

Research Article

Sero-Prevalence Study of Bovine Brucellosis and Its Risk Factors in Dairy Farms in and Around Adama Town, Oromia Regional State, Central Ethiopia

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Abstract

Background: Brucellosis is a contagious, zoonotic and economically important bacterial disease of worldwide distribution. Bovine brucellosis usually caused by *Brucella abortus* and occasionally by *B. melitensis* and *B. Suis*, is one of the economically and publically important diseases for dairymen among others.

Methods: A cross-sectional study was conducted in selected dairy farms in and around Adama town, central Ethiopia, from December, 2017 to May 2018 to determine sero-prevalence of *Brucella* infection in dairy cattle, identify associated risk factors for the seropositivity and to evaluate the knowledge and practice of the owners. A total of 384 dairy cattle from 42 herds were included in the study. Mixed design of purposive and random sampling method were used for sampling at each farm during blood collection, and risk factors accessed via interviewee of the farm owners, animal handlers and others. Serum samples collected were screened by Rose Bengal Plate test, and sera found positive were confirmed by complement fixation test.

Results: An overall individual animal and herd level sero-prevalence was estimated to be 1.04% (95% CI: 0.02, 2.62), 9.5% (95% CI: 0.26, 18.78) respectively. Univariable analysis indicated that abortion history and retained fetal membrane were the major risk factors significantly associated ($p < 0.05$) with bovine brucellosis seropositivity. The odds of having brucellosis increased by 18.6 times in cow with history of abortion compared to cow without the history, increased by 13.6 times in the animal suffered from retained placenta compared to cow with no retained placenta. Fisher's exact test statistics indicated that herd size and culling reason were significantly associated with bovine brucellosis ($p < 0.05$) at herd level. The result of questionnaire survey revealed that the overall awareness about brucellosis (16.7%) and its zoonotic importance (14.28%) among owners was considerably low.

Conclusion: the overall prevalence obtained in the area is low (1.04%); however, there is probable risk of spread of the disease in the unaffected cattle population, and personnel exposure is high, since there is no awareness about the disease and precaution measures taken in the areas. Thus, the need for implementing feasible and sustainable control measures, and awareness creation about zoonotic brucellosis in the public at the study site were recommended.

ABBREVIATIONS

AI: Artificial Insemination; CFT: Complement Fixation Test; CI: Confidence Interval; CSA: Central Statistical Agency; KAP: Knowledge, Attitude and Practice; NMSA: National Meteorology Service Agency; NVI: National Veterinary Institute; OIE: Office of International Des Epistotes; OR: Odds Ratio; PAHO: Pan American Health Organization; RPBT: Rosbengal Plate Test; RFM: Retained Fetal Membrane; SRBC: Sheep Red Blood Cells; WHO: World Health Organization

BACKGROUND

Brucellosis is highly contagious, public and economically important bacterial disease of animals worldwide and it is considered as one of the most widespread zoonoses in the world [1,2]. The disease has wide host range including cattle, swine, sheep, goats, camels and dogs. Besides to these, it also infects other ruminants, marine mammals and humans [3]. It has been virtually eliminated from the majority of the developed countries [4], but it is still endemic in Africa, the Middle East, Central and Southeast Asia, Central and South America and in most of the

Southern European countries [5]. Despite of its endemic nature in many developing countries, brucellosis remains under diagnosed and under-reported [6,7]. Since brucellosis is an important cause of abortion especially in first calf heifers, the disease can also cause important economic losses in developing countries; this actually implies that the public health and economic impact of brucellosis remains of concern in many, particularly developing countries [8].

Bovine brucellosis usually caused by *Brucella abortus* and occasionally by *B. melitensis* and *B. suis*, all of which are Gram-negative, facultative, intracellular coccobacillary bacteria [9,10]. The disease is characterized by trimester abortion at first gestation, weak calves, still birth and is mainly caused by biovars (mainly biotype -1) of *B. abortus*, infertility and reduced milk production [9], whereas bulls can develop orchitis and epididymitis; these consequences implied that the economic importance of bovine brucellosis is of paramount [11,12].

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 [13]. Whereas the most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth; aborted fetuses, uterine discharges and new born calves, which contains large doses of infectious organisms and constitute a very important source of infection [14]. The disease is an occupational risk for farmers, veterinary surgeons and workers within the meat industry, known as risky group. Thus, prevention and control of the disease in animal is a key in affirming safety of public health [15].

Hence, brucellosis easily perpetuate and remains widespread in the animal population and has great impacts both on animal and human health, as well as economic consequences, especially in developing countries where livestock production plays the greatest economic role [16]. The disease could seriously impair socio-economic development for livestock owners, which represent a vulnerable sector in rural populations in general and pastoral societies in particular [17].

There have been many reports of the brucellosis in animals and humans form different areas in sub-Saharan Africa, including Ethiopia. However, many of them relied/adhered on the Rose Bengal plate test and few confirmatory tests [18]. The climatic and agroecological diversities of Ethiopia may allow a wide range of livestock production systems and therefore, different management systems, multiple livestock species per holding, stock density and social organizations to handle livestock may account for the widespread risk factors for maintenance and transmission of bovine brucellosis [19]. Although the livestock sector in Ethiopia has a significant contribution to the national economy, productivity (meat and milk) per animal is very low, majorly due to technical constraints, disease like brucellosis [20].

Brucellosis was first reported in Ethiopia in the 1970s [21,22] since then, the disease has been noted as one of the important livestock diseases in the country [23,24]. A large number of studies on bovine have been reporting brucellosis seroprevalence ranging from 1.1% to 22.6% in intensive management

systems [25-27] and 0.1% -15.2% in extensive management system [19,28-30].

Although sero-prevalence of bovine brucellosis had been established in different parts of the country, still there is no online information on status of the disease in and around Adama town. Further assessment of the status of the disease, both the knowledge of the prevalence and the associated risk factors, and understanding the awareness and practice of the community has paramount importance that can be used for research, control and prevention of brucellosis in Ethiopia as general and specifically in the study site. Hence, the current study was undertaken to know the status of the disease and understand the awareness of the community toward this disease in the study area. Therefore, based on these key statements and the study gap, the following were the objectives of the current study to, (i) determine seroprevalence of bovine brucellosis, (ii) assess the risk factors associated with bovine brucellosis and, (ii) understand the knowledge (awareness), attitude and practice of the community towards zoonotic bovine brucellosis in the study area.

METHODS

Study Area and design

This cross-sectional sero-prevalence study and questionnaire survey was conducted from December, 2017 to May, 2018 in and around Adama two, Oromia regional state, central Ethiopia, which is located 100 km South-east of Addis Ababa, at an altitude of 1,650 meters above sea level. Its annual temperature ranges from 13.9°C to 29°C and the mean annual rainfall is 1024 mm [31]. The livestock population of the area in 2004 estimated to be 70,622 cattle, 36,142 sheep, 42,968 goats and 2,193 equine [32,33].

Geographically, the study areas cover latitude and longitude ranges of 8° 24'305" to 8° 34' 662" North and 39° 14' 720" to 39° 20' 107" East, respectively. The district having the study area found is briefly sketched as illustrated in (Figure 1).

Study Populations

The target populations were dairy cattle comprised of

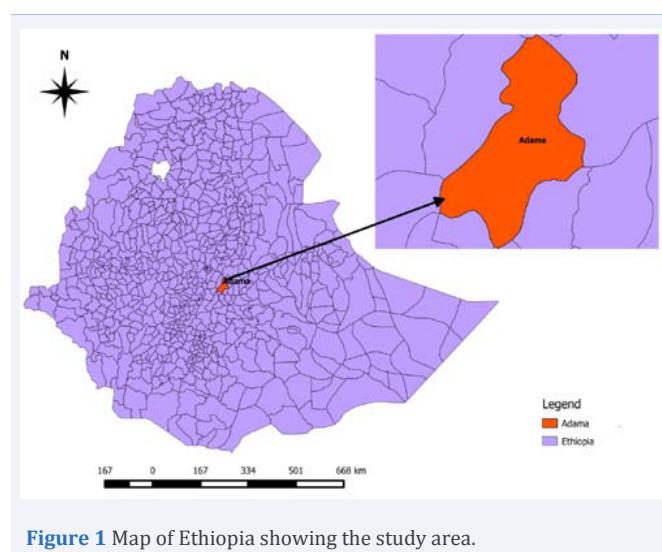


Figure 1 Map of Ethiopia showing the study area.

Holstein Frisian, crossbreed and local cows. The cattle under study were categorized into two age groups young (6-17 months) and adult (> 17 months) according to [34].

Sampling types and Sample Size Determination

A list of 67 dairy farms was prepared in collaboration with the districts' livestock health department and the town livestock and fisheries office to construct a sampling frame of the dairy farms. The farms were classified according to herd size and level of production into smallholder farm (1-15 animals), medium farms (16 to 30 animals) and large farms with more than 30 animals according to [19]. The included farms were selected randomly from sampling frame. Then, the study unit at each farm was selected based on non-probability sampling which means purposively (all cows with history of abortion sampled) followed by simple random sampling until the required sample size was reached.

The sample size was determined according to the formula described by [35].

Accordingly, a total of 384 dairy cattle were considered for this study from 42 farms in the study area.

Blood sample collection, transportation and storage

About 5-7 ml of blood samples were collected aseptically, using plain vacutainer tubes, from jugular vein of individual animals selected. The collected blood samples were kept overnight to allow clotting in slant position at room temperature at Adama Woreda veterinary clinic. The sera were carefully decanted into 1.8 ml labeled cryovials without mixing with the clotted blood. The harvested sera were then taken to National Veterinary Institute (NVI), serology laboratory by using icebox, and stored at -20°C until further processing was held.

Questionnaire survey

Parallel to collection of serum samples, a pretested semi-structured questionnaire survey was administered to willing respondents whose dairy cattle were included in the study through interviewee by local language. The questionnaire was focused on demographic characteristic of the owners such as age, gender and educational level, and herd-level covariates such as herd size, categorized as small, medium, and large, the presence of separated maternity pen, source of replacement stock (from own, market), abortion history, history of retained fetal membrane, selling of dairy breed cow, stray animals and visitors control, were recorded. Farm management methods were ascertained in details. Moreover awareness (knowledge), attitude and practice of the respondent about zoonotic diseases with great emphasis on brucellosis were assessed at the study area.

Study methodology

Rose Bengal Plate Test: the sera samples were first screened using Rose Bengal Plate Test (RBPT) using RBPT Brucella antigen strain 99 (from Lillidale Diagnostics, pig Oak Farm, Holt, Wimborne, Dorset, BH21 7DG, United Kingdom) according to [36] and OIE [37] procedures. Complement Fixation Test: RBPT positive sera samples were further tested using complement fixation test (CFT) as indicated by [38].

Data Analysis

Data were analyzed using standard software programs (STATA version 13.0 for windows (Stata Corp, College Station, TX). The sero-prevalence at individual animal level was calculated by dividing the number positive animals by the total number of animals tested. Herd prevalence was calculated by dividing the number of herds with at least one reactor by the number of all herds tested. Fisher's exact test was utilized to measure the association between the putative risk factors and the seropositivity. Odds ratio (OR) was utilized to measure the degree of association between risk factors and sero-prevalence of bovine brucellosis. For statistical inference, p-value < 0.05 (at 5% level of significance) was considered as statistically significant.

RESULTS

Sero-prevalence of Anti-Brucella Antibodies at Animal and Herd level

In the current study, among 384 dairy cattle tested for bovine brucellosis, 12 (3.12%) and 4 (1.04%) were tested positive for Brucella antibody by RBPT and CFT, respectively. Thus, since CFT is the confirmative test used, the animal level sero-prevalence of bovine brucellosis was 1.04% (95% CI: 0.02- 2.62%). The herd level sero-prevalence was 16.67% and 9.52% (95%CI: 0.26, 18.78) by RBPT and CFT, respectively.

Comparison of Serological Tests for Bovine Brucellosis

The kappa statistics showed that there was moderate agreement between RBPT and CFT, taking CFT as gold-standard test (Table 1). The kappa statistic was used to test interrater reliability, and in this low value of kappa (0.49) was obtained.

The Risk Factors for Brucella Infection at Animal level

According to the result obtained from the present study, 0.0% and 1.27% of sero-prevalence were recorded in animals of age less than 17 months (young) and older than 17 months (adult) respectively. Fisher's exact test showed that the presence of history of abortion and retained fetal membrane were statistically significantly associated with the seropositivity of bovine brucellosis ($P < 0.05$). Herd size, breed type, parity and management system were insignificantly correlated ($p > 0.05$) with the seropositivity of Brucella infection at individual animal level (Table 2).

Table 3 shows the result of animal level firth logistic regression analysis of the associated risk factors and their magnitude for Brucella infection seropositivity. It revealed that the presence of abortion history and retained fetal membrane were significantly associated with the animal level seropositivity of bovine brucellosis ($p < 0.05$). Risk of infection is 18.6 times higher (OR=18.6) in individuals that had history of abortion than those animals didn't have the history. Likewise animals with history of retained fetal membrane (OR=13.6) were suggested to be 13.6 times at higher risk of being infected with Brucella infection.

Result of Questionnaire Survey

Socio-demographic characteristics of the respondents interviewed.

Table 1: Kappa test for agreement between RBPT and CFT for bovine brucellosis.

		CFT		Kappa value	Kappa interpretation	p-value
		+ve	-ve			
RBPT	Positive	4	8	0.49	Moderately agree	0
	Negative	0	372			
Total		4	380			

Interpretation of kappa statistic: > 0.8-1: excellent agreement; > 0.6-0.8: substantial agreement; >0.4-0.6: moderate agreement; >0.2-0.4: fair agreement; > 0-0.2: slight agreement; 0: poor agreement; <0: disagreement (source: (77)).

Table 2: The results of animal level sero-prevalence of bovine brucellosis in association with putative risk factors by using Fisher's exact test.

Variable	Number of animal studied	Number tested positive	Prevalence%(95% CI#)	p-value
All animals	384	4	1.04(0.02,2.62)	
Age				
Young	69	0	0(-)	1
Adult	315	4	1.27 (0.03,3.21)	
Breed type				
HF	87	3	3.45(0.71, 974)	
CR	240	1	0.42(0.01, 2.29)	0.06
Local	57	0	0(-)	
Herd size				
Small	292	0	0(-)	0.052
Medium	73	3	4.11(0.85, 11.54)	
Large	19	1	0.53(0.13, 26.02)	
Management system				
Intensive				
Semi-intensive	179	1	0.56(0.01, 3.07)	0.348
Extensive	83	2	2.41(0.29, 8.43)	
	122	1	0.82(0.02, 4.48)	
Parity				
Non parturated	67	0	0(-)	1
Primiparous	97	1	1.03(0.02, 5.61)	
Pluriparous	220	3	1.36(0.28, 3.93)	
Abortion history				
Yes	21	4	19.05(5.44, 41.90)	0.000*
No	363	0	0(-)	
Pregnancy status				
Pregnant	134	3	2.26(0.4, 6.40)	0.122
Non-pregnant	250	1	0.40(0, 2.20)	
RFM				
Yes	26	4	15.38(4.35, 34.86)	0.000*
No	358	0	0(-)	

implied confidence interval; * statistically significant at $p < 0.05$; RFM= Retained Fetal Membrane; HF=Holstein Friesian, CR=Cross breed.

Among those 42 farmers interviewed during field survey, the age of respondents range between 21-60 years. About 35.7% of respondents' age lies within the productive age as well as majority (20%) of the positive test herd was owned by this age group that suggested as a great risk for them (Figure 2). Majority (59.5%) of the respondents' educational level surveyed in this study was of primary and secondary school. However none these socio-demographic factors included into the present study was show significant association with the sero-prevalence of bovine brucellosis ($P > 0.05$).

Farm Characteristics and activities in relation with herds level seropositivity

Risk factors associated with herd level sero-prevalence of bovine brucellosis were presented in (Table 4). Out of 42 farms assessed by questionnaire survey, about 47.6% ($n=20$) of respondents reared cross breed (local-Holstein Friesian), whereas 33.3% ($n=14$) and 19.1% ($n=8$) reared Holstein Friesian and local breeds respectively. In the study area, majority (71.4%) of farms uses Artificial Insemination (AI), exceptionally 4.7% use bulls and 23.8% attempts both options for breeding purpose.

Table 3: Magnitude of association of potential risk factors with the Brucella infection in dairy cow by firth logistic regression.

Variables	Coefficient	SE	95% CI	OR(95% CI)	P-value
Breed type					
HF	0.19	1.05	(-2.92,2.88)	0.82(0.53,17.86)	0.879
CR					
Local	0.67	1.33	(-4.17,4.23)	1.95(1.10, 2.03)	0.700
History of abortion					
Yes	2.92	1.09	(0.46,7.01)	18.6(4.59,111.2)	0.020* ^a
No					
History of RFM					
Yes					
No	2.61	1.12	(-1.73,6.80)	13.6(1.77, 90.25)	0.018* ^a
Management system					
Intensive	0.57	1.25	(-3.85,2.46)	0.56(0.21,1.67)	0.691
Sem-intensive	1.05	1.16	(0.47,5.102)	2.87(0.16,11.8)	0.475
Extensive					
Pregnancy status					
Pregnant	1.56	0.89	(-0.61,4.56)	4.76(0.54, 9.58)	0.157
Non-pregnant					

*^a indicates significant at $p < 0.05$; CI: Confidence Interval; CR: Cross Breed (HF-local); HF: Holstein-Fresian; RFM: Retained Fetal Membrane; OR: Odds Ratio; SE: Standard Error; Yes: Presence of The Factor; No: Absence of the Factors.

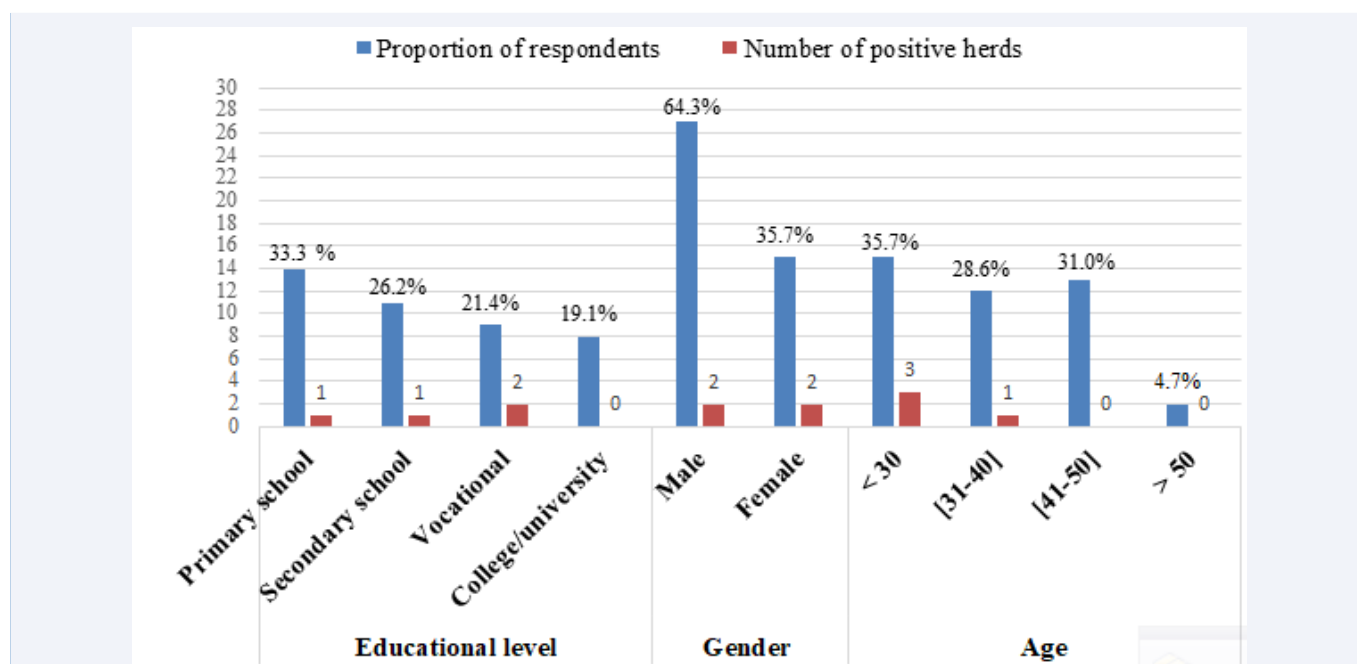


Figure 2 Graphical illustration of age, sex and educational level of respondent with herds tested positive.

As to the respondent, reproductive problems accounts more for culling of dairy cow (54.8%) and relatively less culled due to non reproductive problems (45.2%). Majority of hygienic status of the farms were poor (61.9%), only handful of the farmers keep the farm in good hygiene (11.9%) whereas 26.2% were at fair level. Nevertheless, the hygiene status of farm was statistically not associated with brucellosis seropositivity ($p > 0.05$) at herd level.

The occurrence of abortion in dairy herds were presented by 28.6% ($n=12$) respondents and similarly about 33.3% ($n=14$) of the respondent were replied as their farm encountered RFM during normal parturition and/or abortion. The presence of abortion history and RFM were statistically show significant association with herd level seropositivity to brucellosis ($P < 0.05$). Moreover, herd size and culling reason ($P < 0.05$) were significantly associated with herd level brucellosis seropositivity.

However, whether activity of controlling stray animals and visitors present or not, absence or presence of maternity pen, and selling dairy cattle were not associated with herd level seropositivity to brucellosis ($P > 0.05$) as depicted in (Table 4).

Knowledge, attitude and practice (KAP) of farm owners towards zoonotic infections with great focus on Brucellosis.

The questionnaire survey has provided information regarding the knowledge, attitude and practice of livestock keepers about

zoonotic disease, with more emphasis on brucellosis in the study area. On basis of the questionnaire survey attributed, the majority (83.3%) of the 42 respondents had never heard of brucellosis. Of those who had heard of the disease, 14.28% ($n = 3$), 15.79% ($n=3$) and 50% ($n=1$) were from small, medium and large herd size keeper, respectively. Almost all interviewees who had heard of brucellosis knew that human could become infected (Table 5).

Concerning the attitude of the community at the study site, the majority, 88.1 % ($n=37$) of the respondents wanted more information about brucellosis whereas 11.9 % ($n=5$) claimed that they did not need more information (Table 6).

On bases of farmers practice, it was found that about 80.95% of the farm owners of the study area were disposes aborted materials to open dump, while 11.91 % exercise burying of aborted materials, and even some (7.14%) feed to dogs (Table 6).

DISCUSSION

The present study showed that the overall individual animal level sero-prevalence of bovine brucellosis was 1.04% as determined based on the recommended confirmative test, CFT. This finding is in agreement with the earlier report of 1% [39] in the Benishangul Gumuz region of north-western Ethiopia, and 1% [40] in Nairobi, Kenya. It is comparable with other previous reports from different part of Ethiopia; 1.38% [41] in Jijjiga zone of Somalia regional state, 1.4% [13] in Bishoftu and Asella, central Ethiopia, 1.5% [27] in Addis Ababa, 1.66% [28] in Sidama Zone, Southern Ethiopia, 1.49 % [42] in Tigray region, and 1.4 % [43] in Southeastern pastoral livestock of the country.

On the other hand, there were reports with a relatively higher sero-prevalence rate of bovine brucellosis in other parts of the country; 11.2% [30] in pastoral and agro pastoral areas of East Showa Zone, 3.5% (20) in Southern and Eastern Ethiopia, Oromia region, 3.1% [34] in Jimma zone of Oromia region, 4.9% [44] in Western Tigray, Northern part of the country, 8.0% [45] pastoral region of the country; 2.9% [46] in three agro ecological areas of central Oromia, 3.19% [27] in the extensive cattle production system of Tigray region, and 4.3 % [47] in Adami Tulu, central Ethiopia. However, most of these reports were from the area where herds were managed under extensive system, where cattle from different owners were mingled at communal grazing and watering points. Hence, the low prevalence observed in the present serological investigation could possibly be due the using of AI services, culling of infected animals and, and the prevailing management systems differences among intensive, semi-intensive and extensive production system [5,48]. Similarly, relatively higher sero-prevalence were reported in other African countries; 24.5% [49] from Sudan; 24.0% [50] from Nigeria, 5.5% [51] from Zimbabwe.

On the other hand lower sero-prevalence were recorded in certain part of the country: 0.49% [52] from Western Shewa; 0.4% [24] from urban dairy farms of Northern Ethiopia and Sebeta while [53] from Nazareth, Gondar, and Mekele (possibly due to low sample size in their study); [54] from central Ethiopia were unable to find even a single positive reactor in intensive dairying farms.

It is well known that sexually mature cows are more

Table 4: The result of herd level putative risk factors association with bovine brucellosis ($n=42$) by using Fisher's exact test.

Type of risk factors	Number of herd tested ($n=42$) (%)	Number of herd positive to CFT (%)	p-value
Herd size			
Small	21(50)	0(0.0)	0.033*
Medium	19(45.2)	3(15.8)	
Large	2(4.8)	1(50)	
Breed types			
HF	14(33.3)	3(21.4)	0.231
HF cross	20(47.6)	1(5)	
Local	8(19.1)	0(0.0)	
Reason of culling			
Reproductive problem	23(54.8)	4(17.4)	0.023
Non-reproductive problem	19(45.2)	0(0.0)	
Breeding system			
AI	30 (71.4)	2(6.6)	0.081
Bulls	2(4.7)	0(0.0)	
Both**	10(23.8)	2(20)	
Source of replacement stock			
Raise from own farm	15(35.7)	3(20)	0.384
From market	19(45.2)	1(5.3)	
Both**	8(19.1)	0(0.0)	
Selling of dairy breeding cattle			
Yes	11(26.2)	2(18.2)	0.277
No	31(73.8)	2(6.4)	
Farm hygiene			
Poor	26(61.9)	2(7.8)	0.744
Fair	11(26.2)	1(9.1)	
Good	5(11.9)	1(20)	
Stray animal control			
Yes	20(47.6)	1(5)	0.608
No	22(52.4)	3(13.6)	
Visitor control			
Yes	18(42.9)	1(5.5)	0.623
No	24(57.1)	3(12.5)	
History of abortion			
Yes	12(28.6)	4(33.3)	0.004*
No	30(71.4)	0(0.0)	
RFM			
Yes	14(33.3)	4(28.6)	0.009*
No	28(66.7)	0(0.0)	
Calving pen after parturition			
Flushing with water	28(66.7)	1(3.8)	0.053
Disinfecting	12(28.6)	2(16.7)	
Both**	2(4.8)	1(50)	

Both** implies the two given alternatives; *indicates significant at $p < 0.05$; RFM: Retained Fetal Membrane.

Table 5: Knowledge and understanding about brucellosis among small, medium and large herd sized owners in the study areas (n=42).

Variables	Proportion of respondents(n)				
	Herd size				
	Small (n=21)	n(%)	Medium(n=19)	Large(n=2)	Total(n=42)
			n (%)	n (%)	n (%)
Knowledge of the owners					
Awareness about brucellosis					
Yes	3(14.28)		3(15.79)	1(50)	7(16.7)
No	18(85.7)		16(84.2)	1(50)	35(83.3)
Whether human infected with brucellosis or not					
Yes	3(14.28)		2(10.53)	1(50)	6(14.28)
No	18(85.72)		17(89.47)	1(50)	36(85.72)
About zoonotic disease transmitted through milk consumption					
Yes	10(47.62)		11(57.89)	1(50)	22(52.38)
No	11(52.38)		8(42.11)	1(50)	20(47.62)
About disease transmitted during handling of infected animal and its product					
Yes	11(52.38)		7(36.84)	2(100)	20(47.62)
No	10(47.62)		12(63.16)	0(0.0)	22(52.38)
About disease transmitted during delivery assist					
Yes	10(47.62)		8(42.11)	2(100)	20(47.62)
No	11(52.38)		11(57.89)	0(0.0)	22(52.38)

N= number; Yes means presence of the factor; No means absence of the factor.

Table 6: Attitude, and practice posing risk for brucellosis transmission within and between herds responded by dairy cattle owners at the study area (n=42).

Variables	Proportion of respondents(n)			
	Herd size			
	Small (n=21)	Medium(n=19)	Large(n=2)	Total(n=42)
	n(%)	n (%)	n (%)	n (%)
Attitude of the community				
Need more information on brucellosis				
Yes	19(90.48)	16(84.21)	2(100)	37(88.1)
No	2(9.52)	3(15.78)	0(0.0)	5(11.9)
Practice of the dairy cattle owners				
Brucella infected animal				
Test and slaughter	2(10.53)	1(5.27)	0(0.0)	3(7.14)
Sell	0(0.0)	16(84.21)	2(100)	18(42.86)
Culling	19(90.47)	2(10.52)	0(0.0)	21(50.00)
Handling of aborted fetus				
Burying	3(14.29)	2(10.52)	0(0.0)	5(11.91)
Open dump	17(80.95)	15(78.96)	2(100)	34(80.95)
Feed to dog	1(4.76)	2(10.52)	0(0.0)	3(7.14)
Fate of frequently aborted cow				
Selling	15(71.42)	15(78.95)	2(10.0)	32(76.00)
Retaining	6(28.58)	3(15.79)	0(0.0)	9(21.42)
Test and slaughter	0(0.0)	1(5.26)	0(0.0)	1(2.38)
Visitor control				
Yes	12(57.14)	6(31.57)	0(0.0)	18(42.86)
No	9(42.86)	13(68.43)	2(100)	24(57.14)
Calving pen after parturition				
Flushing with water	15(71.43)	11(57.90)	2(100)	28(66.67)
Disinfecting	6(28.57)	6(31.57)	0(0.0)	12(28.57)
Both**	0(0.0)	2(10.53)	0(0.0)	2(4.76)

N= number; Yes means presence of the factor; No means absence of the factors.

susceptible to *Brucella abortus* infection, which could be explained by the fact that susceptibility increased during sexual maturity and pregnancy due the influence of sex hormones and placental erythritol on the pathogenesis of brucellosis [55]. Accordingly a higher sero-prevalence (1.27%) in older age category (greater than 2 years) and seronegativity in younger age category (6 months - 2 years) was found in present study. This finding was in consistent with report of [24,56,57].

The existence of previous history of abortion and retained fetal membrane was, as expected, significantly associated with animal level seropositivity (Table 2) in the present study. This could be explained by the fact that abortions or /and retained placenta are typical outcomes of brucellosis [58,59] Other studies have also shown a significant association between seropositivity, and history of abortion and RFM [24,60,39,61] Similarly, a number of studies in different African countries also show that individual animal brucellosis sero-prevalence correlates with the presence of abortions [1,6,62,63].

With regard to serological test comparison, moderate agreement with significant association was observed between RBPT and CFT ($k=0.49$). This finding is agreed with [23] who reported a moderate agreement ($k=0.44$) between the tests. However, it was discrepant with finding of [64] ($k=0.758$) who obtained substantial agreement between the tests. The variation might be due to lack of repeatability of the test between laboratory and technician.

In the recent study, the overall sero-prevalence of bovine brucellosis recorded at herd level was 9.5 % (4/42), and the finding is briefly demonstrated by (Figure 2). According to different authors varied range of herd level sero-prevalence (between 2.9 and 45.9%) were reported in different parts of Ethiopia. Of these, the current result was relatively comparable with findings of [28] 10.6% in Sidama zone, Southern part of the county; [65] 11% in Southeastern part; [66] 10.2%; [42] 11.2% in pastoral and agro pastoral areas of East Showa zone.

The recent finding was higher in contrast to the findings of [60] 2.9% in Jimma zone; [39] 4.9% in Benishangul Gumuz region of Northwestern Ethiopia; [44] 7.7 % in Northern Tigray). On the other hand there was also higher report of herd level sero-prevalence: [46] 13.6% in three agro- ecological areas of central Oromia; [24] 15% in Jimma zone; [67] 24.1% in Western part of Tigray; [29] 42.3% in Tigray region; [68] 45.9% in Wuchale-Jida districts, central Ethiopia. The prevailed discrepancy might be due to variation in cattle production and management systems [69].

Different putative risk factors on sero-prevalence at herd level were studied at the farms through questionnaire survey that presented in (Table 4). Herd level sero-prevalence of brucellosis was higher in herds that had history of abortion (33.3%) and RFM (28.6%), compared to those herds without the history of abortion or retained placenta, seronegative. These reproductive disorders showed statistically significant association with occurrence of bovine brucellosis at herd level. This result was in consistent with the findings of researchers who had reported significant association of with reproductive disorders like abortion, RFM and still birth [70,71] On the other hand, the result was contrary

to the finding of [68] where reproductive disorders were not showed significant association with the occurrence of herd level bovine brucellosis. This might be due to presence of non-specific causes of abortion and/ or retained fetal membrane, or possibly information bias from record- keeping by the herd owners.

Reason for culling and herd size were factors revealed to have a significant effect on herd level sero-prevalence. All positive herds were from those farms did culling majorly due to reproductive problem. This might be supported by the fact that reproductive problem is typical outcome of brucellosis infection [44] In fact, 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 [72] But, in the study the higher sero-prevalence of the disease occur in medium herds probable was due a large sample size taken from medium herd size relative to large herd size

Factors related to socio-demographic characteristics including age, sex and education level of respondents were studied to see their effect on herd prevalence (Figure 2). Although education level have been recognized as protective factors [73], in present study herd level sero-prevalence was not showed significant variation across level of education of the farm owners ($p > 0.05$). In support of the recent result, some authors concluded that having higher level of education will not ensure protection against brucellosis but having the right information (regardless of education level) about brucellosis as well as taking heed to all precautionary measures against brucellosis is very important to guard against the infection [74].

The current study revealed that the knowledge and perception of brucellosis in the assessed farms is limited. Majority (83.3%) of the respondent had never heard of brucellosis (Table 5). The finding was in contrary with that of [13] whose study shows a high awareness (77%) of brucellosis among participants in Bishoftu and Asella, Ethiopia. Lower rate of awareness is of great risk for these risky groups. Thus, poor knowledge and high-risk behaviours strengthens the logic for including health education as part of control programmes.

Concerning the farmers/owners practice, (66.67%) of farmers flush the parturition vicinity with water whereas only 28.6 % disinfect with detergents and the rest perform both. More than half (57.1%) of the farms were open for visitors, such malpractice exposes the farms to infection as the fact is visitor play a role in reducing the spread of infection [75,76]. Majority (90.5%) of the farmers need more information about brucellosis. The positive attitudes of the farmers towards learning more, sets good foundation for including information campaigns for brucellosis as part of a future control programme in the study area.

CONCLUSION

The present study revealed that the overall sero-prevalence of bovine brucellosis in dairy farms of Adama town and its surroundings, was relatively low at individual animal and low to moderately high prevalent at herd level. There was strong association between abortion and retained fetal membrane and seropositivity for bovine brucellosis in the study area. Moreover, the finding demonstrated a poor understanding of brucellosis

among communities and a high level of risky practice being undertaken on farms. Therefore, feasible and effective prevention and control measures should be undertaken to protect the cattle populations from *Brucella* infection in order to reduce its economic impact to the dairy industry and the risk of zoonoses in risky human population, awareness creation among the people about the impact of the diseases, modes of transmission, risk factors and methods of prevention of the diseases should be undertaken in the study area, and further study to establish the disease situation in other domestic animals and humans in the study area, in addition to cattle, is recommended and this will also provide guidance on the control measures that need to be implemented.

Furthermore, usage of personal protective equipment whenever they came in contact with their herds is specifically required for farmers and cow keepers.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was granted an ethical approval from the College of Veterinary Medicine and Agriculture Institutional Research and Review Committee. Human samples were collected following prior explanation of the objectives of the study to the farm workers, and sampled based on their consent. Confidentiality of the participants was maintained using unique code.

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