Sero-Prevalence of Small Ruminant Brucellosis in and around Kombolcha, North-Eastern Ethiopia

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Abstract: A cross sectional study was conducted in and around Kombolcha town of Amhara Regional State, North-Eastern Ethiopia from October 2009 to March 2010 with the objectives of determining the sero-prevalence and to identify likely potential risk factors of brucellosis in small ruminants. A total of 714 sheep and goats above six months of age with no previous history of vaccination against brucellosis were randomly sampled. Serum samples collected from small ruminants were screened using the Rose Bengal Plate Test (RBPT). Positive sera were further subjected to the Complement Fixation Test (CFT). All the subsequent test analysis was based on the sera that were positive to both RBPT and CFT. Fisher's exact was used to test the association between group of each risk factor and infection with Brucella. The overall sero-prevalence of 0.7% (5 out of 714) small ruminant brucellosis is recorded. Although statistically not significant (p>0.05) difference were observed between categories of each variable considered as risk factors, the prevalence in goats (0.79%), female (0.96%), adult (=2 years) (0.74%) and extensively managed animals (0.75%) were found higher than the prevalence found in sheep (0.48%), male (0.34%), young (<2 years) (0%) and sheep and goats managed under semi-intensive system (0%), respectively. In conclusion, even though the overall prevalence that was found during the study period is low, the findings still have the power to indicate the presence of the problem in the area. The existence of the disease in the study area has possible risk of spread in the future. Accordingly, elimination of positive reactors will provide better considerable success in the control of brucellosis in the area and small ruminant producers should also be aware of the risk of its zoonotic importance.

Key words: Brucellosis · Sero-Prevalence · Small Ruminant · Kombolcha · Ethiopia

INTRODUCTION

Small ruminants are important domestic animals in tropical production systems. About 21% of the world small ruminant population is found in Africa. The population of sheep in Africa represents 17% of the total world population while goats represent 30%. Small ruminants provide a number of advantages to the producer. They are source of food (Milk and meat), fiber (Wool and skin) and cash in a form of saving, their adaptability to a broad range of environments. Sheep and goats have a high reproductive rate that leads to high production efficiency of small ruminant production. This in turn has an attractive inter price. Under pastoralist and agro-pastoralist production systems, both species of small ruminants could be kept as a source of investment and as insurance against diseases [1, 2].

Ethiopia hosts over 24 million heads of sheep and 18 million heads of goats. Of these, the high lands support about 75% of sheep and 27% of goats while the low land (Mostly pastoral areas) are exhibited by about 25% sheep and 75% of goats [3].

In spite of large population of small ruminants in Ethiopia, the comparative huge resources that the country possesses and the economic return gained from this sub-sector does not seem to coincide. The facts that are attributed to these are under nutrition, malnutrition, low productivity, age-old traditional management system and diseases. Brucellosis is one of the infectious diseases considered as major constraints for animal productivity [4].

Brucellosis occurs worldwide in domestic animals such as cattle, sheep, goats, camels and pigs and creates a serious economic problem for both the intensive and
extensive livestock production system in the tropics and a threat to public health [5, 6]. It has been shown that brucellosis causes heavy economic losses in livestock industry. Economic losses stem from breeding efficiency, loss of offspring, reduced meat and milk production as well as impediment to free animal movements and export of animals and their products [7].

Despite the presence of large populations of small ruminants in different regions of Ethiopia, very limited researches are done on brucellosis even if it is said to be endemic in the country. Previous studies on the sero-prevalence of brucellosis in sheep and goats were variable depending on geographical areas. Prevalence of 4.8% from Afar region [8] 9.7% from Afar and Somalia region [9] 1.5% from Somalia region [10] 1.9% from Southern and Central Ethiopia [11] 2.6% from Arsi-Negele District [12] 3.5% from Tigray Region [13] and 1.5% in sheep and 1.3% in goats were reported from Central highland of Ethiopia [14]. Therefore; this study was undertaken with the objectives of determining the sero-prevalence of small ruminant brucellosis in and around Kombolcha and to identify risk factors that are likely to influence the occurrence of the disease.

**MATERIALS AND METHODS**

**Study Area:** The study was conducted on small ruminant brucellosis in and around Kombolcha town and is 376 kms from Addis Ababa. Kombolcha is a tow in North Central Ethiopia and it is located immediately South East of Dessie, with an altitude of less than 1,880 meters above sea level and an altitude and longitude of 11°43’7’’N, 39°44’42’’E, respectively. It has minimum and maximum mean annual rain fall of 750-900mm. The daily temperature can reach 11.7°C to 23.9°C with relative humidity of 23.9% to 79% [15]. This is known by its mountains, topography, plateaus, hilly and sloppiness. 14% of the zone is highland, 34% midland and the rest 52% is lowland. It has bimodal rainfall. The short rainy season being from March 15 to May and long rainy season similar to the rest part of the country. Mixed crop-livestock is the farming system in the area. Small ruminant production of the area is an integral component of the traditional farming system. In this area, extensive management system is dominant. Semi-intensive system of production is practiced to a lesser extent [16].

**Study Population:** The study animals were sheep and goats at the field areas of in and around Kombolcha town kept by individual farmers for subsistence and reserved as a means of capital for family members. These animals were managed almost under extensive farming system mixed with other species. The total number of small ruminant population in the study area were 44,018 from which 12,975 sheep and 31,043 goats [16]. All of the study animals were non-vaccinated against Brucella and all were above six months of age. According to Gatenby [17] the study population was grouped into two age groups as young those which are < 2 years and adult those which are ≥ 2 years.

**Study Design:** Cross sectional study was carried out on indigenous breeds using serological tests of Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) from October 2009 to March 2010 in and around Kombolcha town.

**Sampling and Sample Size Determination:** The sampling method employed was random sampling and the sample size was determined according to Thrusfield [18] as indicated below. Previous study conducted by Shimeles [19] on the Prevalence of sheep Brucellosis in selected Woredas of the Eastern Amhara Regional State revealed a prevalence of 4.89%. Therefore, using 4.89% expected prevalence and 5% absolute precision at 95% confidence level, the number of animals needed in the study was 357. However, to increase the level of accuracy of determining the prevalence, the sample size was increased by one fold. Accordingly, a total of 714 small ruminants (210 sheep and 504 goats, according to their relative population) were sampled and examined.

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n = \frac{1.96^2 \times \text{Prep}(1-\text{Prep})}{\delta^2}
\]

**Data Collection:**

**Blood Sample Collection:** Ten milliliters of blood was collected from the jugular vein of each randomly selected animals using plain vacutainer tube and allowed to clot overnight in a slant position at room temperature. The serum from each blood sample were separately taken for serological examination and stored at -20°C until it had been tested for Brucella [20]. The species, sex, origin of the animal, management condition and age of the animals were recorded in respect to each sample collected.

**Serological Test Procedure**

**Rose Bengal Plate Test (RBPT):** Epidemiological survey indicated that *B. abortus* was the most prevalent among *Brucella* serotypes in the study area. Antigen and sera required for each day for *B. abortus* serological testing
was taken out from the cold storage and brought to room temperature before testing was undertaken. The RBPT test was carried out according to the method recommended by OIE [21]. The antigen used for RBPT, considered a suspension of *B. abortus* (obtained from Institute Pourquer 326, Rue de la Galera 34097 MONTPELLIER CEDEX 5, France, inactivated by heat and phenol, adjusted to P3.65 and colored with Rose Bengal. This test was carried out at Kombolcha Animal Health and Diagnostic Research Center.

**Complement Fixation Test (CFT):** The CFT is a widely used and accepted confirmatory test although it is complex to perform, requiring good laboratory facilities and adequately trained staff to accurately titrate and maintain the reagents. Numerous variations of the test occur, but each may be most conveniently carried out using micro-titration plates [22]. Sera that are positive to RBPT were further tested by CFT for confirmation. Complement fixation test has been undertaken at the National Animal Health Diagnostic and Investigation Center (NAHDIC) and preparation of the reagents was performed according to the protocols recommended. Standard *B. abortus* antigen S 99 (CVL, New Haw Weybridge and Surry KT153NB, UK) was used. Antigen control sera and complement were obtained from the BgVV, Berlin, Germany. Two percent sheep red blood cells suspension was prepared before the beginning of the test.

**Data Management and Analysis:** Data collected from laboratory assay was stored on Microsoft Excel spread sheet program and analysis was done by using Intercooled STATA 7.0 version software program. The total prevalence was calculated by dividing the number of CFT positive animals by the total number of animals tested. Fisher's exact test was used to assess the association and P values < 0.05 considered as statistically significant.

**RESULTS**

A cross sectional study was made to determine the prevalence of small ruminant brucellosis between October 2009 to March 2010 in and around Kombolcha. A total of 714 sera (n = 210 serum sample from sheep and n = 504 serum sample from goats) were tested for the presence of serum antibodies against *Brucella* infection in sheep and goats. Fifteen (15) sera were found positive by RBPT. Up on further testing of this RBPT positive sera with CFT, only 5 sera were found positive (Table 1). All the sero-prevalence estimates presented here in the paper are with reference to CFT results.

Sero-prevalence of 0.48% (1 of 210) and 0.79% (4 of 504) was observed in sheep and goats, respectively. However, the difference in prevalence between species was not statistically significant (p = 0.354).

Prevalence comparison was also made on the basis of sex and only 1 (0.34%) male and 4 (0.96%) female were found seropositive. However, there was no statistically significant difference between the two sex groups (p= 0.708) (Table 1).

Age supposed to have some association with the recovery of antibodies against brucellosis. Sero-prevalence of 0.74% (5 out of 673) and 0% (0 out of 41) of *Brucella* infection were observed in small ruminants of ≥2 years and <2 years of age, respectively.

<p>| Table 1: Prevalence of Brucella infection in small ruminants in relation to species, age, sex, and management systems: |</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>No of Animals Tested</th>
<th>RBPT</th>
<th>CFT</th>
<th>Fisher's exact</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td></td>
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</tr>
<tr>
<td>Sheep</td>
<td>210</td>
<td>6 (2.86%)</td>
<td>1 (0.48%)</td>
<td>0.354</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Goats</td>
<td>504</td>
<td>9 (1.79%)</td>
<td>4 (0.79%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 M-2 years</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>≥2 years</td>
<td>673</td>
<td>15 (2.23%)</td>
<td>5 (0.74%)</td>
<td>0.592</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td><strong>Sex of Animals</strong></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>296</td>
<td>5 (1.69%)</td>
<td>1 (0.34%)</td>
<td>0.708</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Female</td>
<td>418</td>
<td>10 (2.39%)</td>
<td>4 (0.96%)</td>
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<tr>
<td><strong>Management System</strong></td>
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<tr>
<td>Extensive</td>
<td>666</td>
<td>15 (2.25%)</td>
<td>5 (0.75%)</td>
<td>1.000</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Semi-intensive</td>
<td>48</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>714</td>
<td>15 (2.1%)</td>
<td>5 (0.7%)</td>
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</tr>
</tbody>
</table>
However, the difference between the sero-prevalence of the two age groups was not statistically significant (p=0.592) (Table 1).

On this study, 48 serum from the semi-intensive and 666 from extensively managed animals were also examined for Brucella antibodies. No antibodies were detected in semi-intensive management systems. The sero-prevalence of brucellosis in small ruminants under extensive management system was estimated to be 0.75% (Table 1). In the study area, sero-prevalence was not statistically significant in the extensive managed animals as compared to animals in the semi-intensive management system (p= 1.00).

**DISCUSSION**

In the present study, the overall prevalence of small ruminant brucellosis in and around Kombolcha was found 0.7%. In contrast to this study, previous studies conducted indicated that the sero-prevalence of small ruminant brucellosis varies from region to region in Ethiopia. A sero-prevalence study carried out in small ruminants in Afar and Somalia regions in 2005, clearly demonstrated that the disease exists in Ethiopia. Brucellosis in Kenya 6.01% in sheep [23] in Eritrea 1.4% in sheep and 3.8% in goats [24] and in Somali 7.2% in small ruminants [19] was also recorded. Similarly, in central highlands of Ethiopia 1.5% in sheep and 1.3% in goats [14] in Oromia and SNNPR states 1.65% in small ruminants [25] in Somali 1.6% in small ruminants [9] and in Eastern Amhara Regional State 4.89% Brucella infection in sheep [19] was also recorded. According to Ashenafi et al. [8] 4.8% in small ruminants in Afar, Teshale et al. [9] 9.7% in small ruminants in Afar and Somali and Yibeltal [26] 16.2% in goats and 14.6% in sheep in Afar region were also reported.

The 0.7% sero-prevalence appears generally to be low when compared with most previous reports. This could be mainly due to various factors including geographical location, production system and sample size and management system. In Afar and Somali regions, large numbers of different species of animals are raised on communal pastures under limited watering areas where as the livestock management in the North Western Ethiopia is characterized by mixed farming in which fewer animals are raised separately and it is possible that this might be due to variation in animal management and production systems.

The study demonstrated that from a total of 714 sera tested, only 1 sheep and 4 goats were found sero-positive for CFT. This higher prevalence of brucellosis in goats as compared to sheep might be mainly due to the difference in the pattern of grazing between both species. The difference in sero-positivity between sheep and goats might also be because of the difference in the number of the animals which were sampled and tested for brucellosis. Partly it could be due to the fact that sheep were not excreting the organism for longer period of time unlike goats. This could reduce the potential of the spread of the disease among sheep population [24].

Comparisons of sero-prevalence between the two age groups as well as sexes of small ruminants were also done during the study period. Accordingly, a prevalence of 0.34% (1 out of 296) in male and 0.96% (4 out of 418) in female as well as 0.7% (5 out of 673) in adults (Small ruminants = 2 years of age) were recorded. While no sero-positive sheep and goats were found under the age of < 2 years. However, the analysis revealed that there was no statistically significant difference among sex as well as age groups. It has been reported that brucellosis is essentially a disease of sexually mature animals [27]. Sexually mature and pregnant animals are more prone to Brucella infection than sexually immature animals of either sex [24].

On the other hand, younger animals tend to be more resistant to infection and frequently clear the established infections, although latent infection could occur [28]. This may be due to the fact that sex hormone and erythritol (A polyhydric alcohol which acts as growth factors for brucellosis) which stimulate the growth and multiplication of Brucella organism tend to increase in concentration with age and sexual maturity [24].

According to the present study, female sheep and goats were found more reactive than male reactors. It could probably due to the small number of males (n = 296) sampled and tested as compared to the large number of females (n = 418). However, female are more susceptible than male [29]. In B. melitensis infection, males of sheep and goats are less susceptible than females [30]. Hirsh et al. [31] have also reported that male animals are less susceptible to Brucella infection due to the absence of erythritol. Different factors are probably involved in the variation in sex susceptibility including physiological and behavioral difference between males and females [32].

In the present study, the management system was also considered to evaluate whether the type of management system contributes to brucellosis. Even though no statistically significant difference was recorded between the prevalence of the disease between the two management systems, 5 (0.7%) in extensive and no positive sero-reactors were found in semi-intensive system. The difference in the prevalence rate that were
found between the management systems might be due to the proportion of number of animals tested for semi-intensive system was relatively small as compared to extensive system. An extensive management system (Free grazing) which allows unrestricted contact between animals might also have contribution to the spread of brucellosis in animals in the extensive system. In case of semi-intensive management system it could be associated with better management practices like introducing sheep and goats after being tested by rose Bengal plate test for brucellosis and the routine cleaning of the house may decrease the establishment of infection in the semi-intensive production system and this makes small ruminants not being exposed more by brucellosis.

In this study, the prevalence of brucellosis in small ruminants was found only 0.7%. This low rate of *Brucella* infection might be associated with the availability and administration of regular vaccination against the disease and. Similarly, it might be due to that the awareness of small ruminant producers about the disease and their continual rearing system of small ruminants by separating young’s from that of adults. The other possible reason might be associated with breeding of small ruminants by healthy rams that are free from disease and mating of one ewe or due to only one ram strategy.

**CONCLUSION**

In the present study, relatively low numbers of sero-reactors in small ruminants were identified in the study area. Even though no statistically significant difference were recorded in the prevalence rates between the categories of each risk factor tested for brucellosis, goats, those which have 2 and above age groups, females and those which raised under extensive system have showed higher prevalence rate than the respective opposite groups.

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**REFERENCES**


