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Seroprevalence of Bovine Brucellosis in Eastern Showa, Ethiopia

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Abstract: A cross-sectional study was undertaken to determine the seroprevalence of bovine brucellosis in 300 dairy cows and to assess risk factors that could facilitate the transmission of the disease in Debre-Zeit, Central Ethiopia using questionnaire survey, Rose Bengal Plate Test (RBPT) and Complement Fixation Test. Potential risk factors for seropositivity were analyzed using Chi-square test. The present study revealed an overall 2.0% seroprevalence of bovine brucellosis in the study area in which difference in breed and parity were found to be potential risk factors. The seroprevalence was higher in animals above two years than younger animals. History of abortion and retained fetal membrane were found to be significantly ($P < 0.05$) associated with occurrence of bovine brucellosis. A statistically significant difference ($P < 0.05$) was observed in cross breeds than local dairy cattle. The result of the present study showed that prevalence of bovine brucellosis in the study area was low and hence, test and slaughter policy can be used in order to control the disease in the study area.

Key words: Bovine Brucellosis · Dairy Farms · Debre-Zeit · Seroprevalence · RBPT · CFT

INTRODUCTION

Ethiopia depends on natural resources for its economic and social stability. Among these resources, livestock is the primary agricultural economic sector to which the farmers live is directly or indirectly interrelated. However, the presence of diseases makes the livestock industry less efficient, especially diseases having negative impact on livestock export trade [1].

Brucellosis is an infectious disease caused by bacterial species of the genus *Brucella*. The genus *Brucella* is considered to contain six species: *Brucella abortus*, *B. suis,B. melitensis, B. ovis, B. canis and B. neotome* [2]. *Brucella* organisms are gram negative, small coccobacilli, non-motile, non-capsulated and non-spore forming [3]. Farm animals primarily affected include cattle (*B. abortus, B. melitensis* and rarely *B. suis*), sheep (*B. ovis*and *B. melitensis*), goats (*B. melitensis*) and camels (*B. abortus* and *B. melitensis*) [3, 4].

Bovine brucellosis is primarily a reproductive disease characterized by abortion in the last trimester or birth of unthrifty newborn in the female and orchitis and epididyimitis with frequent sterility in male [5]. Animals of both sexes could harbor brucellosis mainly through ingestion, moreover via direct or indirect contact with contaminated environment by secretion and excretion of aborted animals [6].

Bovine Brucellosis is a major zoonotic disease widely distributed in both humans and animals especially in the developing world causing undulant fever in human [7]. According to FAO/WHO [8], brucellosis is still one of the most important wide spread zoonotic diseases in the world. Thus, from a public health point of view, the sources of brucellosis are either food related or dependent on contact with infected animal either in an occupational or recreational contact. Airborne or contact infection through environmental contamination may be significant problems when infected animal pass through densely occupied areas [9].

Concerning losses in animal production, brucellosis reduces milk production in aborting cows, increases the period between lactationandprolongsthe inter-calving period and loss of the calf crop that affects beef herd industry [10, 11]. Moreover, it results in culling of infertile cows and bulls results in economic loss in dairy and beef farming enterprises [4].

The first serological test for brucellosis was used by Wright and Smith [12]. Since then, a considerable number of serological tests have been developed and modified in various ways to increase their sensitivity and specificity

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[11]. OIE [2] described that no single serological test is appropriate in all epidemiological situations; all have their own limitations. Circulating *Brucella* antibodies have been demonstrated by the Rose Bengal plate test (RBPT), standard tube agglutination test (STAT), Coombs test, complement fixation test (CFT), 2-mercaptoethanol test and enzyme-linked immunosorbent assay (ELISA) [8] of which CFT has been used as a gold standard anda prescribed test for international trade. The important risk factors for brucellosis include herd size, retained placenta, abortion, number of parity and transhumance at herd level and age at animal population level [13, 14].The control of brucellosis depends on preventing the exposure of susceptible animals to infection, increasing resistance of the population through vaccination and applying test and slaughter policy when prevalence of the diseases is low in a given farms [14, 15]. The objective of the current study was to determine the seroprevalence of bovine brucellosis and its associated risk factors in Debre-Zeit, Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: Debre-Zeit is situated in Southeast from Addis Ababa included in Oromia Regional State. The altitude of the zone is about 1850 meters above sea level and is located between 38,57°E longitude and 8,43°N latitude. The mean annual rain fall obtained from the monthly rain fall on the basis of 53 years of records at Debre-Zeit Research Center meteorological station gauge is about 866.6mm. The highest amount of rain fall occurs between June and September and the lower between February and May. The mean annual temperature on the basis of 53 years of records at Debre-Zeit Research Center meteorological station gauge is about 18.9°C. The hottest season is March, April, May and June and the maximum temperature is recorded in April and May. The mean annual relative humidity is 61%. The most humid month is August (76%) and the least is February (53%). The soil type of mthe study area is Vertisol or Alfisol with pH of 6.3-7.1 [16].

Study Animals and Management: The target population was dairy cows which consist of breeding females and replacement heifers. A total of 300 animals of above six months of age were sampled. Both cross and local Borana breeds of cattle were included in the study. All of farms included in the study were managed by intensive system.

Sample Size Determination and Sampling Methodology: A cross sectional study design was employed in this study. Farms were selected by purposive sampling method based on the willingness of the owners and the animals within the farm were selected using simple random sampling method. The sample size of the dairy cattle was calculated on the basis of 1.9% prevalence of bovine brucellosis in Debre-Zeit determined by Bisrat [17] and computed with the expected precision of 5% and at 95% confidence interval. The sample size was calculated by using Thrusfield formula [18] and it was 280 but to increase the precision level a total of 300 dairy cows were sampled.

Sampling and Data Collection: Herd level data that included herd structure, size, history of purchases of animals and farm management practices were collected by interview administered questionnaire. Information on individual animal variables like age, sex and history of abortion were recorded separately on sample data sheets. These herd data were envisaged for further use in studying the herd-level risk factors for brucellosis. Approximately 10 ml of blood sample was obtained from the jugular vein of each animal using plain vacutainer tubes and needle. 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 removed from the clot and then Rose Bengal test was conducted. Finally, serum samples were kept at-20°C at Addis Ababa University, School of Veterinary Medicine and Microbiology Laboratory until the positive sera were submitted for complement fixation test to the National Veterinary Institute (NVI), Debre-Zeit.

Serological Tests: The Rose Bengal plate test (RBPT) was used as screening test of all serum samples for the presence of *Brucella* antibodies by using procedure described by Alton *et al.* [19]. All sera which tested positive to RBPT were further tested using CFTatthe National Veterinary Institute Debre-Zeit, Ethiopia.

Statistical Analysis: Data were coded entered into SPSS, version 15. Analyzing the effects of different potential risk factors on the seroprevalence of brucellosis at both the individual level was performed by logistic regression. The seroprevalence was calculated by

dividing the number of CFT and RBPT positive animals by the total number of animals tested. Chi- square test was utilized to measure the association between the seroprevalence and categorical variables. For statistical inference, P value less than 0.05 considered as significant.

RESULTS

In dairy cows of above 6 month ageinvestigated, 24 (8%) were local breed whereas 276 (92%) were cross-breeds of indigenous zebu and Holstein Friesian dairy cows. In addition, 177 (59%) of the animals were lactating cows, 76 (25.3%) were pregnant and the remaining 47 (15.7%) were heifers. In 13 (4.3%) of the studied animals, there was a history of retained fetal membrane and 8 (2.7%) were with a history of abortion. Generally, the frequency distribution of breed, parity, age group, physiological status, retained fetal membrane and calf status were summarized in the following table (Table 1). Out of the 300 serum samples, 10 (3.3%) were positive for brucellosis using RBPTandonly 6 (2%) sera samplesfurther were confirmed positive using CFT.

The present study attempted to look into the existence of any association between seropositivity and breeds of the animals. Thus, the prevalence of local and the cross breed animals was compared in Table 2. The sera prevalence of local and cross breed cattle was calculated as 0.3% and 1.7% having a significant variation with OR of 5.1 and P-value of 0.008. The association of brucellosis with abortion and retained fetal membrane was tested using Chi-square. It was found that brucellosis was significantly associated with abortion and retained fetal membrane with Chi- square value of 22.5 and 12.86 and P-value of 0.000 and 0.002 respectively (Table 3). The study also revealed that older animals were highly infected than younger animals and parity of individual animals was found to have direct association with the number of parity (Table 4).

Table 1. Frequency distribution of variables and percent

Variables	Group	Frequency	Percent
Breed	Local	24 8%	24 8%
	Cross	276	92%
Parity	No parity	47	14.3%
	Parity one	53	16.7%
	>one parity	200	69%
Age	6 month- 2 years	43	14.3%
	>2 years	257	85.7%
Physiological status	Lactating	177	59%
	Pregnant	76	25.3%
	Heifers	47	15%
Retained fetal	Encountered	13	4.3%
Membrane	Not encountered	259	83%
	Not gave birth	37	12.7%
Calf status	Dead	8	2.7%
	Alive	255	85%
	Not gave birth	37	12.7%
Rose Bengal plate	Negative	290	96.7%
test result	+	5	1.7%
	++	4	1.3%
	+++	1	0.3%
Complement fixation	Negative	294	98%
Test	Positive	6	2%

Table 2. Breed wise seroprevalence of bovine brucellosis

Breed	No. tested	CFT Positive	Prevalence (%)
Local	24	1	0.3%
Cross	276	5	1.7%
Total	300	6	2.0%

Pearson x^2=6.96, P- value=0.008,Odds Ratio=5.1

From the questionnaire survey administered, it was discovered that the vast majority of farm owner (71.40%) used natural mating for breeding purpose. On the other hand, most of the animals were fed install (85.7%). The details of the questionnaire results were summarized in the following table (Table 5).

Table 3: Association of brucellosis with abortion and retained fetal membrane

Result	History of abortion			History of retained fetal membrane		
test	Aborted	Not aborted	Total	Present	Not present	Total
CFT^+	2(0.7%)	4(1.3%)	6(2%)	2(0.7%)	4(1.3%)	6(2%)
CFT^-	6(2%)	251(83.7%)	257(85.7%)	11(3.7%)	245(81.7%)	256(85.4%)
Total	8(2.7%)	255(85%)	263(87.7%)	13(4.4%)	250(83%)	263(87.4%)

x^2 =22.5, df=2, P-value=0.00 x2= 12.86, df= 2, P- value=0.002

Table 4: Prevalence of retained fetal membrane and abortion across parity in the study area parity

	Parity 0	Parity1	Parity2	Parity3	Parity4	Parity5	Parity6	Parity7	Total
No.Examined	43	50	89	47	32	20	18	1	300
RFM	0	3	4	4	1	0	1	0	13
Prevalence	0%	6%	4.5%	8.5%	3.1%	0%	5.5%	0%	27.6%
Examined	43	50	89	47	32	20	18	1	300
Abortion	0	2	4	1	1	0	0	0	8
Prevalence	0%	4%	4.5%	2.1%	3.1%	0%	0%	0%	13.7%

No.- Number, RFM-retained fetal membrane

Table 5: Summary of result of questionnaire survey

Farm management activities	Number		Percent
Presence of maternity pen	4/7		57.14%
Breeding system	Natural mating	5/7	71.43%
	AI	1/7	14.3%
	Both	1/7	14.3%
Farm replacement	Own farm	3/7	42.86%
	Market	2/7	28.58%
	Both	2/7	28.58%
Method of disposal of placenta	Burn	0/7	0%
	Burry	7/7	100%
Feeding system	Grazing	1/7	14.3%
	Stall feeding	6/7	85.71%
Have awareness about brucellosis	Yes	3/4	42.86%
	No	4/7	57.14%
Animal tested for brucellosis	Yes	2/7	28.58%
	No	5/7	71.43%

DISCUSSION

The overall seroprevalence of *Brucella* antibodies determined with CFT and RBPT in Debre-Zeit, Ethiopia were 2.0% and 3.3% respectively. Since CFT is the recommended confirmatory test for brucellosis with high specificity [3, 4], the overall seroprevalence of bovine brucellosis in the study area is 2.0%. This low seroprevalence is in agreement with previous findings of Bisrat [17] who reported 1.9% prevalence in the same area and Asmare *et al*.[20] documented a 2.46% in Sidama Zone of southern Ethiopia. In Ethiopia, even a lower seroprevalence which contradict the current study were documented in previous findings of Tolosa [21] who reported 0.77% in southwestern, Yayeh [22] who observed a prevalence of 0.14% in north Gondar Zone, Hailu *et al*. [23] who reported a seroprevalence of 1.38% in Jig-jiga zone of Somali Regional State and Gebreyohans [24] who reported 1.5% seroprevalence in Addis Ababa dairy farms. In the country, so far higher seroprevalence which in contrast with the current findings are 39% in western Ethiopia [25], 22% in a dairy farm in northeastern Ethiopia [26], 11-15% in dairy farms and ranches in southwestern Ethiopia [27], 11.0% in Wuchale-Jida district [28], 10% in different private dairy farms of Addis Ababa [29], 8.2% in Arsi area [30], 8.1% in dairy farms in and around Addis Ababa [31], 7.7% in Tigray region [32], and 3.1% in Jimma zone of Oromia regional state [33].

The difference in seroprevalence of bovine brucellosis is due to the difference in management systems, age and sex among dairy farms [28, 34]. It has been reported that susceptibility of cattle to *B. abortus* infection is influenced by age of an individual animal. Thus, sexually matured and pregnant cattle are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex. On the other hand, younger animals tend to be more resistant to infection and frequently clear infections, although latent infection may occur. This may be due to the fact that sex hormones and erythritol, which stimulates the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity [14].

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 [23, 34, 35].

Using questionnaire survey, a prevalence of 2.7% abortion and 4.4% retention of fetal membranes were recorded respectively. This indicated that history of abortion or still birth and retained fetal membrane were significantly associated with brucellosis seropositivity. This could be explained by the fact that abortion or still birth and retained fetal membrane are typical outcome of brucellosis [4]. The prevalence rate of abortion in the current study was in agreement with other investigators [34, 36].

CONCLUSION

The current cross-sectional study of bovine brucellosis in Debre-Zeit, Ethiopia showed a low prevalence. Though its seroprevalence is low, it can still be a potential risk for both susceptible animals and humans. All the risk factors in the study site showed a statistical significance that played a role for the existence of the disease in the study area. There was an association between abortion and retained fetal membrane and seropositivity for bovine brucellosis in the study area. Finally, considering the economic and public health importance of brucellosis, avoiding mixing of cattle without screening for brucellosis, promoting the use self-contained units instead of shared facilities and test and slaughter policy was recommended to control the disease in the study area.

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