sci. 6:508-512.
Ibrahim N, Belihu K, Lobago F, Bekana M (2010). Seroprevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-Western Ethiopia. Trop. Anim. Health Prod. 42:34-40.
Jergefa T, Kelay B, Bekana M, Teshale S, Gustafson H, Kindahl H (2009). Epidemiological study of bovine brucellosis in three agroecological areas of central Oromia, Ethiopia. Rev. Sci. Technol. Off. Int. Epiz. 28:933-943.
Kebede T, Ejeta G, Ameni G (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Rev. Med. Vet. 159:3-9.
Köppel C, Knopf L, Ryser MP, Miserez R, Thür B, Stärk KDC (2007). Serosurveillance for selected infectious disease agents in wildboars (*Sus scrofa*) and outdoor pigs in Switzerland. Eur. J. Wildl. Res. 53:212-220.
Land O'Lakes Inc. (2010). The next stage in dairy development for Ethiopia dairy value chains. In: End markets and food security cooperative agreement 663-A-00- 05-00431-00. Land O'Lakes Inc., Addis Ababa, Ethiopia.
Leuenberger R, Boujon P, Thür B, Miserez R, Garin-Bastuji B, Rüfenacht J, Stärk KD (2007). Prevalence of classical swine fever, Aujeszky's disease and brucellosis in a population of wild boar in Switzerland. Vet. Rec. 160:362-368.
Lopes LB, Nicolino R, Haddad JPA (2010). Brucellosis risk factors and prevalence: A review. Open Vet. Sci. J. 4:72-84.
Magona JW, Walubengo J, Galiwango T, Etoori A (2009). Seroprevalence and potential risk of bovine brucellosis in zero-grazing and pastoral dairy systems in Uganda. Trop. Anim. Health Prod. 41:1765-1771.
Matope G, Bhebhe E, John B, Muma JB, Oloya J, Madekurozwaa RL, Lund A, Skjerve E (2011). Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. Trop. Anim. Health Prod. 43:975-979.
McDermott JJ, Arimi SM (2002). Brucellosis in Sub-Saharan Africa: Epidemiology, control and impact. Vet. Microbiol. 90:111-134.
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.
Megersa M, Feyisa A, Wondimu A, Jibat T (2011). Herd composition and characteristics of dairy production in Bishoftu town, Ethiopia. J. Agric. Ext. Rural Dev. 3:113-117.
Muma JB, Samui KL, Oloya J, Munyeme M, Skjerve E (2007). Risk factors for brucellosis in indigenous cattle reared in livestock wildlife interface areas of Zambia. Prev. Vet. Med. 80:306-317.
Muñoz PM, Marín CM, Monreal D, González D, Garin-Bastuji B, Díaz R, Mainar-Jaime RC, Moriyón I, Blasco JM(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. Clin. Diagn. Lab. Immunol. 12:141-151.
Navarro E, Casao MA, Solera J (2004). Diagnosis of human brucellosis using PCR. Expert Rev. Mol. Diagn. 4:115-123.
Nicoletti P (2002). A short history of brucellosis. Vet. Microbiol. 90:5-9.
OIE (2004). Manual of the diagnostic tests and vaccines for terrestrial animals, 5th edition. Office International des Epizooties, Paris, France; pp. 409-438.
OIE (2009a). Bovine brucellosis. In: Manual of Diagnostic Tests and Va ccines for Terrestrial Animals. World Organization for Animal Health, Paris, France, http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.03_BOVINE_BRUCELL.pdf.
OIE (2009b). *Ovine epididymitis* (B.ovis).In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals". World Organization for Animal Health, Paris, France,http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.09_OVINE_EPID.pdf.
Poester P, Nielsen K, Samartino E (2010). Diagnosis of brucellosis. Open Vet. Sci. J. 4:46-60.
Radostits OM, Blood DC, Gay CC (1994). Brucellosis caused by *B. abortus*. In: Textbook of Veterinary Medicine. 9th edition. Bailliere Tyndall, London. pp. 786-802.
Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007). Veterinary Medicine. A text book of diseases of cattle, sheep, pigs, goats and horses. 10th edition. London: W.B., Saunders. pp. 963-985.
Radostits OM, Gay CC, Hinchcliff W (2000). Veterinary Medicine. Textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th edition, New York: W.B. Saunders Company Ltd. pp. 867-882.
Rahman MS, Nuruzzaman M, Ahasan MS, Sarker SS, Chakrabartty A, Nahar A, Uddin MJ, Sarker MAS, Akhter L (2012). Prevalence of brucellosis in pigs: The first report in Bangladesh. Bangladesh J. Vet. Med. 10:75-80.
Rashid M (1993). Reproductive wastage in cattle due to bovine brucellosis. In: Proceeding of the fourth livestock improvement conference. Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia. pp. 270-272.
Seifert HS (1996). Brucellosis. In: Tropical Animal Health., 2nd edition. Kluwer Academic Publishers group, Dordrecht. pp. 358-362.
Seleem MN, Boyle SM, Sriranganathan N (2010). Brucellosis: A re-emerging zoonosis. Vet. Microbiol. 140:392-398.
Swell MM, Brocklesby DW (1990). Handbook of Animal Diseases in the

Tropics. 4th edition. BaillièreTindall, London. pp. 1-41.
Tesfaye G (1996). Survey of major prepartum and postpartum reproductive problems of dairy cattle in Mekele and its environment. DVM Thesis. Faculty of Veterinary Medicine, Addis Ababa University, DebreZeit, Ethiopia.
Tesfaye G, Tsegaye W, Chanie M, Abinet F (2011). Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms. Trop. Anim. Health Prod. 43:1001-1005.
Tessaro SV, Forbes LB (2004). Experimental *B. abortus* infection in wolves. J. Wildl. Dis. 40:60-65.
Thrusfield M (2007). Sample size determination. In: Veterinary Epidemiology. 3rd edition. Blackwell Science Limited, Oxford, UK. pp. 185-189.
Tolosa T, Yohannes M, Mersha T, Degefu H, Woyesa M (2012). Bovine Brucellosis: Serological Survey in Guto-Gida District, East Wollega Zone, Ethiopia. Glob. Vet. 8:139-143.
Tolosa T (2004). Seroprevalence study of bovine brucellosis and its public health significance in selected sites of Jimma Zone, Western Ethiopia. MSc Thesis. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. P 9.
Tolosa T, Bezabih D, Regassa F (2010). Study on seroprevalence of bovine brucellosis, and abortion and associated risk factor. Bull. Anim. Health Prod. Afr. 58:236-247.
Yayeh T (2003). A survey of bovine brucellosis in selected areas of North Gondar Zone, Ethiopia. DVM Thesis. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
Yohannes M, Mersha T, Degefu H, Tolosa T, Woyesa M(2012). Bovine brucellosis: Serological survey in Guto-Gida district, East Wollega Zone, Ethiopia. Glob. Vet. 8:139-143.