Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.7, No.9, 2017

Seroprevalence of Caprine Brucellosis and Its Associated Risk Factor in Mirab Abay district, South Eastern Ethiopia

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Abstract
Brucellosis is an infectious bacterial zoonotic disease resulting in a serious economic loss in animal production sector and deterioration of public health. There is no previous study information on Seroprevalence of caprine brucellosis in Mirab Abay district. The cross sectional study was conducted with objective of determining prevalence and associated risk factors for caprine brucellosis from four selected peasant association (PAs) in Mirab Abaya districts through February to April in 2009. A total of 389 blood samples collected from goats and tested for the presence of brucella antibodies. Sera were first screened for brucella antibodies by modified Rose Bengal Plate Test (mRBPT) and positive sera were further subjected to test using the Complement Fixation Test (CFT) for confirmation. Out of 26 (6.7%) mRBPT positive sera subjected to retest by using CFT confirmed that 20 (5.1%) were positive for the caprine brucellosis. Among variety of factors considered, age of goats was found to be significantly associated with seropositivity ($P<0.05$). On the other hand, statistical analysis of the data showed no significant difference in seroprevalence to *Brucella* antibodies with that of herd size and sex of animal examined ($P>0.05$). The results revealed that established circulation of the organism in the study area. Thus, appropriate control measures needs due consideration.
Keywords: Brucellosis, Caprine, CFT, mRBPT, seroprevalence, Mirab Abaya, Ethiopia.

INTRODUCTION

Ethiopia is a country with different agro-ecological zones where considerable populations of small ruminants are raised. The small ruminants of the country are estimated to be 29.33 million heads of sheep and 29.11 million heads of goats of these low lands mostly pastoral areas have 25% sheep and 73 % of goats (CSA, 2015).

Even though small ruminants represent huge resources the level of production of this important asset is not commensurate with its potential and number owing to inadequate feed resources, poor management and disease (Ibrahim, 1998). Among these factors, which limit the economic return from small ruminant production, diseases stands in the front lines (Hirsh and Zee, 1999). One of such infectious disease that hampers small ruminant production causing increased gap between demand for and supply of ruminant services is brucellosis (Renukaradhya *et al*., 2002).

Brucellosis is a bacterial zoonotic infection and is amongst the most important diseases, in terms of loss to economy that affects sheep and goat population in the developing countries (Lone *et al.,* 2013).The genus Brucella is now considered to contain six species: namely, *B. abortus*, *B. melitensis, B. suis, B. ovis, B. canis* and *B. neotomae*. *Brucella melitensis* (biovars 1, 2 or 3) is the main causative agent of caprine and ovine brucellosis and it is highly pathogenic for humans causing one of the most serious zoonoses in the world (OIE, 2000; Ferede *et al*., 2011).

In animals, brucellosis mainly affects reproduction and fertility, reduces the survival of new-borns, and diminishes milk yield. The mortality of adult animals is insignificant. In human beings, the symptoms of disease are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, night sweats and chills, marked asthenia and anorexia (Hugh-Jones, 2000; Rahman *et al*.,2011).

In humans, brucellosis can be a serious and debilitating disease with spectrum of non specific multiple symptoms. These symptoms could be due to nephritis, dermatitis, vasculitis, lymphadenopathy, deep vein thrombosis, granulomatous hepatitis, osteomyelitis, or formation of abscesses in internal organs (Gwida *et al.,* 2012; Robayo and Esubalew, 2017). Occasionally, this disease might also lead to death due to the development of endocarditis (Sprague *et al.,* 2012; Robayo and Esubalew, 2017).

Caprine brucellosis in sub-Saharan Africa is less commonly reported than brucellosis in cattle (McDermott and Arimi, 2002; Asmare *et al.,* 2013). Similarly published information on goat brucellosis is scarce and is limited to few districts in Ethiopia (Tekelye and Kasali, 1990; Yibeltal, 2005;Teshale *et al*., 2006; Ashenafi *et al*., 2007; Ashagrie *et al*., 2011; Megersa *et al.,*2011;Asmare *et al*., 2013).

In view of this, the study was conducted to determine seroprevalence and associated risk factor for occurrence of caprine brucellosis in Mirab Abaya district in Gamo Gofa zone.

MATERIALS AND METHODS

Study area:

The study was conducted in Mirab Abaya district, South Eastern Ethiopia, which is located at about 405 km and

62 km from Addis Ababa and Arbaminch respectively. According to the CSA (2004) the district has a total surface area of 1, 182, 73 km^2 and supports 232, 432 residents whose main occupation is subsistence farming. District's agricultural planning office classified the study area into agro climatic zones "Kolla" (lowland) "Weyna dega" (Mid land) and "Dega" (High lands) accounting to 37%, 22% and 41% respectively. Altitude ranges from 1,100- 3,000 meters above sea level with irregular topography of mountain, marshy areas, steeply slope and water covered parts. Arbaminch state farm meterological station recorded two rainy seasons in the area the long rainy season that extends from February to April with mean annual rainfall of 673- 934 mm and mean annual temperature 15- 31^0C.

The vegetation cover of the area includes various bush formation and deciduous forest. The soil type of the area is also classified as sandy and clay-sandy soil. According to the record by district agricultural planning office the livestock population of the area is estimated about 70,098 cattle; 11, 713 sheep; 17, 351 goats; 2, 775 equines, 34, 684 chickens and 5, 982 beehives are also registered in the district. Almost all people in the area engaged in mixed crop livestock production with exception of lakes shores pastoral community (CSA, 2004).

Study Animals

The study was carried out from February to April 2009 on randomly sampled goat poulation from selected four different peasant associations in Mirab Abaya district. Serum samples were collected from a total of 389 heads of goat that are greater than or equal to six months of age. Since no brucella vaccine has been used in the study area, all of the study animals were unvaccinated.

Study design

A cross-sectional study was conducted in selected peasant associations in Mirab Abaya district of Gamo Gofa Zone to determine the seroprevalence of caprine brucellosis from February to April 2009. Blood sample were collected from local breed of goats that were above 6 months of age.

Sample size and sampling methodology

The sample size required for this study was determined depending on the expected prevalence of caprine brucellosis and the desired absolute precision by the formula given by Thrusfield (2005). Therefore, using 95% confidence interval, 5% precision and 50% expected prevalence, the number of goats needed to demonstrate sero prevalence of caprine brucellosis in Mirab Abay district were 384 goats, but 389 goats were included for this study. Simple random sampling was used to sample individual animals from selected herds of PAs, Mirab Abay.

Sample collection and serological test

Approximately 8 ml of blood sample was collected from the jugular vein for serological examination, using plain vacutainer tubes and needles. The tubes were labelled and left tilted overnight at room temperature to allow for clotting. Next morning, sera was decanted into single sterile cryovial, labeled, and transported in cold chain to the laboratory where Modified Rose Bengal Plate Test (mRBPT) and complement fixation test (CFT) was conducted. Separated sera were stored at -20°C until serological test undertaken. The mRBPT was performed at Soddo Regional Veterinary Laboratory and CFT was done at National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia.

To improve the sensitivity of the mRBPT, simple modification, increasing the volume sera to be tested has been recommended and used in this study. It is the use of serum of 75 and 25 µl of antigen and named mRBPT. In the screening, mRBPT was used, that 75 µl of serum and 25 µl of antigen on white enamel plate and mixed thoroughly with the applicator stick. It was shacked and waits for four minutes and any agglutination reaction was graded as positive (OIE, 2004).

The sera that had been positive to mRBPT were retested by CFT for confirmation at NAHDIC. Preparation of the reagents and CFT test proper were done according to protocols recommended by OIE (2004).

Data analysis

The data collected in the field were entered into a computer on a Microsoft Excel spreadsheet. Statistical analysis was performed using 'Statistical package for the social sciences' (SPSS), version 11.5 for windows. Categorical variables (sex, age and PAs) were expressed in percentages. The prevalence proportion was calculated as the number of animals testing positive by the mRBPT/CFT, divided by the total number of animals tested. The association between each risk factor and the outcome variable was assessed using the Chi-square ($\chi 2$) test. For all analyses, a *p*-value of less than 0.05 was taken as significant.

Results

Serum sample of 389 goats were analysed and the prevalence of brucellosis was recorded by mRBPT (Table-1) and CFT (Table-2). Since mRBPT is highly sensitive and can pick many false positive, CFT used as

confirmation. Therefore, the overall prevalence calculated based on the later test was considered as the seroprevalence of caprine brucellosis (Table-2). Based on this, the overall seroprevalence of caprine brucellosis was recorded in this study as 5.1% by CFT.

On sex distribution, none of male samples (0 %) out of 33 samples screened were positive while 20 (5.6%) female samples were positive out of the 356 screened. There was no significant association between *Brucella* infection and sex (Table-3).

On age, 7 (3.9%) out of 180 screened samples were positive in the age band of 6-36 months (young) while 19(9.1%) out of 209-screened samples were positive in the age band of greater than 3 years. There was a significant association between *Brucella* infection and age (Table-3).

On herd size distribution, 5(3.01%) out of 166 screened samples were positive in the herd size band of <5. In the herd size band 5-10, 13 (6.8%) out of 191-screened samples were positive while 8(25%) out of 32 samples were positive in the herd size band of goats above ten. In this study, there was no a significant association between *Brucella* infection and herd size (Table-3).

Table-1: Individual seroprevalence of brucellosis for goats subjected to modified Rose Bengal Plate Tests in selected peasant associations in Mirab Abaya district.

Finding	Peasant Association			
	Omo Lante, n=96	Faragosa, n=98	Yake, n=97	Korga, n=98
Positive	5	0	2	19
Negative	91	98	95	79
Prevalence	5.2%	0%	2.1%	19.4%

Table-2: The overall seroprevalence of caprine brucellosis in Mirab Abaya.

Nr of sera tested	mRBPT positive	CFT positive	mRBPT $^{+}$/CFT^{+}	Prevalence
389	26 (6.7%)	20 (5.1%)	20 (5.1%)	5.1%

Table 3: Seroprevalence of caprine brucellosis according to age, sex and herd size in Mirab Abaya .

Risk factor	**Total Number**	**mRBPT positive**	**CFT positive**
Sex			
Male	33	-	-
Female	356	26 (7.3%)	20 (5.6%)
Age			
Young(6 month- 3 years)	180	7 (3.9%)	3 (1.7%)
Adult(>3 years)	209	19 (9.1%)	17 (8.1%)*
Herd size			
< 5	166	5(3.01%)	4(2.4%)
5-10	191	13(6.8%)	10(5.2%)
>10	32	8(25%)	6(18.75%)

*P<0.05

DISCUSSION

The seroprevalence of caprine brucellosis was determined based on the results of CFT which is recommended confirmatory test for brucellosis. Vaccination against brucellosis had never been implemented in the country. Therefore, any seropositvity was considered as previous exposure to infection. The results of sera sample subjected to mRBPT from Mirab Abaya district in four selected peasant association (PA's) *i.e.* Omo Lante, Faragosa, Yake and Korga have 5.2 %, 0%, 2.1% and 19.4% respectively. Among PA's, Korga had higher prevalence than other which was also confirmed similarly by CFT.

The overall seroprevalence of 5.1% indicates that there has been an active brucella infection circulating in the study area. The 5.1% prevalence of brucellosis in goats observed in the study was fair consent with result of many of the previous studies in karnatake in India (Renukaradhya *et. al*, 2002) in United Arab Emirate (Benkirane, 2006) and Yemen (Refai, 2002). This finding was also comparable to results observed in East Africa. The prevalence reported in Eritrea is 3.8% and in Somali 5.29% (Falade and Hussein, 1997). In Ethiopian scenario, again the overall seroprevalence is in agreement with work done in Afar pastoral areas of 5.8 % (Ashenafi *et al*., 2007) and 4.7% in SNNPR (Mengistu, 2007). However, it was lower than the observation recorded by Gumi *et al*.(2013) and higher than report by Megersa *et al*. (2011) and that of Tekelye and Kasali (1990) who have reported prevalence rate of 1.3% from central high lands. The difference in seroprevalence between this study and previous studies might be due to agro ecological differences of study areas, sample size, animal management and production systems.

Most PA's of South, Nation and Nationalities People's Regional State had low prevalence of caprine brucellosis (Mengistu, 2007). In these study, the highest prevalence was recorded in Korga PA's in Mirab Abaya district although the difference was not statistically significant. This could be attributed due to husbandry practices that allow the mixing of flocks of different origin at communal watering and grazing areas coupled with possibility of having unrestricted animal movement in trade onto adjacent pastoral areas and mixing of animals in market where accentuating infection. Moreover, it might also be an indicator for the established circulation of the organism in the flock with no control measures taking place and relatively large herd size. This is not to say large herd size and other related factor always result in higher seropositivity of taken sample during brucellosis surveillance. However, it clearly indicates that during circulation of the organisms in the flocks if no control measures are taking place, it will enhance inter and intra species spread of brucellosis with in flock which commingling up higher prevalence in the area or peasant association. It was also evidenced that during laboratory analysis if the existence of anti-*Brucella* anti bodies was detected the probability of obtaining other sero positive result increases within a given herd.

It has been reported that brucellosis is essentially a disease of sexually mature animals (Quinn *et.al,* 2000). Sexually mature and pregnant animals are more prone to infection and brucellosis than sexually immature animals of either sex (Radostits *et.al,* 2000). On the other hand, younger animals tend to be more resistant to infection and frequently clear the established infections, although latent infections could occur (Walker, 1999). This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of brucella organisms tend to increase with age and sexual maturity (Walker, 1999). In this study, statistically significant difference was recorded in the prevalence of brucellosis between young and adult goats. Higher prevalence was found in adults than in younger ones ($P<0.05$). Similar findings were also reported by Teshale *et al*.(2006), Ashenafi *et al*. (2007), Ferede *et al*.(2011) and Ashagrie *et al*. (2011).

This study revealed that mRBT positive sera were found only in female goats. The absence of male seroreactor animals in this study could probably be due to the small number of males (N=33) tested as compared to the number of females (n=356). It has also been reported that males are usually resistant than female animals to *Brucella* infection (Radostitis *et al.,* 2000). Hrish and Zee (1999) have described that male animals are less susceptible to brucella infection due to the absence of erythritol. The absence of statistical difference between the two sex groups was similarly reported from Bahir Dar (Ferede *et al*., 2011), pastoral region of Afar (Teshale *et al*.,2006) and South Omo (Ashagrie *et al*., 2011). Although herd size is documented by Radostits *et al*. (2000) as a main factor for transmission of Brucella infection , the observed difference among herd size was not statistically significant in these study ($P>0.05$).

CONCLUSION

In conclusion, sero survey of brucellosis in goat revealed the existence of sero positive reactors in Mirab Abaya district with overall seroprevalence rate of 5.1%. In line with absence of hygienic measures like use of isolated kidding areas, appropriate disposal of aborted materials and removal of foci of infection can accentuate the situation of brucellosis as contamination of pasture and water occurs. Thus, it is justifiable to recommend that putting in place measures to minimize the risk and control of the brucellosis in goat in the study area.

ACKNOWLEDGEMENTS

The author is grateful for the technical and material support of the staffs of Soddo Regional Veterinary Diagnostic Laboratory and National Animal Health Diagnostic and Investigation Center (NAHDIC).

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