

Sero-Prevalence of Small Ruminant Brucellosis in Three Selected Districts of Somali Region, Eastern Ethiopia

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Abstract

A cross-sectional study was conducted from November, 2011 to March, 2012 in three selected districts of Jijiga zone, Somali region, Ethiopia, aimed at determining the sero-prevalence and to identify potential risk factors of small ruminant Brucellosis. A total of 291 serum samples (100 from sheep and 191 from goats) were collected from extensive management system with no history of vaccination. All serum samples were initially screened by RBPT and positive reactors to RBPT were further tested by CFT for confirmation. Accordingly, the overall prevalence of Brucellosis in small ruminants was 1.37% (4/291). Rose Bengal Plate Test detected 1.72% (5/291) of the samples as sero-positive. Up on further testing by CFT only 1.37% (4/291) was positive. Comparison of seroprevalence of small ruminant Brucellosis was carried out for different age, species, animal origin and sex groups. Sero-prevalence was higher in male compared to female. Brucellosis was also detected in all three selected districts of the region. Nevertheless, no statistically significance difference of small ruminant Brucellosis was observed among species, sex, age groups as well as animal origin ($P > 0.05$). The result of the present study revealed that the seroprevalence of small ruminant Brucellosis in the study area was very low. However, the existence of the disease in the study area has possible risk of spread in the future. Accordingly, elimination of positive seroreactors has been recommended to control the spread of Brucellosis in these species of animals.

Keywords: Brucellosis; Small Ruminants; Sero-prevalence; Risk Factors

Introduction

Ethiopia is an agricultural based country and owns huge number of small animals which is estimated to be over 48.2 million head of sheep and goats. Majority of these animals are largely concentrated in pastoralists areas of the country [1]. The livestock subsector contributes some 45% of agricultural GDP, 15-18% of national GDP and 5-17% total exports [2]. Sheep and goats are highly adaptable to broad range of environmental conditions. Moreover, low cost of production, requirement of little land and higher prolificacy made them attractive asset for development. Investment in sheep and goats avoid losses due to high inflation rates that are found in unstable economies of many developing countries like Ethiopia [1].

In spite of these large small ruminant population sizes, the country fails to optimally utilize these resources as the sector is suffering lower productivity due to various factors in which diseases stand front line. One of the diseases that hamper the productivity of small ruminant is Brucellosis [3]. The economic and public health impact of Brucellosis remains of particular concern in developing countries. The disease poses a barrier to trade of animals and animal products, represents a public health hazard, and is an impediment to free animal movement [4].

Brucellosis is a bacterial disease, caused by members of the genus *Brucella*. *Brucella* is facultative intracellular gram negative cocco-bacilli. The *Brucella* genus is composed of 12 recognized species after isolation and identification of novel species from mandibular lymph nodes of the red fox [5] and in small ruminants *B. melitensis* is the most common species in developing countries and is associated with clinically apparent disease in humans [6]. Most breeds of goats are readily infected by *B. melitensis* but sheep breeds vary greatly in susceptibility [7]. Small ruminants infected by ingestion of contaminated feed or water and the consequences of the infection are determined by the virulence of the bacteria, resistance and reproductive status of the host [8].

Brucellosis considered an neglected zoonosis by the world health organization and has been identified as having the highest public health burden across all sections of the community [9]. In human, Brucellosis is always caused by *B. melitensis* (cause Undulant or Malta fever) followed by *B. suis*, *B. abortus* and *B. canis* [10]. The disease is transmitted to man mainly by direct contact with infected livestock or through consumption of raw or uncooked animal products. It causes a systemic infection with clinical manifestations as fever, sweats, fatigue and joint pain [11].

Clinically small ruminant Brucellosis is characterized by abortion and birth of weak offspring (in all females) and acute orchitis and epididymitis with later infertility in males. *B. ovis* is also an important cause of orchitis and epididymitis in sheep but it is not recognized as a cause of natural infection in goats [12]. In chronic cases animals generally abort once, although reinvasion can occur in subsequent pregnancies where the organisms are shed with the fetal membranes and fluids. On the other hand non pregnant animals of sheep and goats develop self-limiting or become latent carriers [13]. Despite the advances made in surveillance and control, the prevalence of Brucellosis is increasing in many developing countries due to various sanitary, socio-economic and political factors [14].

Despite the huge population of small ruminants in different regions of Ethiopia, the research conducted in small ruminates Brucellosis is not adequate to support and represent the various aspect of the diseases in Ethiopia. Therefore, the objectives of this study is to estimate the sero-prevalence of brucellosis in small ruminants and to investigate the importance and degree of involvement of host related possible risk factors in the occurrence of Brucellosis in the study area.

Materials and Methods

Study Area

The study was conducted in three purposively selected districts of Jijiga zone, namely Awbarre, Jijiga and Gursum. Jijiga is the capital city of Somali regional state which is found in the eastern part of Ethiopia about 630 km from Addis Ababa. Jijiga is situated at an altitude ranging from 1,660 to 1,710 meter above sea level at geographic coordinates of approximately 9°20' North latitude and 45°56' East longitude. The climate of Jijiga is semi-arid type which is characterized by high temperature and low rainfall. The mean annual temperature and mean annual rainfall is about 22 °C and 543mm respectively. Farming system of the area includes mixed crop livestock production [15].

Study Population

There are about 503,881 goats and 666,130 sheep in Jijiga zone including jijiga district [16]. The study population consisted of small ruminants kept under extensive management system in three purposively selected districts of Jijiga zone. The animals under study comprised the indigenous Somali goats and black Ogaden sheep. Sheep and goats which were above 6 months of age, with no history of vaccination against brucellosis were included in the study. Then individual animal age, species, sex category were recorded. Based on their sexual maturity animals were classified into ≤ 2 years, between 3-4 years and >4 years, respectively.

Study Design

A cross sectional study was carried out from November, 2011 to March 2012 to determine the sero-prevalence of Brucellosis in small ruminants kept under extensive management system and to investigate potential risk factors associated with the occurrence of the disease in the study areas. Multistage sampling technique was used in the survey of sheep and goat brucellosis. The Peasant association (PAs) was considered as primary unit, the herds as secondary units and individual animals as tertiary units. Sheep and goats herds in 9 PAs from three districts were sampled during the study based on the livestock population of each district.

Sample Size Determination and Sampling Method

Sample size was determined using a method recommended by Thrusfield [17]. Accordingly, the estimated sample size was 230 animals, based on the expected Brucellosis prevalence of 15% from previous preliminary reports by Megersa *et al.* [18] and 0.05 desired absolute precision at 95% level of confidence.

$$n = \frac{1.962 \times p_{\text{exp}}(1 - p_{\text{exp}})}{d^2}$$

Where: n= required sample size, p_{exp} = expected prevalence and d^2 = desired absolute precision

However, to increase the precision 61 sera samples were added and a total of 291 sera samples were collected from a randomly selected 100 sheep and 191 goats from purposively selected three districts (Awbarre, Jijiga and Gursum) of Jijiga zone. During sampling, both sexes and different age groups with no history of previous vaccination were considered so as to make the sampling representative with uniform distribution.

Study Methodology

Sample Collection, Transportation and Storage: Approximately 5-10ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tubes, needle holder and needles. The blood from each animal was labeled and left tilted overnight at room temperature to allow for clotting. Next morning, sera were removed from the clots by siphoning them in to sterile cryovials. The sera samples were then shipped to Jijiga regional veterinary diagnostic and research laboratory, in an ice box and stored at -20 °C, until required for testing.

Laboratory Tests

Rose Bengal Plate Test: For the Rosen Bengal plate test, brucella antigen from Veterinary laboratories agency, Addle Stone, United Kingdom was used and positive as well as negative control sera were used during the test. All serum samples collected were screened using the RBPT. Briefly 30µl of stained rose Bengal antigen was dispensed on to card plate and then 75µl of sera samples were dropped alongside the stained rose Bengal Brucella antigen. By using the tip of the automatic micropipette tips, the sera were mixed and examined for agglutination. Positive and negative controls were employed for interpretation of the results. Agglutinations were recorded as 0, +, ++ and +++ according to the degree of agglutination [19]. A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination and +++ indicates coarse agglutination. Those samples with no agglutination (0) were recorded as negative while those with +, ++ and +++ were recorded as positive.

Complement fixation test: All sera which tested positive by the RBPT were further retested, using the CFT, for confirmation. Standard *B. abortus* antigen for CFT (from the Veterinary Laboratories Agency, Addle stone, United Kingdom), Amboceptor and sheep red blood cells (SRBCs) obtained from National Veterinary Institute (NVI), Debre Zeit, Ethiopia were used to detect the presence of Brucella antibodies against Brucella antigen in the sera. Similarly, the control sera and complement used in this test were also obtained from NVI, Debre Zeit, Ethiopia. The following sera were classified as positive ([7] OIE, 2009): sera with a strong reaction of approximately 100% fixation of the complement (4+), at dilution of 1:5; sera with about 25% fixation of the complement (+3), at dilution of 1:20; sera with about 50% fixation of the complement (+2), at a dilution of 1:10.

Data Analysis

All the data obtained from the study were entered in to Ms excel spreadsheets and analyzed using STATA® 12.0 statistical software program. The sero-prevalence were calculated by dividing the proportion of sheep and goats whose sera was found positive to CFT by the total number of sample size, multiplied by 100. The association between each risk factor and the outcome variable was assessed using chi-square (χ^2) test. For all analysis a *p*-value of less than 0.05 was taken as significant.

Results

A total of 291 serum samples (100 from sheep and 191 from goats) were collected from small ruminants with no history of vaccination, kept under extensive management system. All serum samples were initially screened by RBPT and positive reactors for RBPT were further tested by CFT for confirmation. Accordingly, the overall prevalence of Brucellosis in small ruminants was 1.37%. Rose Bengal plate test detected 5 (1.72%, 95% CI: 0.22-3.22) of the samples as sero-positive. Upon further testing by CFT only 4 (1.37% 95% CI: 0.02-2.72) were positive (Table 1).

Comparison of seroprevalence of small ruminant Brucellosis was carried out for different Species, animal origin, Sex and Age groups. The study showed a higher sero-prevalence (by CFT) of Brucellosis in goats than in sheep. The prevalence was 1.5% in goats while it was showing 1% in sheep (Table 1).

In this study, Brucellosis was detected in all three districts, the highest prevalence was recorded in Gursum (2%) while the lowest prevalence were in Awbarre (1.1%) followed by Jijiga (1.0%) (Table 1). Based on sex, the highest prevalence was found in male (1.57%) than female (1.32%). On the other hand a prevalence of 1.9% was observed in the age group of 3-4 years and 1.41% in younger animals (<2 years) (Table 2).

Variables		Sera tested	No RBPT + (%)	No CFT + (%)	95% C. I.	χ^2	P-value
Species	Sheep	100	1 (1.00)	1 (1.00)	0.02-5.4	0.1577	0.691
	Goats	191	4 (2.09)	3 (1.57)	0.32-4.5		
	Total	291	5 (1.72)	4 (1.37)			
Districts	Awbarre	91	2 (2.19)	1 (1.10)	0.02-6.0	0.4430	0.801
	Jijiga	100	1 (1.00)	1 (1.00)	0.02-5.4		
	Gursum	100	2 (2.00)	2 (2.00)	.002-.0704		
	Total	291	5 (1.72)	4 (1.37)			

Table 1: Confirmed sero-prevalence of Brucellosis based on species and district of small ruminants in jijiga zone

Variables		Sera tested	No RBPT + (%)	No CFT + (%)	95% C. I.	χ^2	P-value
Sex	Female	224	3 (1.32)	3 (1.32)	0.02-3.9	0.0214	0.884
	Male	63	2 (3.17)	1 (1.56)	0.04-8.5		
	Total	291	5 (1.72)	4 (1.37)			
Age	≤ 2 years	71	1 (1.41)	1 (1.41)	0.03-7.6	1.1843	0.553
	3-4 years	157	4 (2.55)	3 (1.9)	0.3-5.5		
	>4 years	62	0 (0)	0 (0)	-		
	Total	291	5 (1.72)	4 (1.37)			

Table 2: Confirmed sero-prevalence of Brucellosis based on the sex and age of the small ruminants

Analysis for the association of different risk factors with sero-prevalence of Brucellosis in the study area, such as species, animal origin, sex and age groups were performed. Nevertheless, statistically significance difference were not observed between species, animal origin, sex and age groups as well ($p>0.05$).

Discussion

Based on the result obtained from the study, the overall sero-prevalence of small ruminant Brucellosis was 1.72% (95% CI: 0.22-3.22) by RBPT and 1.37% (95% CI: 0.02-2.72) by CFT. This result is in line with the report of Tsegaye *et al.* [20] which report 1.99% and 1.76% Brucellosis using RBPT and CFT, respectively. Similarly Dabassa *et al.* [21], indicate the respective Brucellosis sero-prevalence of 2.34% and 1.56% by RBPT and CFT.

In the study, there was no statistically significant ($p>0.05$) difference in the prevalence of Brucellosis within the species of small ruminants (Table 1). However, the reports of Adugna *et al.* [22] and Teshale *et al.* [23] indicates higher ($p<0.05$) sero-prevalence of Brucellosis in goats than in sheep. Likewise, Radostitis *et al.* [24] explain the possible higher prevalence of Brucellosis in goats than in sheep is due to the greater susceptibility of goats to *Brucella* infection than sheep and the fact that unlike goats sheep do not excrete the *Brucella* organisms for longer periods of time which in turn can reduce the spread potential of the disease among sheep flock. The inconsistency among various studies could be due to the different sample proportion of sheep and goat which could affect the prevalence of the diseases.

The individual Sero-prevalence in the study was indicate 1.00% (95% CI: 0.02-5.4) in sheep and 1.57% (95% CI: 0.32-4.5) in goats (Table 1). This level of sero-prevalence is in agreement with some of previous studies in Ethiopia. Megersa *et al.* [18] in Jijiga, reported a sero-prevalence of 1.2% in sheep and 1.9% in goats; similar finding is reported by Teshale *et al.* [23] which indicate a sero-prevalence of 1.6% in sheep and 1.7% in goats in Somali region. Likewise, Yesuf *et al.* [25] and Megersa *et al.* [26] reported sheep Brucellosis of 1.5% and goat Brucellosis of 1.6%, respectively.

On the other hand, the overall sero-prevalence of small ruminants in this study was relatively higher than 0% in sheep and 0.5% in goats Brucellosis sero-prevalence by Ferede *et al.* [27] in and around Bahirdar and 0.5% in sheep and 0.87% in goats by Amenu *et al.* [28] in Arsi Negele district. On the contrary, it is lower than 4.6% and 9.7% of Brucellosis prevalence from other parts of the country by Deddefo *et al.* [29] and Teshale *et al.* [23] respectively. This difference could be due to difference in agro-ecological location, sample size and animal management practices. Besides these, lack of hygienic measures against disease occurrence in the other study areas may contribute to the relatively higher prevalence of small ruminant Brucellosis.

Similarly, the sero-prevalence of this study was relatively lower than that of Adugna *et al.* [22] with respective prevalence 7.1 and 13.6% in sheep and goats. In addition, Teshale *et al.* [23] reported sero-prevalence of 14.6% in sheep and 16.45% in goats in Afar region. This difference might be due to the variation in herding practices. In afar region mixing of the animals from various areas is common at communal grazing and watering areas Teshale *et al.* [23] while in the current study area of Somali region only animals belonging to a given clan are allowed to be mixed and there is a strong clan-based segregation of animals and use of rangeland.

When the sero-prevalence of small ruminant Brucellosis was compared between males and females, higher sero-positivity was observed in males over females in this study. However, no statistically significance ($p>0.05$) difference was found between males and females (Table 2). This should not be overemphasized since very few numbers of positive cases observed which makes it difficult to make comparisons. However, Yesuf *et al.* [25] report significantly ($p<0.05$) higher prevalence of Brucellosis in female sheep than in male sheep. Similarly, it is an established fact that male animals are less susceptible to *Brucella* infection, due to the absence of erythritol [30]. On the other hands, Ashanafi *et al.* [31] and Dabassa *et al.* [21] have reported no observable difference in the prevalence of Brucellosis in male and female small ruminants similar to the present finding. Again, the very small number of positive result recorded in the current study might have contributed for not observing statistically significant difference between the two sexes.

The study also revealed slightly a higher sero-prevalence of small ruminant Brucellosis in the adult age groups than in the younger ones even though the difference is statistically ($p>0.05$) insignificant (Table 2). Likewise, the work of Ashagrie *et al.* [32] and Megersa *et al.* [33] indicate the absence of significant variation in the seroprevalence of Brucellosis between different age groups of goats. However, Adugna *et al.* [22] report the significantly ($p<0.05$) higher sero-prevalence of Brucellosis in small ruminant more than 2 years than the other age categories. Similarly, it has been reported that sexually mature and pregnant animals are more prone to *Brucella* infection than sexually immature animals of either sex [24,34]. On the other hand, younger animals are more resistant to infection and frequently clear an established infection, although latent infection can occur [35]. This may result from the fact that sex hormones and erythritol which stimulate the growth and multiplication of *Brucella* organisms tend to increase in concentration with the age and sexual maturity. Nevertheless, in this study no statistically significance ($p>0.05$) difference was observed between age groups (Table 2). However, it is difficult to made firm conclusion, as number of the positive animals is low in the current study.

In line with assessing and evaluation of potential risk factors that are associated with the occurrence of small ruminant Brucellosis in the study area, the districts from where the animals came from is taken in to consideration. Hence, a higher sero-prevalence of

the disease was observed in sheep and goats from Gursum (2%) while the lowest prevalence were in Awbarre (1.1%) followed by Jijiga (1.0%) (Table1). However, there was no statistically ($p>0.05$) significant difference in the sero-prevalence of small ruminant Brucellosis within the study areas (Table 1). The lack of significance difference between the districts might be due to similarities in agro-ecological location or condition and management as well as production systems of district in the study area.

Conclusion

In conclusion, the finding of the study revealed that sero-prevalence of small ruminant Brucellosis in the study area was very low. However, the existence of the disease in the study area has possible risk of spread in the future and can pose great public health and economic threat since the livelihood of pastoralist's community is mainly depends on these species animals, providing milk, meat and cash income to cover family expenses for food and other essential consumers' goods. Therefore, appropriate prevention and control method should be put in place to minimize the risk posed by this disease including elimination of sero-positive animal, vaccination and working on improving the awareness of the community toward the diseases.

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