Prevalence and risk analysis of bovine brucellosis in Asella organized dairy farm, Oromia Regional State, South East Ethiopia

Eyob Eticha¹*, Hani Solomon², Diriba lemma¹ and Birhanu Abera¹

¹Asella Regional Veterinary Laboratory, Asella, Ethiopia.
²Arsi University School of Agricultural and Environmental Science, Asella, Ethiopia.

A cross-sectional study was conducted on organized Dairy Farm at Asella, Oromia Regional State of Ethiopia to determine seroprevalence and risk analysis of bovine brucellosis in May, 2016. A total of 304 samples were collected; all were tested and confirmed serologically using Rose Bengal plate test (RBPT) and complement fixation test (CFT). Out of 304 samples tested, overall seroprevalence of RBPT and CFT results was 12.48% (38) and 9.87% (30) respectively, which was higher in animals above two years age than younger one. History of abortion and retained fetal membrane were found to be significantly (p<0.05) associated with occurrence of bovine brucellosis. A statistically not significant difference (p >0.05) was observed between cross and local dairy cattle. The result showed the high prevalence of bovine brucellosis in the farm. Hence, culling of the positive dairy cattle and practicing good management should result in a control and prevent of the brucellosis.

Key words: Asella, brucellosis, dairy cattle, seroprevalence, risk factors.

INTRODUCTION

Brucellosis is a highly contagious, zoonotic, and economically important bacterial disease of animals worldwide (OIE, 2009). It is endemic in many developing countries and caused by Brucella species that affect man, domestic and some wild animals, and marine mammals (Seleem et al., 2010). It is primary reproductive disease clinically characterized by abortion in the last trimester and retained placenta in the female whereas orchitis and epididymitis with frequent and sterility occur in male (Radostits et al., 2007). Sources of infection for isolation of bacterial include aborted fetuses, fetal membranes, vaginal discharges and milk from infected cows. The most common route of transmission in cattle is through direct contact with an aborting cow and the aborted foetus or by indirect contact with contaminated fomites. Ingestion of contaminated pasture, feed, fodder and water may also play a secondary role (Godfroid et al., 2010). Susceptibility of animals to brucellosis depends on their natural resistance, level of immunity and environmental stress (Radostits et al., 2007). Mature animals are much more susceptible to infection, regardless of sex. In female animals, pregnancy has

*Corresponding author. E-mail: eyoba20000@gmail.com.
positive contribution to the degree of susceptibility than their age. Bulls are relatively resistant than sexually mature heifers and less resistant than sexually immature heifers (Godfroid et al., 2010). A precise diagnosis of *Brucella* spp. infection is important for the control of the disease in animals and consequently in man. Clinical diagnosis is based usually on the history of reproductive failures in livestock, but it is a presumptive diagnosis that must be confirmed by laboratory methods (Poester et al., 2010). Laboratory methods also help to differentiate from other infectious causes of abortions (Juyal et al., 2011). No single test is appropriate in all epidemiological situations; all have their own limitations. The first serological test for brucellosis was used by Wright and Smith (OIE, 2009). Compliment fixation test is a standard method for the epidemiological surveillance of brucellosis (Köppel et al., 2007). Antibodies anti-*Brucella* 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 immunosorbentassay (ELISA)(OIE, 2009). In Ethiopia, the prevalence of bovine brucellosis has been intensively investigated in state owned dairy farms (Bekele et al., 2000). In smallholder farms in some parts of the country (Berhe et al., 2007) and in the central highlands of Ethiopia (Kebede et al., 2008). Thus, this study was carried out to determine the seroprevalence of bovine brucellosis and its associated risk factors in Asella organized dairy farm.

**MATERIALS AND METHODS**

A cross sectional study was conducted in May, 2016 at Asella organized dairy farm managed under intensive system which is located at 175 km South East of Addis Ababa. In this study a bout 5-10 ml of blood was collected from the jugular vein of 304 cattle which are more than six month of age using plain vacationer tube to collect a serum samples. Information on individual animal such as age, sex, breed and history of abortion was recorded in separate sheet. The collected sera samples were screened for the presence of antibody against *Brucella* using the Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) as a confirmatory test were used in detecting antibody against *Brucella* antigen. RBPT undertaken at Asella regional veterinary laboratory and CFT was undertaken at the National Animal Health Diagnostic and Investigation Center, serology laboratory, sebata, Ethiopia. The procedure and interpretation of results described by OIE (2008) were followed. Finally, the collected data and the results of laboratory tests were analyzed by statistical package for social science (SPSS), to determine those variable that were significantly associated with seropositivity to *Brucella*.

**RESULTS**

In dairy animals investigated during the study were above six month of age and 76(25%) and 228 (75%) were local Borena and cross-breeds of indigenous zebu and Holstein Friesian, respectively. In addition 95 (31.25%) of the animals were lactating cows, 30(9.87%) were pregnant, 42(13.82%) were bulls and the remaining 137(45.10%) were heifers. From the studied animals there was 45(14.8%) history of retained fetal membrane and 28(9.2%) abortion. Generally, the frequency distribution of breed, age group, and sex were summarized in Table 1. Out of 304 serum samples, 38 (12.5%) were positive for brucellosis using RBPT. The present study attempted to look into the existence of any association between seropositivity and breeds, age and sex of the animals. Thus, the prevalence of local Borena, and to cross breed animals was compared in Table 2. The sera prevalence of local Borena, and cross breed cattle was calculated as 1.32 and 8.55% having not a significant variation with P-value of 0.265, the sera prevalence of age for animals 6 month-3 year, 3-6 year and above 6 year which assess in Table 3 was intended as 2.3, 2.63 and 4.93% respectively which have significant variation with p-value 0.011 the prevalence of male and female which assess in Table 4 was intended as 0.99 and 8.88% have not a significant variation with p-value of 0.523. 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 p-value of 0.000 and 0.002, respectively (Table 5).

**DISCUSSION**

The present study revealed that the seroprevalence of anti-*Brucella* antibodies determined with CFT and RBPT was 9.87 and 12.48%, respectively. The overall seroprevalence of bovine brucellosis in the study area was 9.87%. This high seroprevalence is an agreement with previous finding of (Kebede et al., 2008) with 11% in central highland, (Hunduma and Regassa, 2009) with 11.2% in east show and (Megersa et al., 2012) with 8% in pastoral region.

On the other hand, there were reports with a relatively higher sero-prevalence of bovine brucellosis in other parts of the country, (Sintaru, 1994) with 22% in a dairy farm in northeastern Ethiopia and (Bekele et al., 2000) with 11-15% in dairy farms and ranches in southwestern Ethiopia. Other investigator 0.14% in selected area of north Gondar (Tadese, 2003), 0.77% in selected site of Jimma Zone (Tolosa et al., 2008), 0.45% in central highlands of Ethiopia (Lidia, 2008) and 0.05%, in Arsi Zone (Degefa et al., 2011) indicates lower overall prevalence when compared to our present study. The level of brucellosis infection tends to be relatively high in intensive farm than in extensive farm (Matope et al., 2011).

There is still disagreement between different authors among breed susceptibility to brucellosis. In this study breed has supposed one of the risk factors, consequently seroprevalence was found to be higher in cross breed animals (8.55%) than local (1.32%). Nevertheless, this difference was statistically not significant which is similar
Table 1. Distribution of variables with percent.

| Variable                        | Group       | Frequency | Percent |
|---------------------------------|-------------|-----------|---------|
| Breed                           | Local Borena| 76        | 25      |
|                                 | Cross       | 228       | 75      |
| Age                             | 6 month -3 years | 166       | 54.6    |
|                                 | 3-6 years   | 86        | 28.29   |
|                                 | >6 years    | 52        | 17.1    |
| Sex                             | Male        | 42        | 13.81   |
|                                 | Female      | 262       | 86.18   |
| Rose Bengal Plate Test result   | Negative    | 266       | 87.5    |
|                                 | +           | 3         | 0.98    |
|                                 | ++          | 7         | 2.3     |
|                                 | +++         | 28        | 9.21    |
| Compliment Fixation Test result | Positive    | 30        | 9.87    |
|                                 | Negative    | 274       | 90.13   |

Table 2. Breed wise sero prevalence of bovine brucellosis.

| Breed   | n     | CFT positive | Prevalence (%) |
|---------|-------|--------------|----------------|
| Local   | 76    | 4            | 1.32           |
| Cross   | 228   | 26           | 8.55           |
| Total   | 304   | 30           | 9.87           |

$\chi^2 = 2.66$, df=2 p value = 0.265.

Table 3. Age wise seroprevalence of bovine brucellosis.

| Age      | n     | CFT positive | Prevalence (%) |
|----------|-------|--------------|----------------|
| 6 month-3year | 179  | 7            | 2.3            |
| 3-6year   | 73    | 8            | 2.63           |
| Above 6 year | 52   | 15           | 4.93           |
| Total     | 304   | 30           | 9.87           |

$\chi^2 = 9.035$, df=2 p value = 0.011.

Table 4. Sex wise seroprevalence of bovine brucellosis.

| Sex     | n     | CFT positive | Prevalence (%) |
|---------|-------|--------------|----------------|
| Male    | 42    | 3            | 0.99           |
| Female  | 262   | 27           | 8.88           |
| Total   | 304   | 30           | 9.87           |

$\chi^2 = 0.407$, df=1 p value = 0.523

to reported in GutoGidadistrict (Moti et al., 2012) and in central highland of Ethiopia (Lidia, 2008). On the other hand Minda et al. (2016) and Jergefa et al. (2009) reported significant variation on serological prevalence of
brucellosis with higher prevalence in cross-bred than in local ones. Age have association with occurrence of brucella. This could be explained by sexual maturity and pregnancy due to the influence of sex hormones and placenta erythritol on the pathogenesis of brucellosis (Radostits et al., 2007). This result in agreement with report of Lidia (2008) central highland of Ethiopia and Nuraddis et al. (2010) in selected site of Jima Zone. The presences of statistically significant contradict with the previous finding of Minda et al., (2016) and Magona et al. (2009). Even if there is high prevalence in adult animals there was seropositive reactor in less than 3 years of age this is an indication of variations in the management practices (level of intensification and hygienic practices).

Even though sex is not significantly associated with Brucella seropositivity (p> 0.05), high seroprevalence was found among female animals which is 8.88% in female and 0.99% in male animals. This finding was in agreement with the report done by Asfaw et al. (1998) in and around Addis Ababa, Tolosa et al. (2008) in Jima Zone and Desalegn et al. (2011) in Asella dairy farm. The lower prevalence of male reactors in this report could be as a result of smaller number of males tested as compared to female and it was also reported that the serological response of male animal to Brucella infection is limited (Mohammed et al., 2009). Female animals are more susceptible to Brucella organism in gravid uterus of pregnant animals than in testis due to the presence of erythritol in female reproductive tract which stimulates the growth of the organism (Godfroid et al., 2010).

In our study, individual animal sero-prevalence was positively associated with the occurrence of abortion and retained fetal membranes. 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 (Radostits et al., 2007). This result was in agreement with other investigators Desalegn et al. (2011) in Asella dairy farm and Berhe et al. (2007) in Tigray Region.

**Conclusion**

The study reflected higher prevalence of bovine brucellosis about 9.87% in the target dairy farm. The current findings indicated that the age, history of abortion and retained fetal membrane were the risk factors statistically significant associated with Brucella seropositivity for this study. Therefore, considering the economic and public health importance of brucellosis, regular screening of brucellosis for newly introduced and the whole farm animals, and culling of those positive one and practicing good farm management were recommended to reduce the risk incidence of bovine brucellosis in dairy farm and surrounding population.

**TABLE 5. Association of brucellosis with abortion and retained fetal membrane.**

| Test result | Aborted | Not aborted | Total | Present | Not present | Total |
|-------------|---------|-------------|-------|---------|-------------|-------|
| CFT+        | 21(6.9%)| 214(70.4%)  | 235(77.3%)| 38(12.5%)| 197(64.8%)  | 235(77.3%)|
| CFT-        | 7(2.3%) | 20(6.6%)    | 27(8.9%)| 7(2.3%) | 20(6.6%)    | 27(8.9%)|
| Total       | 28(9.2%)| 234(77%)    | 262(86.2%)| 45(14.8%)| 217(71.4%)  | 262(86.2%)|

\( \chi^2 = 22.5, df=2, p-value=0.000 \)

\( \chi^2 = 12.86, df= 2, p-value=0.002 \)

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGMENTS**

Authors would like to thank Arsi University for their financial and material support of this work. We also express our deepest gratitude and appreciation to Asella Regional Veterinary Laboratory and National Animal Health Diagnostic and Investigation Center (NAHDIC) for their providing us valuable support and assistance during sample processing in the laboratory.

**REFERENCES**

Asfaw YB, Molla HKZ, Tegene A (1998). The epidemiology of bovine brucellosis in intra and peri urban dairy production system in and around Addis Ababa. Bulletin of Animal Health and Production in Africa 48:217-224.

Bekele A, Molla B, AsfawY, Yigezu L (2000). Bovine brucellosis in ranches and farms in southeastern Ethiopia. Bulletin of Animal Health and Production for Africa 48:13-17.

Berhe G, Kelay B, Yilkal A (2007). Seropidemiological investigation of bovine brucellosis in the extensive production system of Tigray Region of Ethiopia. The International Journal of Applied Research in Veterinary Medicine 5:65-71.

Degefa T, Duressa A, Duguma R (2011). Brucellosis and Some Reproductive Problems of Indigenous Arsi Cattle in Selected ArsiZone’s of Oromia Regional State. Ethiopia. Global Veterinaria 7:45-53.

Desalegn TB, Gangwar SK (2011). Seroprevalence study of bovine brucellosis in Assela government dairy farm of Oromia Regional State, Ethiopia. International Journal of Science and Nature 2:692-697.

Godfroid J, Nielsen K, Saegerman C (2010). Diagnosis of brucellosis in...
livestock and wildlife. Croatian Medical Journal 51:296-305.
Hunduma D, Regassa C (2009). Seroprevalence study of bovine brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. Am. American-Eurasian Journal of Agricultural & Environmental Sciences 6:508-512.
Jerjefa T, Kelay B, Bekana M, Teshaile S, Gustafson H, Kindahl H (2009). Epidemiological study of bovine brucellosis in three agro ecological areas of Central Oromia, Ethiopia. Revue Scientifique et Technique 28:933-943.
Juyal PD, Bal MS, Singla LD (2011). Economic impact, diagnostic investigations and management of protozoal abortions in farm animals. In: All India SMVS’ Dairy Business Directory 11:39-46.
Kebede T, Ejeta G, Ameni G (2008). Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia. Revue MédVét 159:3-9.
Köppel C, Knopf L, Ryser MP, Miserez R, Thür B, Stärk KDC (2007). Serosurveillance for selected infectious disease agents in wildboars (Sus scrofa) and outdoor pigs in Switzerland. European Journal of Wildlife Research 53:212-220.
Lidia B (2008). Seroprevalence study of bovine brucellosis in Central High Land of Ethiopia, DVM Thesis, Jimma University, Jimma, Ethiopia.
Magona JW, Walubengo J, Galiwango T, Etoori A (2009). Seroprevalence and potential risk of bovine brucellosis in zero-grazing and pastoral dairy systems in Uganda. Tropical Animal Health and Production 41:1765-1771.
Matope G, Bhebhe E, John B, Muma JB, Oloya J, Madekurozwa RL, Lund A, Skjervé E (2011). Seroprevalence of brucellosis and its associated risk factors in cattle from smallholder dairy farms in Zimbabwe. Tropical Animal Health and Production 43:975-979.
Megersa B, Bifta D, Abunna F, Regassa A, Godfroid J, Skjerve E (2012). Seroepidemiological study of livestock brucellosis in a pastoral region. Epidemiology and Infection 140:887-896.
Minda AG, Gobena A, Tesfu K, Getachew T, Angella A, Gezahegne MK (2016). Seropositivity and risk factors for Brucella in dairy cows in Asella and Bishoftu towns, Oromia Regional State, Ethiopia. African Journal of Microbiology Research 10(7):203-213.
Mohammed H (2009). Seroprevalence of small ruminant brucellosis in and around Jijiga. DVM Thesis. School of Veterinary Medicine, Jimma University, Jimma, Ethiopia.
Moti Y, Tesfaye M, Haile D, Tadele T, Mezene W (2012). Bovine Brucellosis: Serological Survey in Guto-Gida District, East Wollega Zone, Ethiopia. Global Veterinary 8(2):139-143.
Nuraddis I, Kelay B, Fikre L, Merga B (2010). Seroprevalence of bovine brucellosis and its risk factors in Jimma Zone of Oromia region, southwest Ethiopia. Tropical Animal Health and Production 42:35-40.
OIE (2008). Bovine Brucellosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Office International des Epizootics, Paris. Chapter 2.4.3:624-629.
OIE (2009). Bovine brucellosis. Manual of diagnostic Tests and Vaccines for Terrestrial Animals, Office International Des Epizootics. Paris. pp. 409-435.
Poester P, Nielsen K, Samartino E (2010). Diagnosis of brucellosis. The Open Veterinary Science Journal 4:46-60.
Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007). Veterinary Medicine A text book of diseases of cattle, sheep, pigs and horses. 10th edition. London: W.B., Saunders. pp. 963-985.
Seleem MN, Boyle SM, Sriranganathan N (2010). Brucellosis A re-emerging zoonosis. Veterinary Microbiology 140:392-398.
Sintaru T (1994). The impact of brucellosis on productivity in improved dairy herd of Chaffa State Farm, Ethiopia. M.Sc Thesis, Frei University, Berlin, Germany.
Tadese Y (2003). A survey of brucellosis in sleeted area of North Gonder Zone, DVM Thesis, Addis Ababa University, Debre Zeit, Ethiopia.
Tolosa T, Regassa F Belihu K (2008). Seroprevalence study of bovine brucellosis in extensive management system in selected sites of Jimma Zone, Western Ethiopia. Bulletin of Animal Health and Production in Africa 56:25-37.