Seroprevalence and Public Health Significance of Small Ruminant Brucellosis at Two Districts of Dollo Zone in Ethiopian Somali Regional State

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Abstract: A cross-sectional study was conducted on small ruminants in two selected districts of Dollo zone of the Somali region of Ethiopia, in an attempt to determine the seroprevalence of brucellosis and identify potential risk factors. A total of 384 serum samples (181 from sheep and 203 from goat) were collected from those small ruminants that were kept in extensive management system and that have no history of vaccination. All serum samples were initially screened by Modified Rose Bengal Plate Test (mRBPT) and positive reactors to mRBPT were further tested by Complement Fixation Test (CFT) for confirmation. Accordingly, the seroprevalence of brucellosis in goats and in sheep was found to be 1.97 and 1.1%, respectively. Comparison of seroprevalence of small ruminant brucellosis was carried out for species, age groups and sex of the study animals. Although the seroprevalence was higher in goats than sheep, in females as compared to males and in adult small ruminants than young ones, no statistically significant difference (P >0.05) of small ruminant brucellosis was observed among species, sex as well as age groups. In conclusion, this study revealed the presence of small ruminant brucellosis in the study area which can have a high possibility of spreading in the future. Hence, well organized disease prevention and control methods should be implemented to reduce the risk posed by the disease. In addition, public health education should be provided for improving the awareness of the pastoral community towards the disease.

Key words: Brucellosis • Dollo zone • Ethiopia • Seroprevalence • Small ruminant

INTRODUCTION

Ethiopia, one of the developing countries in sub-Saharan Africa, stands first in livestock population in Africa and tenth in the world [1]. The country hosts large number of small ruminants that are raised under extensive pastoral production system or in adjunct to crop production. Majority of these animals are largely concentrated in pastoralist areas of the country. In spite of the presence of huge small ruminant population, Ethiopia fails to optimally utilize this resource as a sector. This is because small ruminant production is constrained by the compound effect of diseases, poor feeding, poor management and low genetic endowment. Among many factors that limit economic return from small ruminants, production diseases stand in the front line. One of such diseases that hampers the productivity of small ruminants is brucellosis [2, 3].

Brucellosis is a disease of worldwide importance and affects a number of animal species resulting in reproductive inefficiency and abortion caused by infectious bacterial disease of the members of the genus Brucella. Brucella is composed of 12 recognized species and in small ruminants, brucellosis is mainly caused by Brucella melitensis. Brucella species are obligate parasites, requiring an animal host for maintenance. The host range includes humans, ruminants, swine, rodents, canines and marine mammals. High numbers of the organism are shed in urine, milk, vaginal discharge, semen and through discharges of birth of infected animals; and infection occurs through inhalation or ingestion of the organisms [4].

In Somali region of Ethiopia, in spite of the presence of huge population of small ruminants, studies carried out on brucellosis are confined in the districts of Jijiga zone [5-9], which showed a prevalence ranging from 1.37 to
1.9%. However, very few efforts have been made so far to determine the prevalence of this disease in small ruminants in the other zones of the Somali region. Hence, this study was conducted in order to determine the seroprevalence of small ruminant brucellosis at two districts of Dollo zone in Somali region of Ethiopia as well as to assess the associated risk factors for brucellosis infection in small ruminants in the study area.

MATERIALS AND METHODS

The current study was conducted in two randomly selected districts of Dollo Zone, namely, Galladi and Bookh, Ethiopian Somali Regional State.

Study animals: The study animals consisted of small ruminants kept under extensive management system in the two districts of Dollo zone. The animals under this study comprised of indigenous breeds of Somali goats and black headed Ogaden sheep. Sheep and goats which were above 6 months of age of both sexes, with no history of vaccination against brucellosis were included in the study. Based on age, the animals were classified into young (6 months < 2 years) and adult (≥2 years). Relevant individual animal information including animal species, age, sex and district origin were recorded.

Study design and sampling method: A cross sectional study was carried out to determine the seroprevalence of brucellosis in small ruminants kept under pastoral production system and to investigate the potential risk factors associated with the occurrence of the disease in the study area.

Simple random sampling technique was used in selecting the districts of Dollo zone and the pastoral kebeles by using lottery system. Individual animals were selected based on systematic sampling to represent equal proportion in terms of species, sex and age in each flock of small ruminants.

Sample size determination: The total number of animals to be sampled was calculated using the formula given by Thrusfield [10].

\[
n = \frac{1.96^2 \times P_{\text{exp}} (1 - P_{\text{exp}})}{d^2}
\]

where: - n = required sample size; P_{\text{exp}} = expected prevalence; and d = desired absolute precision

A 5% desired level of absolute precision, 95% confidence interval (CI) and a 25% expected prevalence of small ruminant brucellosis in the study area were used to obtain the maximum sample size. Accordingly, the calculated sample size was 288 animals. However, to increase precision, 96 animals were added & a total of 384 small ruminants (203 Goat & 181 sheep) were included in the study.

Sample collection, transport and storage: About 10 ml of blood was collected from the jugular vein of small ruminants using plain vacutainer tubes. The blood from each animal was properly labelled and left at room temperature for overnight to allow clotting. The serum was then removed from the clot and stored in sterile tubes. These sera samples were shipped to district veterinary clinic in cold chains and stored at -20°C before it is finally transported to microbiology laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University, until serological analysis was performed.

Serological diagnosis: Serological tests of mRBPT and CFT were carried out at the College of Veterinary Medicine and Agriculture of Addis Ababa University and at National Veterinary Institute (NVI), respectively, in Bishoftu, Ethiopia.

Modified Rose Bengal Plate Test: All serum samples collected were screened using mRBPT according to the procedures described by Alton [11] and the OIE World Organization for Animal Health [12]. For this test, brucella antigen from Veterinary laboratories agency (Addle Stone, United Kingdom) was used. Additionally, brucella positive as well as negative control sera were also used and were tested along with the test sera to guide in the reading of the results. Rose Bengal antigen, constituted of a suspension of *Brucella abortus* and *Brucella melitensis*. For this method, 75 µl of serum and 25 µl of antigen were mixed on a test plate and shook for 4 minutes. After four minutes of shaking, visible agglutination was considered as positive. Agglutinations were recorded as 0, +, ++ and ++++, according to the degree of agglutination. A score of 0 indicates the absence of agglutination, + indicates barely visible agglutination, ++ indicates fine agglutination and +++ indicates coarse clumping. The presence of agglutination (+, ++ & ++++) was considered positive reaction, while the absence of agglutination (0) was considered negative [11].
Complement Fixation Test: All sera which tested positive by the mRBPT were further retested with CFT for confirmation. Standard Brucella abortus antigen for CFT, Amboceptor and sheep red blood cells (SRBCs), obtained from NVI, 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.

Preparation of the reagent was evaluated by titration and performed according to protocols recommended by OIE World Organization for Animal Health [12]. Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive and lack of fixation was considered as negative.

Data analysis: All the data obtained were entered into Microsoft excel and finally transferred to STATA 13.0 for statistical analysis. The seroprevalence was calculated by dividing the proportion of sheep and goats whose sera were found positive to combined mRBPT-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 ($\chi^2$) test. For all analysis a $P$-value less than 0.05 was taken as significant.

Table 1: Seroprevalence of small ruminant brucellosis based on species, district, sex and age by mRBPT and by combined mRBPT-CFT tests in Dollo zone.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Categories</th>
<th>Tested sera</th>
<th>mRBPT +ve</th>
<th>Prevalence by mRBPT</th>
<th>Combined mRBPT-CFT +ve</th>
<th>Prevalence by combined mRBPT-CFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Goats</td>
<td>203</td>
<td>9</td>
<td>4.43%</td>
<td>4</td>
<td>1.97%</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>181</td>
<td>12</td>
<td>6.63%</td>
<td>2</td>
<td>1.10%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>384</td>
<td>21</td>
<td>5.47%</td>
<td>6</td>
<td>1.56%</td>
</tr>
<tr>
<td>District</td>
<td>Galladi</td>
<td>256</td>
<td>14</td>
<td>5.47%</td>
<td>4</td>
<td>1.56%</td>
</tr>
<tr>
<td></td>
<td>Bookh</td>
<td>128</td>
<td>7</td>
<td>5.47%</td>
<td>2</td>
<td>1.56%</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>144</td>
<td>4</td>
<td>2.78%</td>
<td>4</td>
<td>2.78%</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>240</td>
<td>17</td>
<td>7.08%</td>
<td>2</td>
<td>0.83%</td>
</tr>
<tr>
<td>Age</td>
<td>Young</td>
<td>144</td>
<td>6</td>
<td>4.17%</td>
<td>1</td>
<td>0.69%</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>240</td>
<td>15</td>
<td>6.25%</td>
<td>5</td>
<td>2.08%</td>
</tr>
</tbody>
</table>

Table 2: Analysis of risk factors of brucellosis in small ruminants in Dollo zone

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Category</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>$x^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Goats</td>
<td>2.99</td>
<td>0.59-15.1</td>
<td>0.495</td>
<td>0.466</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>1.702</td>
<td>0.37-7.6</td>
<td>0.56</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Adult</td>
<td>1.588</td>
<td>0.31-8.0</td>
<td>0.288</td>
<td>1.129</td>
</tr>
<tr>
<td></td>
<td>Young</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

In the current study, of the 203 goats that were tested 4 were found to be positive by combined mRBPT-CFT indicating the prevalence in goats to be 1.97%. On the other hand, in sheep 2 were positive by combined mRBPT-CFT out of the 181 tested and the prevalence in sheep was 1.1% (Table 1).

As it is additionally shown in Table 1, out of the total of 384 small ruminants’ sera tested, 21 were found to be positive for brucella infection by mRBPT alone and upon further testing by CFT only 6 were found to be positive. Thus, the overall seroprevalence of brucellosis in small ruminants in both districts of Dollo zone was found to be 1.56%. It was 5.47% using the mRBPT test and 1.56% by the combined mRBPT and CFT tests.

Based on Table 1, as it can be seen in species, sex and age groups of small ruminants, higher prevalence was observed in goats (1.97%) than sheep (1.1%), in female small ruminants (2.78%) than in males (0.83%) and in adult small ruminants (2.08%) than in young ones (0.69%).

Analysis for the association of different risk factors with seroprevalence of brucellosis in the study area, such as species, sex and age groups was performed (Table 2). However, statistically significant influence was not seen for the species, sex as well as age groups ($P > 0.05$).
DISCUSSION

This study revealed the seroprevalence of brucellosis in goats and in sheep to be 4.43% and 6.63%, respectively, by mRBPT and 1.97 and 1.1%, respectively, by combined mRBPT-CFT. In both species, more than half of the sera which tested positive for mRBPT, tested negative for CFT. This could be due to cross-reactions between brucella and other bacteria which share similar epitopes. The study also demonstrated the overall seroprevalence of small ruminant brucellosis in the study area to be 1.56% by combined mRBPT-CFT.

The overall 1.56% seroprevalence of small ruminants observed in this study is comparable to the reports of Mohammed et al. [13] (1.37%), Dabassa et al. [14] (1.56%) and Tsegaye et al. [15] (1.76%). However, the prevalence of this study is lower than those recorded in previous studies carried out in different parts of the country: 4.8% in Afar [16], 9.7% in Afar and Somali [5], 1.5% in sheep and 1.3% in goats in the central highlands of Ethiopia [17], 16% in Afar Region [18] and 3.37 and 3.94% from Afar Region and Borena Zone of Ethiopia, respectively [19]. The difference in the prevalence of brucellosis between the current study and previous other reports might be attributed to the differences in geographical location, sample size and management systems.

In the current study, although higher prevalence of brucellosis was reported in goats than sheep, the difference was not statistically (P > 0.05) significant. However, Adugna et al. [20] and Teshale et al. [5] reported significantly (P < 0.05) higher seroprevalence of brucellosis in goats than in sheep. The higher prevalence of brucellosis in goats than in sheep might be due to the greater susceptibility of goats to brucella infection than sheep and the fact that unlike goats, sheep do not excrete brucella organisms for longer periods of time which in turn can reduce the spread potential of the disease among sheep flock [21].

The seroprevalence of small ruminant brucellosis in this study was higher in female small ruminants than males, but no statistically significant (P > 0.05) difference was found between the two sexes. Previous studies also showed no significant difference in the prevalence of brucellosis in male and female small ruminants [14, 16]. However, Yesuf et al. [7] reported significantly (P < 0.05) higher prevalence of brucellosis in female sheep than in male. Generally, it is an established fact that male animals are less susceptible to brucella infection, due to the absence or little amount of erythritol [22].

In the current study, the seroprevalence of small ruminant brucellosis was higher in the adult age groups than in the younger ones, but the difference was not statistically significant (P > 0.05). Similarly, Ashagrie et al. [23] and Megersa et al. [9] observed that there was no significant variation in the seroprevalence of brucellosis between different age groups of goats. However, significantly (P < 0.05) higher seroprevalence of brucellosis in small ruminants more than 2 years of age than the other age categories was reported by Adugna et al. [20]. It is a known fact that sexually mature and pregnant animals are more prone to brucella infection than sexually immature animals of either sex [21, 24]. On the other hand, younger animals tend to be more resistant to infection and frequently clear an established infection, although, latent infections can occur [22].

CONCLUSIONS

This study showed a low seroprevalence of small ruminant brucellosis in the two selected districts of Dollo zone of the Somali region of Ethiopia. However, the presence of the disease in the study area indicates the possibility of spreading in the future that can have great zoonotic and economic impact, since these animals are sources of milk, meat and cash income to the pastoral society.

Hence, the following recommendations are forwarded:

- Well-organized disease prevention and control methods should be undertaken to reduce the risk posed by the disease
- Public health education should be provided for improving the awareness of the pastoral community towards the disease

REFERENCES


