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Sero-Prevalence and Associated Risk Factors of Bovine Brucellosis in Selected Dairy Farms in Bishoftu Town, Oromia, Ethiopia

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Abstract: A cross-sectional study was carried out from December 2015 to April 2016 to determine sero-prevalence and associated risk factors of bovine brucellosis in dairy farms in Bishoftu town. A total of 400 blood samples were collected from cross breed dairy cattle and the Rose Bengal plate test (RBPT) was used as a screening test. Those serum samples reacting positively to RBPT were subjected to the complement fixation test (CFT) for confirmation. Accordingly, RBPT detected 15 of 400(3.75%) of the samples as brucellosis positive. The positive sera when further retested using CFT, 12 out of the 15 RBPT positive sera were confirmed to be positive. The prevalence of brucellosis based on CFT in the study area was 3% and all positive sera were from female cattle. A Chi-square computed statistical analysis indicated that abortion history (χ^2=10.67; P<0.001), abortion period (χ^2=29.24; P<0.000), retained fetal membrane (χ^2=14.25;P< 0.00) and parity (χ^2=5.69; P<0.05) were the major risk factors for *Brucella* infection in the study area. In addition, result of the questionnaire survey revealed that percentage of 16.14% history of abortion and 17.72% history of retained fetal membranes. A total of 66 cattle attendants and farm owners were interviewed and 30.3% were found to have no knowledge of brucellosis, only 18.18% were wear protective gloves during handling of aborted material and 34.85% responded that they consume raw milk. Therefore, in order to control spread of brucellosis implementation of better management practices like isolation of aborted animals, provision of separate parturition pen, proper disposal of aborted fetuses and fetal membranes should be practiced.

Key words: Bishoftu · Bovine brucellosis · Dairy cattle · Risk factors · Sero-prevalence

INTRODUCTION

Ethiopia has huge livestock population, yet, they were affected by different diseases which greatly affect the economy and public health within the country. Among these diseases brucellosis is one of the major diseases affecting the dairy industry responsible for low productivity [1]. It is an economically important disease of livestock causing reproductive wastage through infertility, delayed heat, loss of calves, reduced meat and milk production, culling, death from secondary infection from abortion and economic losses from international trade bans [2].

Brucellosis is the most widely spread zoonoses disease in the world [3]. It is an infectious bacterial disease caused by genus *Brucella* which is Gram-negative; intracellular coccobacillary comprised of species based upon biochemical features and their correlation with preferred host species [4]. Currently ten species are recognized including the better known six classical species comprised of *B. abortus* (cattle, biovar 1-6 and 9), *B. melitensis* (goats, sheep, biovar 1-3), *B. suis* (pigs, reindeer and hares, biovar 1-5), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (desert wood rats). More recently, new members to the genus include *B. ceti* and *B. pinnipedialis* (dolphins/porpoises and

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seals respectively), *B. microti* (voles) and *B. inopinata* (reservoir undetermined). Of these species, *B. melitensis* has the greatest risk for human infection followed by *B. suis* and *B. abortus*, however several of the other species have been shown to be virulent for human [5].

Among the animal brucellosis, bovine brucellosis is the most important disease in many countries around the world due to its economic importance [2, 6 & 7]. Bovine brucellosis is an infectious and contagious disease known for its impact on reproductive performance of cattle and is predominantly a disease of sexually mature animals [8, 9]. 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 and is mainly caused by biovar (mainly biotype-1) of *B. abortus* [10, 5]. Chronic infection of the mammary glands due to *Brucella suis* has also been reported [11]. Clinically bovine brucellosis is characterized by impaired fertility specifically with abortion, metritis, orchitis and epididymitis [12].

Since the first report of brucellosis in the 1970s in Ethiopia [13, 14] the disease has been noted as one of the important livestock diseases in the country [15-18]. A large number of studies on bovine have been reporting individual brucellosis seroprevalence ranging from 1.1 to 22.6% in intensive management systems [19-22] and 0.05 -15.2% in extensive management system [23-27].

Cross breeding 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 and moreover in Ethiopia, despite several researches have been undertaken in the area of bovine brucellosis in different parts of the country the disease is still a major problem demanding much research and investigation. This manuscript was put forward with the objectives of determining the seroprevalence of bovine brucellosis and its associated risk factors in dairy farms in Bishoftu town and to assess knowledge, attitude and practices of the farm owners and attendants regarding to brucellosis.

MATERIALS AND METHODS

Study Area: The study was conducted in Bishoftu town from December 2015 to April 2016. Bishoftu is located 45kms South East 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 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°C and 14°C respectively, with mean relatively humidity of 61.3% [28]. Study population: The target population was dairy cattle that were managed under the intensive production system, which consists of breeding females, replacement heifers and available bulls. Breed of cattle in dairy farms were crosses of local Zebu with Holstein Frisian breed. A total of 400 animals of above six months of age were selected of which 254 were breeding females, 134 were heifers and 12 were serving bulls. None of the animals tested were vaccinated against brucellosis.

Study Design

Sampling Methodology: A cross sectional study design was employed in this study. Farms were selected purposively based on the willingness of the owners and animals within the farm were selected using simple random sampling method. The sample size of the dairy cattle was calculated on the basis of 5.6% prevalence of bovine brucellosis in and around Addis Ababa [15]. Therefore, to determine the sample size of dairy cattle in this area, 11.2% was used as Pexp and 95% confidence interval and 5% required precision [29].

$$n = \frac{Z\ x\ P\exp\ x\ (1 - P\exp)}{d^2}$$

where n = the required sample size, Z =Confidence level (regular value=1.96), P = expected prevalence (5.6%) and d=desired absolute precision (0.05).

$$n = \frac{(1.96)^2\ \text{x}\ 0.056\ \text{x}\ (1\text{-}0.056)}{(0.05)^2} = 81$$

However, a total of 400 dairy cattle were sampled in order to increase the precision and reliability of the data collected and because of the availability of adequate resources.

Collection of Blood Samples: Approximately 10 ml of blood sample was obtained from the jugular vein of each animal using plain vacutainer tubes and sterile needles. After identification, each animal was labeled on the corresponding vacutainer tube; the tubes were set tilted overnight at room temperature to allow clotting.

Next morning, sera were decanted into cryovials and each cryovial containing the serum was labeled. Then Rose Bengal Plate test was conducted. Finally, serum samples were kept at -20°C at Addis Ababa University, College of Veterinary Medicine and Agriculture, microbiology laboratory until the positive sera were submitted for complement fixation test to the National Veterinary Institute (NVI), Bishoftu, Ethiopia.

Serological Tests

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 [3]. 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.

Complement Fixation Test (CFT): Sera that tested positive to RBPT were further tested using CFT for confirmation using standard *B. abortus* antigen S99 (Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, T15 3NB, United Kingdom). Preparation of the reagent was evaluated by titration and performed according to protocols recommended by World Organization for Animal Health [48] Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1: 5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation/complete hemolysis was considered as negative.

Questionnaire Survey: The questionnaire was designed to obtain information from cattle attendants in the farms and data with regard to the farms such as herd size, use of maternity pens, breeding method, disposal of aborted materials and replacement strategies. During each sample collection the sex, age, parity, breed, history of abortion and retained fetal membranes were recorded. Furthermore, knowledge on brucellosis, whether they use protective gloves during handling of aborted materials or not and consumption of raw milk was included in the questionnaire survey.

Data Analysis: Data generated from questionnaire survey and laboratory investigations were recorded and coded and entered in to Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, Texas, USA). The seroprevalence was calculated as the number of seropositive samples divided by the total number of samples tested. To identify association of seropositivity with the potential risk factors (age, herd size, parturition pen, abortion history, abortion period, retained fetal membrane and parity) were computed by Pearson's Chi-square test.

RESULTS

Among the 400 serum samples that were tested by the RBPT for screening of brucellosis, 15 (3.75%) were positive. Out of 15 RBPT positive sera, 12 were positive for CFT and all of them were female cattle. The overall seroprevalence of bovine brucellosis in the study area was 3% (Table 1).

Out of the tested cows of above 2 years age (254), there were 41(16.14%) and 45(17.72%) cows with history of abortion and retained fetal membrane respectively (Table 2).

A Chi-square analysis revealed abortion history, abortion period, retained fetal membrane and parity were significantly associated ($P< 0.05$) with seropositivity of bovine brucellosis than among other factors considered during the study (Table 3).

From 66 farms studied, 64.58, 72.73 and 100% of the farm owners and attendants 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. From a total of 66 cattle attendants and owners interviewed, only 18.18% wear protective gloves during handling aborted material and 34.85% responded that they consume raw milk (Table 4).

The study, based on the questionnaire survey, revealed that all farms in the study area had no frequent contact with other herds. Of the 66 farms assessed by questionnaire survey, it was found that 87.5% of small farms, 63.64% of medium farms and 71.43% of large farms used AI for breeding purpose. The practices of provision of separate parturition pens were 100% in large farms, 54.55% in medium farms and only 6.25% for small farms. Moreover, all of the farms cull their animals based on the reproductive and non-reproductive problems (Table 5).

Table 1: Seroprevalence of overall bovine brucellosis in Bishoftu Dairy farms.

Number of sera tested	RBPT positive	CFT positive	Prevalence
400	15(3.75%)	12(3%)	3%

Table 2: Percentage of examined animals with of history of abortion and retained fetal membrane

Total number of cows	Abortion	Retained fetal membrane
254	41(16.14%)	45(17.72%)

Table 3: Association of risk factors with *Brucella* seropositivity

Variables	Number of tested animals	CFT negative	CFT positive	χ^2(P-value)
Age				
6 months to 2 years	92	92(100%)	0(0%)	3.69(0.055)
>2 years	308	296(96.1%)	12(3.9%)	
Herd size				
Small	20	20(100%)	0(0%)	5.37(0.068)
Medium	101	101(100%)	0(0%)	
Large	279	267(95.7%)	12(4.3%)	
Parturition pen				
No	54	54(0%)	0(0%)	1.94(0.164)
Yes	346	334(96.52%)	12(3.47%)	
Abortion history				
No	213	207(97.18%)	6(2.82%)	10.67(0.001**)
Yes	41	35(85.37%)	6(14.63%)	
Abortion period				
No abortion	213	207(97.18%)	6(2.82%)	29.24(0.000**)
First trimester	12	12(100%)	0(0%)	
Second trimester	8	8(100%)	0(0%)	
Third trimester	21	15(71.43%)	6(28.57%)	
Retained fetal membrane				
No	209	204(97.61%)	5(2.39%)	14.25(0.000**)
Yes	45	38(84.44%)	7(15.56%)	
Parity				
Primiparous	79	79(100%)	0(0%)	5.69(0.017*)
Pluriparous	175	163(93.14%)	12(6.86%)	

* Significant; ** Highly significant

Table 4: Knowledge, attitudes and practices (KAP) of farm owners and attendants about *Brucella* infection in small, medium and large herd size in the study area

	Proportion of respondents(n)			
	Herd size			
Variables	Small(n=48) n (%)	Medium(n=11) n(%)	Large(n=7) n(%)	Total n(%)
Awareness about brucellosis				
No	17(35.42)	3(27.27)	0(0)	20(30.3)
Yes	31(64.58)	8(72.73)	7(100)	46(69.7)
Brucella infected animal				
Culling	48(100)	8(72.73)	0(0)	56(84.85)
Test and slaughter	0(0)	0(0)	0(0)	0(0)
Both	0(0)	3(27.27)	7(100)	10(15.15)
After birth disposal				
Burning	0(0)	0(0)	2(28.57)	2(3.03)
Burying	18(37.5)	5(45.45)	5(71.43)	28(42.42)
Open dump	30(62.5)	6(54.55)	0(0)	36(54.55)
Wearing protective glove				
No	48(100)	10(90.91)	1(14.29)	59(89.39)
Yes	0(0)	1(9.09)	6(85.71)	7(10.61)
Raw milk consumption				
No	30(62.50)	7(63.64)	6(85.71)	43(65.15)
Yes	18(37.50)	4(36.36)	1(14.29)	23(34.85)

n=number, %=percent

Table 5: Summary of the proportion of variables in the three herd (farm) size

Variables	Herd size: Small(n=48) Frequency (%)	Medium(n=11) Frequency (%)	Large(n=7) Frequency (%)	Total No. (%)
Frequent contact with other herd				
No	48(100)	11(100)	7(100)	66(100)
Yes	0(0)	0(0)	0(0)	0(0)
Service type				
AI	42(87.5)	7(63.64)	5(71.43)	54(81.81)
Bull	0(0)	3(27.27)	1(14.28)	4(6.06)
Both	6(12.5)	1(9.09)	1(14.29)	8(12.12)
Parturition pen				
No	45(93.95)	5(45.45)	0(0)	50(75.76)
Yes	3(6.25)	6(54.55)	7(100)	16(24.24)
Cleaning of calving pen				
Flushing with water	34(70.83)	6(54.55)	0(0)	40(60.61)
Both*	14(29.17)	5(45.45)	7(100)	26(39.39)
Replacement stock				
Buy in	2(4.17)	0(0)	0(0)	2(3.03)
Raise own stock	42(87.5)	8(72.73)	5(71.43)	55(83.33)
Both	4(8.33)	3(27.27)	2(28.57)	9(13.64)
Culling criteria				
Reproductive problem	0(0)	0(0)	0(0)	0(0)
NRP	0(0)	0(0)	0(0)	0(0)
Both	48(100)	11(100)	7(100)	66(100)

Both*=Flushing with water and disinfection with detergent, n=number, %=percent NRP=Non-reproductive problem

DISCUSSION

In the present study, the overall prevalence of bovine brucellosis in Bishoftu dairy farms was 3%. This overall seroprevalence of 3% was comparable with the findings of other authors in Ethiopia; 3.2% in Tigray region by Berhe *et al*. [23], 3.1% in Jimma zone by Ibrahim *et al*. [18], 2.9% in central oromia by Jergefa *et al*. [30] and 3.5% in southern and eastern Ethiopia by Megersa *et al*. [27]. Similarly, comparable seroprevalence was reported from some other countries: 4.2% in Eritrea [31], 3.3% in Central Africa [32]. However, higher seroprevalence rates than the present study were reported by Eshetu *et al.* [16], 10% in Addis Ababa by [33], 14.96% in Northwestern parts of Ethiopia; Dinka and Chala [24], 11.2% in East Showa zone of Oromia regional state and Megersa *et al.* [34], 8% in Pastoral region of Ethiopia. The reasons for the low prevalence of bovine brucellosis in this study area might be due to better hygienic practices, use of maternity pen and/or separation of cows during parturition, cleaning and disinfection activities, culling of infected animals, depending on own herds for replacing stock and farm owners knowledge of brucellosis in these intensive farms.

The prevalence of the disease in male was nil compared to female animals. This finding is in agreement with the work done by Abebe [35] in Tigray region, Tolosa [36] in Jimma Zone and Degefu *et al*. [26] in Jijjiga zone who reported only female positive reactors. The explanation for this finding could be that male animals are kept for a shorter time than females and thus the chance of exposure is lower for males [37]. It was also reported that serological response of male animals to *Brucella* infection is limited. It was specified that the testes of infected male animals were usually observed to be non-reactors or showed low antibody titers-[38]. On the other hand, Asfaw *et al*. [15] reported a 0.11% seroprevalence among male animals while Hailemelekot *et al.* [20] reported 2.11% seroprevalence in extensive management system.

Even though age was not significantly associated with *Brucella* seropositivity (P> 0.05) a seroprevalence of 3.9% was found among adult age group (>2 years of age) whereas no *Brucella* seorpositivity was observed in young age group (6 months to 2 years) of dairy cattle in the study site. Several previous reports have indicated that higher seroprevalence of brucellosis in adult age group of cattle [27, 39 & 40] 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 [41].

The present study revealed that a history of previous abortion and retained fetal membrane were significantly associated (P<0.05) with brucellosis seropositivity. Using the questionnaire survey, 16.14% abortion and 17.72% retention of fetal membranes were

recorded. The prevalence of abortion in the study area is in agreement with that of Geresu *et al.* [40] in which he reported a prevalence rate of 17.39% in Asella and Bishoftu towns. This could be explained by the fact that abortions and retained placenta are typical outcomes of brucellosis. In addition, in highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease [42]. In contrary to this findings, a relatively lower prevalence was reported by Tesfaye *et al.* [22] (4.5%) in Addis Ababa dairy farms and [43] (6.7%) in North Gondar, Ethiopia.

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 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 [44, 41].

This study also revealed that there is association between parity and seropositivity of bovine brucellosis with P-value < 0.05 and hence, parity was one of the potential risk factors in the study area. This is probably due to increased contact with fetal materials and vaginal discharge from infected cows there by increasing the chance of being infected by *B. abortus*. This association was in agreement with the finding of other investigators [45, 46].

The questionnaire survey of 66 cattle attendants and owners in the farms indicates that 30.3% have no knowledge of brucellosis, only 10.61% wear protective gloves and 34.85% consume raw milk. Presence of high association between brucellosis and abortion as well as retained fetal membranes (Table 4) is indicative of risk to cattle attendants and professionals working in the area without precautions and protective clothes. Most cases of brucellosis in human are occupational and occur in the farm attendants, veterinarians and butchers [41]. In addition, most of the respondents in this study with the small herd size (54.55%) did not bury afterbirth (aborted fetus, still birth and retained fetal membrane) rather left them on open dump. These factors combined with the poor cleaning practice by the owners could pose a great risk of spread of the disease to unaffected animals [36]. Since 34.85% of cattle attendants have habit of milk consumption without boiling or pasteurization, the risk from the disease could be high. The possibility of infection occurring by drinking milk necessitates the pasteurization or boiling of milk [47, 48].

CONCLUSIONS

Bovine brucellosis caused by *B. abortus* has a major impact on human health, besides causing significant economical losses in dairy industry. In the present study, the seroprevalence recorded revealed that brucellosis is an established disease in dairy farms of Bishoftu town. Even though age was not significantly associated with brucellosis adult animals were highly infected than younger animals and all of the positive reactors were female animals. In addition, history of abortion, abortion period, retained fetal membrane and parity were significantly associated with *Brucella* seropositivity. From questionnaire survey, poor hygienic practices like improper disposal of aborted fetuses and fetal membranes, were identified as potential risk factors which could create favorable condition for the entry and establishment of brucellosis in the dairy farms and consumption of raw milk and absence of habit of using a protective clothes and gloves also act as a risk factor for human brucellosis. In conclusion, the prevailing *Brucella* seropositivity in the dairy farms indicates the importance of brucellosis in dairy cattle industry of the area and potential public health implication for human population in the study area. Therefore, awareness creation among farm owners and attendants about the nature and effect of the disease; relative to large herd size dairy farms, hygienic practices are poor in medium and small herd size dairy farms. So that implementation of better management practices like isolation of aborted animals, provision of separate parturition pen and proper disposal of aborted fetuses and fetal membranes practice; since brucellosis has zoonotic importance, habits of pasteurizing of milk before consumption and using of protective gloves during handling of aborted fetus and fetal membrane and continuous surveillance to detect the presence of infection in the dairy farms and quarantine policy designed and implementation were forwarded as recommendations.

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