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Sero-Prevalence Study of Bovine Brucellosis and its Associated Risk Factors in Debrebirhan and Ambo Towns

Liya Bashitu[1], **Berihun Afera**[2*], **Getachew Tuli**[3] **and Fasil Aklilu**[3]

[1]*Bureau of Agriculture and Rural Development, Addis Ababa, Ethiopia*

[2]*Mekelle University College of Veterinary Medicine, Ethiopia*

[3]*National Animal Health Diagnostic and Investigation Centre, Ethiopia*

[*]**Corresponding author:** Berihun Afera, Mekelle University College of Veterinary Medicine, Mekelle, Ethiopia, Tel: 251 344 40 40 05; Fax: +251-344-40-1090; E-mail: berihun414@yahoo.com

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Abstract

A cross-sectional sero-prevalence study using random sampling was conducted from November 2013 to May 2014 in the selected district of west Shewa zone of Oromia region (Ambo) and semen Showa zone of Amhara region (Debrebirhan) to determine seroprevalence of bovine brucellosis and assess the potential risk factors. In this study, a total of 415 animals aged from 6 month and above were screened for brucella antibodies using Rose Bengal plate test and positive sera were confirmed by complement fixation test. Four sera samples out of the 415 (0.7%) reacted positively for RBPT and one of them reacted positively for CFT (0.2%). In the current finding, the prevalence in male and female and age were not significantly associated with brucellosis sero-prevalence ($p>0.05$) but there is significant association of sero-postivity of bovine brucellosis with history of abortion ($P<0.05$). In conclusion the sero-prevalence of bovine brucellosis in the study area was very low. Thus, slaughter of positive reactors and proper hygienic practices and good husbandry management were recommended.

Keywords: Brucellosis; CFT; RBPT; Sero-prevalence

Introduction

Ethiopia is believed to have largest livestock population in Africa, with the livestock population 53.99 million cattle, 49.56 million small ruminants, about 0.92 million camels and 9.01 million equine and 50.38 million chickens [1]. This livestock has been contributing considerable portion to the economy of the country and still promising to rally round the economic development of the country. Though the country has such huge livestock, 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

Brucellosis, caused by a variety of *Brucella* species, is primarily a disease of domestic livestock and wild animals and major important public health problem in many countries [2,3]. The disease is considered as one of the most widely spread zoonoses in the world [4]. It is an infectious bacterial disease caused by genus *brucella* [5] which are Gram-negative, facultative, intracellular *coccobacillary* comprised of species based upon biochemical features and their correlation with preferred host species [6].

Currently ten species are recognized including the better known six classical species comprised of B. *abortus* (cattle, biovars 1-6, and 9), B. *melitensis* (goats, sheep, biovars 1-3), B. *suis* (pigs, reindeer and hares, biovars 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 seals respectively), B. *microti* (voles) and B. *inopinata* (reservoir undetermined) [7].

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 humans [7].

Among the animal brucellosis, bovine brucellosis, caused by B. *abortus* is the most important disease in many countries around the world due to its economic importance [8-10].

Brucella abortus is mainly infective for cattle, but occasionally other species of animals such as sheep, swine, dogs and horses may be infected. In horses, B. *abortus* together with *Actinomyces bovis* is commonly present in poll evil and fistulous withers. Ingestion of contaminated meat and milk from infected animals are the main source of infection. In addition penetration of the intact skin and conjunctivitis and contamination of the udder during milking grazing on infected pasture or consuming other feed with infected cows and contact with aborted fetuses and infected newborne calves are the most common methods of spread [11].

Following infection with B. *abortus* pregnant adult females develop placentitis usually resulting in abortion between the fifth and ninth month of pregnancy. Even in the absence of abortion, profuse excretion of the organism occurs in the placenta, fetal fluids and vaginal discharges.The mammary gland and associated lymph nodes may also be infected, and organisms may be excreted in the milk. Subsequent pregnancies are usually carried to term, but. Uterine and mammary infection recurs, with reduced numbers of organisms in cyetic products and milk. In acute infections, the organism is present in most major body lymph nodes. Adult male cattle may develop orchitis and brucellosis may be a cause of infertility in both sexes. Hygromas, usually involving leg joints, are a common manifestation of brucellosis in some tropical countries and may be the only obvious indicator of infection. While animals typically recover, and will be able

to have live offspring following the initial abortion, they may continue to shed the bacteria [12].

Control program for the disease include vaccination of young and mature animals and slaughter of infected animals and disposal of animals following serological test [10]. Regulation of the disease in many countries relies on vaccination and culling of infected animals in order to minimize chances for perpetuation of the infection and for protection of consumers and people that are associated with animals keepin [13].

Brucellosis is known to be an endemic and growing problem in domestic livestock (local and cross breed) herds in Ethiopia.Although much work has been done and reports are available, there is limited information on the status of bovine brucellosis in west shewa zone of Oromia region (Ambo) and North Shewa zone of the Amhara region (Debrebirhan). Therefore the objective of the paper was

- To assess the prevalence of bovine brucellosis and its associated risk factors in dairy farms

Materials and Methods

Study area

The study was conducted in central Ethiopia located 126 km west of Addis Ababa in Oromia region (Ambo) and 120 km north east of Addis Ababa (Debrebirhan) in Amhara region. Debrebirhan and Ambo are characterized by has a latitude and longitude of 9°41′N 39°32′E, an elevation of 2,840 meters and 8°59′N 37°51′E and 2101 meters respectively. The maximum and minimum temperature of Debrebirhan and Ambo are 16°C and 31°C and 20°C and 29°C respectively. The average rainfall in Debrebirhan and Ambo was also 1100 mm and 1000 mm respectively.

Study design

A cross-sectional sero-prevalence study using random sampling was used to collect serum sample from the study population.

Study animal

The study consisted of cattle that were managed under the intensive production system. The cattle under study comprised of the cross breeds with no history of vaccination against brucellosis. Both sexes and different age group greater than six month were included in the study as the disease wasn't common in the cattle less than 6 months of age due to maternal antibody.

Sample size determination

For sample size determination expected prevalence of 50% and level of confidence of 95% were used using the formula of Thrusfield [14] as there was no work done before in the region particularly in the zone. Hence the sample size was determined as 384 but to increase the precision of the result 31 more animals were included.

N=1.962 pq/d2

Where

n=sample size

P=expected prevalence

q=1-p

d=standard error

Source: Thrusfield [13]

Sampling procedure

About 10 ml of blood sample was collected from the jugular vein of each cattle using plain vacutainer tubes. The blood was left at room temperature for 24 hours and serum was harvested using cryovials and each cryovials containing the serum was labeled. Relevant risk factor; age, breed, sex, were also recorded simultaneous during blood collection. The collected serum sample was stored at -20°C until tested by both RBPT and CFT tests.

Rose bengal plate Test (RBPT)

The serum samples were screen using RBPT antigen (VLA weybridge, UK).

The test serum and antigen was left at room temperature for half an hour before the test; 30 µl of RBPT antigen and 30 µl of test serum was place alongside on plate, and then mix thoroughly. The plate was shaked for 4 min and the degree of agglutination reactions was record. The samples were considered as positive if any agglutination was observed and negative if no agglutination.

Complement fixation test (CFT)

Complement fixation test (CFT) was used to all sera tested positive by Rose Bengal Plate Test (RBPT) for further confirmation B.abortus antigen for CFT was used to detect the presence of anti-Brucella antibody in the sera like RBPT. Test was done according to the protocol of recommended by OIE [15] at NAHDIC, Sebeta, Ethiopia.

Data entry and analysis

All the data collected was entered in to Microsoft excel spread sheet and coded appropriately. The coded data was transferred in to SPSS version 16 software. For data analysis descriptive statistics was used but to test the association of the risk factors with the disease chi-square test was used and association was considered if P-value is less than 0.05.

Result

The overall prevalence of the current finding was 0.2% with the rates of 0.2% in Ambo and 0.7% in Debrebirhan using the RBPT test while during the confirmation of the sera-positive animals using CFT it was 0% in Ambo and 0.2% in Debrebirhan with no statistical significance variation between these two areas (P>0.05) as indicated in Table 1.

Area	Number of examined animals	RBPT positive	CFT positive	p-value
Ambo	169	1 (0.2%)	0(0%)	0.52
Deberbrihan	246	3(0.7%)	1(0.2%)	

Table 1: The prevalence of bovine brucellosis in Ambo and Debrebirhan.

The prevalence of brucellosis in female and male was 1% 0.0% when tested using RBPT while the CFT test indicated that it was 0.2% and 0% in female and male respectively. But there was no statistical variation in the rate of the disease between male and female (P>0.05) as indicated in Table 2.

Sex	Number of examined animals	RBPT positive	CFT positive	p-value
Female	313	4(1%)	1(0.2%)	0.251
Male	102	0(0%)	0(0%)	

Table 2: Prevalence of bovine brucellosis in female and male.

Age category prevalence of brucellosis indicated that it was higher in those cattle, its age ranged from 6 months to 4 years old compared to the rest of the age groups with no statistical variation among the age groups (P>0.05) as indicated in Table 3.

Age	Number of examine animals	RBPT positive	CFT positive	P-value
6 month-4 years	240	4(1%)	1(0.2%)	0.229
5 year-8 years	135	0(0%)	0(0%)	
>8 years	40	0(0%)	0(0%)	

Table 3: Prevalence of bovine brucellosis in different age group.

The prevalence of brucellosis was associated with their history of abortion on those examined animals (P<0.05) as indicated in Table 4.

History of abortion	Number of examined animals	RBPT positive	CFT positive	P-value
Aborted	81	2(.05%)	1(0.2%)	0.042
Non aborted	334	2(0.5%)	0(0%)	

Table 4: Association of brucellosis with abortion.

Discussion

In this study RBPT as screening test was used due to its fastest, sensitive (97.9%), easy, and allows processing many samples per day [16]. Those RBPT sera test positives were retested using CFT test having specificity of 100% [17] in order to maximize the specificity of the tests. An animal was regarded as sero positive if it was positive by both the RBPT and CFT.

The overall prevalence of the current finding was 0.2%. This indicated that only limited number of dairy cows was affected by the disease. But this doesn't mean that the disease is insignificant as it is very serious disease responsible for reproduction failure of the dairy industry in the area and its zoonotic importance. The overall prevalence of the current finding was lowered compared to the study conducted by Kassahun et al. [18] who reported prevalence of 2.46% in Sidama Zone of Southern Ethiopia. Similarly, the prevalence reported by Mussie et al. [19] (4.63%) in Northwestern part of Amhara Regional State was also higher compared to the current finding. In contrary to the current finding, much higher seroprevalence were reported by Haileselassie et al. [20] with the prevalence of 7.7% in Tigray region. But the current result compared to Alem and Solomon [21] and Belihu [22] who reported 0% due to absence of sero-reactive cattle after screening 564 animals in Eastern and Western Showa zones of central Ethiopia using rose Bengal plate test (RBPT), serum agglutination test (SAT) and complement fixation test (CFT) was higher. At the same time, Belihu [22] could not find positive reactors in intensive dairy farms in Addis Ababa area (n=747). This reports is considerably in agreement with the current study reported (0%) in the region of west showa zone of central Ethiopia.

The reasons for the low prevalence of bovine brucellosis in this study areas 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. Brucellosis has been labeled to be a disease of poor hygienic condition that would exposure animals to aborted fetus, placentas, vaginal discharges or newborn calves from infected cows. Likewise, the use of maternity pens at calving is proved to be associated with a decrease in prevalence of infection, presumably due to decreasing the exposure of infected and susceptible animals [11].

The prevalence of the disease in male was nil compared to female having the prevalence of 0.25%. In agreement with the current result Tesfay [23] and Yayeh [24] reported that only female cattle's were considered as positive reactors. This was also explained by Radostits et al. [11] who stated clearly that sex has been one of the risk factors affecting susceptibility of cattle to *Bruella abortus* infection.

Age wise distribution of the current finding also indicated that, the rate of the disease in this study was 0.2% in those cattle its age ranging from 6 month to 4 years old. In line with the present finding Kassahun et al. [18] reported that the majority (97.87%) of sero-reactors detected were in animals above 2 years of age in both the extensive and intensive management systems. In contrary to the current finding [19] in Amhara Regional State documented a statistically significant variation (p<0.05) among the three age groups of 0.5-1 year (2.70%), >1-3 years (4.22%) and >3 years (4.23%). Moreover, Radostits et al. [11] explained that susceptibility of cattle to *Brucella abortus* infection is influenced by age of the individual animal. Walker [25] also stated that though younger animals tend to be more resistant to infection and frequently clear infections, a latent infection do occur.

In the present finding there was statistical association of history of abortion and the presence of infection in those animals (P<0.05). Radostits et al. [11] also showed that in highly susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature of the disease.

Conclusion and Recommendations

The current study revealed that the sero-prevalence of bovine brucellosis in central Ethiopia is very low. At the same time low prevalence of the disease was observed in different sex of animals and age groups of cattle. The result also revealed that the disease is associated with the history of abortion of the cattle industry in the study sites. The low prevalence of the disease in the study area could serve as source of infection to other cattle of the country as there is free movement of animals from one area to another area of the country. The presence of sero-reactor animals for the disease indicated the presence of foci of infection that could need great attention of the dairy industry in- around the area to safe guard the public health.

Hence based on the current finding the following points were recommended

- Test and slaughter sero-positive reactor should be practiced.
- Animals should be purchased from farms free of the disease
- Proper hygienic practices and good husbandry management should be exercised
- Awareness creation to farm owners is essential
- Isolation of aborted animals and proper disposal of aborted fetuses and fetal membranes, is needed
- Continuous surveillance to detect the presence of the infection in the dairy farms should be designed and implemented.

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