Global Scientific JOURNALS

GSJ: Volume 8, Issue 7, July 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

SEROPREVALENCE STUDY OF BRUCELLOSIS IN SMALL RUMINANTSIN JIBATDISTRICT OF WEST SHOA ZONE, OROMIA REGIONAL STATE

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ABSTRACT

A cross sectional study was conducted from October 2013 to April 2014 to determine the seroprevalence of brucellosis and associated risk factors in small ruminants in Jibat district of west shoa zone. Small ruminants were selected randomly, from purposively selected district. A total 308 blood samples were collected from local breeds of sheep and goats and serum samples from these animals were initially screened by Rose Bengal Plate Test (RBPT) and those samples found positive by RBPT were further tested by Complement Fixation Test (CFT) for confirmation. Out of the total collected serum samples, 9 (2.92%) of them were positive when they screened by RBPT. Among the 9 positive serum samples, 4 of them were confirmed as *Brucella*antibodies positive by CFT. Thus the overall seroprevalence of sheep and goats brucellosis in the study area was 1.29% (4/308). None of the risk factors studied had significant effect on the sero prevalence of the disease in study animals ($P > 0.05$).

Key words: Brucellosis, CFT, Jibat, RBPT,Seroprevalence, Small Ruminant.

INTRODUCTION

The majority of the Ethiopian rural populations are involved in Livestock production for draft power, food, transportation and as source of family income. Small ruminants productions are a sub sector of livestock production and at the farm level small ruminants serves as a sorce of family income, meat, food, wool and insurance due to high fertility, short generation interval, small feed requirement and adaptability to harsh environment conditions (Urgessa*et al*., 2012). Constraints which restrict the potential of extensive farms of small ruminants include the presence of disease of various nature and low input feeding. Among many diseases of small ruminants reproductive disease is most economically important disease (Alemu, 1995). Reproductive disease reduces the benefit which is gained from animals either by abortion or reduces the production capacity of the animals. Brucellosis is one of the reproductive diseases of sheep and goats and usually caused by *Brucellamelitensis*or by *Brucellaovis*in sheep. It can affect almost all domestic and wild mammal species and cross transmission can occur between cattle, sheep, goats, camels and other species of animals (Ghanem*et al.,*2009).

The consequences of this disease in small ruminants include infertility, mastitis and reduced milk production (Seifert, 1996) and causes abortions usually during the two last months of stage of pregnancy, premature births, retained placenta, weak offspring, weight loss, orchitis, epididimits and lameness. Brucellosis also causes economic loss through a major impediment for the trade (Radostitis*et al.,*2000). In addition to the economic losses caused by reproductive failure in a number of important livestock animals, the disease is transmitted to humans by direct contact with infected animals or their carcasses and through inhalation of the organism.

The diagnosis of brucellosis in sheep and goats is based on serological, bacteriological, allergic, and molecular methods (Simsek*et al*., 2004). The rose bengal plate test is the most common screening test for detection of brucellsis (Neilsen and Dunkan, 1990). All positive tests which are observed by screening tests are not always the true positive and there should be confirmatory tests which confirm them.

The existence of small ruminant brucellosis in Ethiopia has been reported by few researchers: 1.5% in sheep and 1.3% in goats in the central highlands (Tekelye and Kasali, 1990), 4.8% in goats and 1.9% in sheep in Adamitulu-Jido-Kombolcha District of Oromia Regional State (Tesfaye*et al*., 2012), 5.8% in goats and 3.2% in sheep in Affar region (Ashenafi*et al*., 2007), 0.87% in goats and 0% in sheep in north west Ethiopia (Ferede *et al.*, 2011*)*, 1.17% in sheep and 1.88% in goats in Borana zone (Dabassa*et al*., 2013), 5.6% in sheep and 13.2% in goats in Affar and Somali region (Teshale*et al*., 2006), 9.39% in goats and 8.77% in sheep in DirreDawa (Negash*et al*., 2012) and 7.1% in sheep and 13.6% in goats in Affar region (Adugna*et al*., 2013). Despite the occurrence of small ruminant brucellosis in different parts of our country there was no report on the occurrence of the disease in small ruminants in Jibat district of west shoa zone. Therefore the objective of this study wasto determine sero-prevalence of small ruminant brucellosis in the study district and to determine the associated risk factors with the occurrence of the disease.

STUDY METHODS

Description of the Study Area

The study was conducted in Jibat district of West Shoa zone, Oromia regional state and this district is located at 147 km to the west of Addis Ababa. It is found at an altitude of 2600 meters above sea level in the central high lands of Ethiopia. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September and a short rainy season from March to May with an average annual rainfall of 1100 mm with average temperature of 20^{o}C. According to the current document of Jibatworeda ministry of agricultural Berea (JWMAB, 2013): The total animal population of the area are: 690000 cattle, 58435 ovine, 18200 caprine, 36000 equine and 450345 poultry, respectively.

Study Population

Local breeds of sheep and goats of greater than six months of age with no previous history of vaccination were included in the study population. The study animals weremanaged under extensive production system. The age of animal was categorized in to young (from half a year to one year) and adult (beyond one year).

Study Design

A cross-sectional study was carried out from October 2013 to April 2014 to determine the sero prevalence of brucellosis and associated risk factors in sheepand goats in the study district. Data on potential risk factors such as species of animal, age, sex, history of abortion and retained fetal membrane and parity status of animals were recorded.

Sampling strategy

Simple random sampling was used to select peasant associations, house hold and individual animals from purposively selected district. The district was selected based on the population of sheep and goats and on the assecciblity of the district. Five peasant associations were randomly selected from 23 peasant associations in the district. A simple random sampling method was used on the lists of house holds that were obtained from the villages. Blood samples were collected from sheep and goats and tested for the presence of antibody against *Brucella*organismsusing serological tests.

Sample size determination

The total numbers of animalsrequiredfor the study was calculated based on theformula given by (Thrustfield, 1995). Since therewas no previous study in the prevalence of small ruminant brucellosis in the area, 50% prevalence was taken to calculatethe sample size and it was calculated at 95 % confidence interval and a desired accuracy level of 5%. Therefore, the calculated sample size was 384. However, due to some owners unwillingness to allows to collect blood samples from their animals a total of 308 blood samples were able to collected.

Study methodology

Serological tests

The Rose Bengal Plate Test (RBPT) was used as ascreening test for detection of *Brucella*antibodies in the serum and complement fixation test wasemployed to confirm whether positive animals by RBPT are the true positive or not. About 5-10 ml of blood was collected from sheep and goats by using plainvacutainer tubes. The blood samples were kept overnight at room temperature to allowed clotting. Then after the serum samples was separated from the clot and transferred to cryovials. The separated serum was stored at -20^0C at Ambo university veterinary laboratory until serological test was performed (Nielsen and Dunkan, 1990). Both the serum samples and rose bengal plate antigen were removed from the refrigerator and left at room temperatureforhalf an hour before the test was performed. Before each screening session the RBPT was validated by using a previously known positive and negative control sera.The RBPT test procedure was performed by adding 30 µl of RBPT antigen and 30 µl of test serum to each circle on the plate and mixed together and the plate was gently rotated for four minutes and after four minutes the degree of agglutination was read and interpreted as 0, +, ++, and+++ with 0 = no agglutination, + =barely visibleagglutination ,++ = fine agglutination, and +++ = coarse agglutination.Thosesamples with no agglutination (0) were recorded as negative (Nielson and Dunkan, 1990). Sera samples proved positive by thescreening test were transported to national veterinary institution for conformation by complement fixation test. The CFT reading was as complete fixation (no hemolysis) with water clear supernatant was recorded as + + + +, nearly complete fixation (75% clearing) as + + +, partial hemolysis (50%) as + + and some fixation (25% clearing) as +.Complete lack of fixation was recorded as 0.The small ruminants were considered positive if the complement test gives positive result (OIE, 2004).

Data Processing and Statistical Analysis

Data obtained from the serological tests were stored in Microsoft excel spreadsheet. These data then were analyzed by descriptive statistic using SPSS 15 version. Sheep and goats tested positive to both RBPT and CFT serially were said to be sero-positive. The individual animal

level sero-prevalence was calculated on the basis of CFT positive results divided by total number of animals tested.Analytical statistics employed was Pearson's chi-square test (x^2) and when necessaryFisher's exact test was used. These tests determine the association of risk factors with the sero-prevalence of brucellosis.

During analysis of the data sero-prevalence was considered as dependent variable and risk factors that would likelypredict the outcome variable in this case sero-prevalence were considered as independent variables.

RESULTS

Over All Prevalence

Out of 308 serum samples collected form sheep and goats,2.92% (9/308) were positive by RBPT. Among those positive samples by RBPT 4 of them were confirmed as *Brucella* positive when they are re tested by Complement Fixation Test. Thus the overall sero prevalence of small ruminant brucellosis in the study area was 1.29% (4/308).

The seroprevalence of small ruminant brucellosis was high in goats (2.38%) than in sheep (1.13%) although, there is no significant variation of seropositivity was observed between these species of animals (Table 2).

Table 2: Sero prevalence of *Brucella* antibodies in relation to species of animals.

Species of animals	No of animal examined	No of positive by CFT	Prevalence (%)	95% CI
Goat	42	1	2.38	0.42 – 12.32
Sheep	266	3	1.13	0.4 - 3.3
Total	**308**	**4**	**1.29**	
Fisher exact	P = 0.445			

There was a fact which indicates that female animals are more prone to affected than male animals. Taking account this fact in consideration, the sex of animals was taken as a risk factor for the prevalence of the disease in the current study. Although the sero prevalence of brucellosis in the female in this study was high (1.5%) as compared to male (0%) there was no statistically significant difference was observed between the sex of animals and sero prevalence of the disease (P>0.05) (Table 3).

Table 3: Sero prevalence of *Brucella* antibodies in sheep and goats in realtion to sex

Sex	No of animals examined	No of positive by CFT	Prevalence (%)	95 % CI
Female	265	4	1.5	0.6 – 3.8
Male	43	0	0	
Fisher exact	P = 0.546			

Among the many risk factors which affect the prevalence of brucellosis in various species of livestock, age of the animal is important as brucellosis is the disease of advanced age and sexually matured animals. Thus age of sheep and goats was assumed as a risk factor for the susceptibility of these animals to the disease in this study. There was no statistically significant difference between the two age groups and the sero prevalence of the disease (P>0.05), although higher sero prevalence of the disease was occurred in young animals (3.7%) as compared to adult animals (0.78%) (Table 4).

Table 4: Sero prevalence of *Brucella* antibodies in sheep and goats in relation to age

Age	No of animals examined	No of positive by CFT	Prevalence (%)	95 % CI
Adult	254	2	0.78	0.2 – 2.9
Young	54	2	3.70	1.2 – 12.55
Total	**308**	**4**	**1.3**	

Fisher exact P = 0.143

There was no pathognomic sign of brucellosis in animals, however certain signs which are shown by the animals which are affected by the disease can give a clue to diagnosis the disease tentatively. Among them history of abortion is more closely related with the prevalence of the disease. Taking account this fact in consideration, the history of abortion in the animal was taken as a risk factor for the prevalence of the disease. Statistical analysis of the result showed that there was no significant difference in the sero prevalence of the disease between animals which had history of abortion and which did not had. However, higher sero prevalence of the disease was occurred in animals which did not had history of abortion than those animals which had history of abortion (Table 5).

Table 5: Sero- prevalence of *Brucella* antibodies in sheep and goats in relation to abortion

Abortion	No of animals examined	No of positive by CFT	Prevalence (%)	95 % CI
Yes	12	0	0	
No	232	4	1.72	0.7 – 4.3

Fisher exact P = 0.816

History of retained fetal membrane was taken as risk factor for the prevalence of the disease in this study. Although, there was no statistical difference was observed between sheep and goats which had history of abortion and which did not had, higher sero prevalence of the disease was

recorded in sheep and goats which had no history of retained fetal membrane than those animals which had history of retained fetal membrane (Table 6).

Table 6: Sero-prevalence*brucella* antibodies in sheep and goats in relation to retained fetal membranes.

Retained fetal membrane	No of animals examined	No of positive by CFT	Prevalence (%)	95 % CI
Yes	4	0	0	
No	240	4	1.72	0.65 – 4.2
Fisher exact P = 0.938				

Although no significant association (P>0.05) was observed between *Brucella*seropositivity and parity status of animals, higher seroprevalence of *brucella* antibody was observed in animals with 5^{th} parity compared to those in their 0 parity (Table 7).

Table 7: Sero-prevalence of *brucella* antibodies in sheep and goats in relation to parity of the animals.

Types of parity	No of animals examined	No of positive by CFT	Prevalence(%)	95 % CI
No birth	40	2	5	1.4 – 16.5
One	76	1	1.3	0.23 – 7.09
Two	91	0	0	
Three	40	0	0	
Five	15	1	6.7	1.19 – 29. 8
Six	3	0	0	
X^2 7.8 P = 0.15				

DISCUSSION

Among a total of 308 blood samples collected from local breed of sheep and goats of above six months of age without history of vaccination, 9 (2.92%) of them were positive when they are initially screened with Rose Bengal Plate Test. Since RBPT is a screening test for the diagnosis of brucellosis in small ruminants all positive result which was obtained by this test may not a true positive. Thus positive samples by RBPT were further tested by Complement Fixation Test (CFT) for confirmation at national veterinary institution in this study, since CFT has high accuracy in the detection of the brucella antibodies (Chin *et al*., 1991).Out of9 (2.92%) serum samples which were positive by RBPT, 4 of them where confirmed as *Brucella* positive by CFT. The over allsero prevalence was calculated by dividing all the sero positive animals by CFT to the total sampled animals. Thus the overall seroprevalence of sheep and goats brucellosis in the study area was 1.29% (4/308).

The 1.29% current recorded sero prevalence of brucellosis in sheep and goats (1.13% in sheep and 2.38% in goats) is lower than the 3.8% sero prevalence of the disease by CFT conducted inAdamitulu-Jido-Kombolcha District of Oromia Regional State by Tesfaye*et al.*,(2012), the seropravelence of 4.8% by CFT in the pastoral region of Affar by Ashenafi*et al.*,(2007), 9.7% sero prevalence by ELISA in Afar by Teshale*et al.*, (2006) and the sero prevalence of 1.56% by CFT in Yabello district of Bornan zone by Dabassa*et al.*, (2013). This lower prevalence of the disease is might be due to the small sample size in the present study or due to the management and animal production system, in which few animals are raised separately in central high land of Ethiopia.

All the above researchers conducted the investigation of the disease in pastoral area of the country by the same test except Teshale*et al.*, (2006), who uses ELISA as confirmatory test. In pastoral areas large flock of animals are grazing together, mass movement of animals from one area to the other in order to get pastures for their animals and commingling of animals at communal pastures and watering areas (Martin and Aitken, 1991).All this character of pastoral

areas of the country facilitates the spread of the disease between the animals and over a large distance of the earth. Thus this condition has an effect role on the increment of the prevalence of the disease in the pastoral part of the country. But in contrast to the nomadic way of life stock production system, in central high land of Ethiopia livestock productions is characterized by mixed farming, in which fewer animals are raised separately.In addition to this farmers did not go with their animals from one place to other place to search feed for their animals in central Ethiopia. This results the low prevalence of the disease in the area in compare to the pastoral part of the country. The higher seroprevalence of the disease in the pastoral area by Teshale*et al*., (2006), than others was due to I – ELISA is higher in specificity and sensitivity in diagnosis of the diseaeas explained by Teshale*et al*., (2006).

In contrast to the above the current sero prevalence recorded is comparable with the seroprevalence of the 1.5% in sheep and 1.3% in goats recorded by Tekelye and Kasali, (1990) in central highland of Ethiopia. This comparable recorded sero prevalence is due to the same animal management practice in the area.

Statistically no significant variation was observed in sero prevalence of brucellosis between the species of animals. This might be due to the smaller sample of goats in this study. However, the higher sero prevalence of the disease in goats than in sheep is due to the fact that the greater susceptibility of goats in *brucella* infection and goats excrete the organisms for a long period of time, unlike sheep, which reduces potential for disease spread among sheep flock (Radostitis*et al*., 2000). Even though there was no statistically significant difference between sheep and goats in susceptible to the disease in the current study this finding is in agreement with the work done by Dabassa*et al*.,(2013) as it reported no observable difference in the prevalence of brucellosis between sheep and goats. On the other hand Ashenafi*et al*.,(2007), Adugna*et al*., (2013) and Tesfaye*et al*., (2012) reported that statistical significant higher sero prevalence of the disease in goats than in sheep.

In the current study all infected sheep and goat were female animals. Statistical analysis showed that there was no significant difference in the sero prevalence of *Brucella* antibodies and sex of animals ($p > 0.05$). The prevalence of the disease according to the sex of animals was 1.5% in

female animals and 0% in male animals.Inspite of statistically insignificant, the higher prevalence of the disease in female animals than male animals could be due to more females were available in samples or it connected with the higher concentration of sugar erythritol in the reproductive organs of female animals than in male reproductive organs, which enhance the growth of *Brucella*organisms (Radostitis*et al*., 2006). This result is in agreement with Teshale*et al*., (2006*),* Ashenafi*et al*., (2007), Dabassa*et al*., (2013) and Adugna*et al*., (2013).

The prevalence of the disease in young and adult animals was 3.9 and 0.78 per cent, respectively. Statistical analysis showed that there was no significant difference in sero prevalence of *Brucella* antibodies and age groups (p> 0.05). The higher sero prevalence of the disease in young animals than adult animals in this finding might be due to the presence of the disease in small ruminants in the study district that animals of all age groups can be are affected from contaminated environment by positive animals, since there was no vaccination given for the animals as part of control and prevention methods in that area. The other reason might be due to young animals acquire the infection through suckling of their infected dams Grillo*et al*., (1997). But, this finding contradicted with the finding of Teshale*et al., (*2006) and Ashenafi*et al*.,(2007) who reported a statistically significant difference between the sero prevalence of the disease and among young and adult age groups. However, this insignificant difference between young and adult animal is in agreement with the finding ofTekelue*et al*., (2013) and Adugna*et al*., (2013).

Statistically no significant variation was observed in sero prevalence of brucellosis between sheep and goats which had experienced history of abortion and retained fetal membrane and animals which did not show such symptoms. Brucellosis is mainly characterized by abortion and retained fetal membrane in animals, however, it is not associated with seropositivity in this study. This finding contradicts with Radiostitis*et al*., (2000). The higher sero prevalence of the disease in those animals with out history of abortion than animals which had history of abortion is might be abortion could be due to other factors which causes abortion in small ruminants. This finding contradicts with the finding of Tesfaye*et al.,* (2012).

CONCLUSION AND RECOMMENDATIONS

The study showed that low prevalence of brucellosis in small ruminants in study area. None of the risk factors studied had significant effect on the sero prevalence of the disease in study animals ($P > 0.05$). The prevalence of the disease in small ruminants in the study area indicated that since brucellosis is a contagious disease and cross transmission can occur between cattle, sheep, goats and other livestock species it have the capability to spread and infect other healthy livestock of the area.

Thus based on the above conclusions the following recommendations were forwarded

- Since the seropositive animals in the study district have the capacity to contaminate the enviroment and cross transmission can occour between domestic animals, control measures should be taken before the disease can be expanded.
- Seroprevalence of the disease in other domestic animals of the study area was not reported, so it is essential to diagnose the status of the disease in other live stock and humans in the study area.
- The prevalence of brucellosis in small ruminants in the study area was low, so it needs to control the disease before it spreads.
- Since, *Brucellamelitensis* is the most pathogenic *brucella*species for humans, it is better if the society, especially those at higher risk groups should be aware of the zoonotic importance of the disease through veterinary extension or through other concerned bodies.
- Further epidemiological studies should be conducted in order to know the exact biovars involved in sheep and goats in the study areas.

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