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Sero-prevalence and associated risk factors for *Brucella* sero-positivity among small ruminants in Tselemti districts, Northern Ethiopia

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A cross sectional study design was employed with the aim to determine sero-prevalence of brucellosis among sheep and goats and identify factors associated with sero-positivity to *Brucella*. A total of 558 sera were collected randomly and aseptically from small ruminants from November, 2015 till October, 2016 in Tselemti district, Northern Ethiopia, following proper restraining. All the sera were primarily screened for the presence of *Brucella* antibodies using Rose Bengal Plate test (RBPT) and then confirmed by Complement Fixation Test (CFT). The overall sero-prevalence of disease in the study area was 1.79% (n=10). Most of the risk factors including peasant association, species, sex, age, parity, herd size, lactation, and pregnancy status had no significant effect on the sero-positivity to *Brucella* (P>0.05), whereas animals with previous history of abortion and retained fetal membrane had significant effect (P<0.05). Hence, the odds of being sero-positive to *Brucella* was found to be 5.68 (COR=5.68; 95% CI: 1.13, 28.53) and 4.05 (AOR=4.05; 95% CI: 1.01, 16.22) times higher in animals with previous history of retained fetal membrane and abortion when compared with animal with no history of retained fetal membrane and abortion, respectively (P<0.05). The results of the current study demonstrated that brucellosis is endemic and the cause for reproductive loss and failure. Hence, the finding suggests that there is a need for implementation of better management practice such as culling of positive animals from the flock, burning/burial of aborted or retained fetal membrane, and also community awareness about zoonotic importance of the disease should be raised.

Key words: Risk factors, small ruminants, sero-prevalence, Tselemti districts.

INTRODUCTION

Ethiopia owns a huge resource of small ruminant population with an estimated number of 27.34 and 28.16 million heads of sheep and goats, respectively (CSA, 2014). Small ruminants provide various benefits particularly to smallholder farmers. They may be used as a source of immediate cash income, meat, milk, skin,

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manure, risk spreading, and various social functions (Berhanu et al., 2006). Besides this, small ruminants are also considered as means of investments and insurance for the small holder farmers in order to provide income for the purchase of food during the seasons of crop failure because sheep and goats have high fertility rates, short generation interval, and small feed requirement and adaptability to harsh environmental conditions as compared to large ruminants which make them best suited for smallholder farming practice in the country (Berhanu et al., 2006; Tsedeke, 2007).

The current levels of contributions of the livestock sector in Ethiopia, at either the macro or micro level are below the country's potential. The levels of foreign exchange earnings from livestock and livestock products are also much lower than would be expected, given the size of the livestock population (Berhanu et al., 2007). This is due to the prevailing animal disease, feed shortage both in quality and quantity, low genetic potential and management problems. Of these, infectious diseases are the major constraints for enhancing small ruminant production all around the globe including Ethiopia (Singla, 1995; Getahun, 2008; Gizaw, 2010; Kaur et al., 2013).

Brucellosis is a zoonotic infectious disease affecting a wide range of species of animals and humans with an estimated half a million human cases reported annually. It is caused by different *Brucella* species of the genus *Brucella*. It is facultative intracellular Gram negative bacteria. The disease is one of the most widespread zoonoses and is endemic in many developing countries (Corbel, 2006; Pal et al., 2013).

Brucella melitensis is the most important cause of brucellosis which primarily affects sheep and goats and also very pathogenic for human beings. The disease is also caused by *Brucella ovis* which severely affects sheep. Although the disease has preferred hosts, the bacteria have an ability to cross infect other domestic animals. Hence, sporadic infections in small ruminants could also be caused by *Brucella abortus* or *Brucella suis*, but such cases are rare (Corbel, 2006; OIE, 2015).

Small ruminant brucellosis mainly affects the reproductive tract of animals which is manifested by late term abortions, retention of placenta in the case of female animals, epididymitis and orchitis in males. Additionally, the disease also poses major constraint to international trading of animal and animal products (Benkirane, 2006; Radostits et al., 2007; Seleem et al., 2010).

As the disease often goes undetected, identification of infected herd and animals is of prime importance for control of the disease. Having huge livestock resource at hand coupled with intermingling of livestock species may cause uninfected animals to easily get exposed to the disease from multiple sources such as abortion discharges and direct contact with infected animals. Mixed farming especially raising goats and sheep along with cattle was also reported by many researchers to be a risk factor for *Brucella* transmission between different animal species (Godfroid et al., 2013; Padilla et al., 2010).

In Ethiopia, the existence of small ruminant brucellosis has been reported from different parts of the country. Most of the authors used serological surveys to determine the prevalence and associated risk factors. Such kind of information on the status of small ruminant brucellosis in Tselemti district, Northwestern Zone of Tigray Regional State is absent, although there are cases of abortion and retained placenta among small ruminants according to oral information given by farmers, local authorities, and experts. This problem is probably because of brucellosis. So far, the existence of the disease in small ruminants is little known in the study area and so is the circulating *Brucella* spp. in sheep and goats. As there are major risk factors for the occurrence of the disease, a detailed study on small ruminant brucellosis is necessary to establish disease effective control program.

Therefore, the aims of this study were to estimate the sero-prevalence of brucellosis in small ruminants in Tselemti district and to determine risk factors associated with *Brucella* sero positivity.

MATERIALS AND METHODS

Study area

The study was conducted from November, 2015 till October, 2016 in Tselemti district of Northwestern Zone of Tigray Regional State, Northern part of Ethiopia. The study area is situated at 38°15' E and 13°48' N and 1178 km away from the capital city of Addis Ababa. The study area is among the six districts of the Northwestern zone of Tigray and border with districts of Asgede Tsimbla on the North, Welkayit on the West, Tanqua Abergelle on the East and Amhara region on the South.

In Tselemti districts, six Peasant Associations (PA) were used for the current study. Geographically, the Medinealem which is located at longitude 13°35′21″ N and 38°8′48″ E with an altitude of 1361 m, Wihdet at 13°33′0″ N and 38°5′48″ with 1156 m, Mayteklit at 13°37′42″ N and 38°3′4″E with 1227 m, Mayayni at 13°40′38″N and 38°9′51″ E with 1413 m, Maytsebri at 13°58′04″ N and 38°14′17″E with 1370, Mayambesa at 13°37′29″N and 38°13′2″E with 1405 m and Mayayni located at 13°40′38″N and 38°9′51″E with altitude of 1413 m. The area coverage of the district is approximately 2702.5 km^2 with an altitude ranging from 800 to 2870 m above sea level. The mean annual temperature of the area ranges from 16 to 38°C. The annual rainfall also ranged from 758 to 1100 mm and has a mono-modal pattern.

In addition to this, the livestock production system is predominated by extensive production system. The dominant ruminant species in the study area are cattle and goats and followed by sheep with an estimated number of 268, 647, 264, 429, and 13,276 thousands of cattle, goats, and sheep, respectively (OoARDT, 2014).

Study design and study animals

A cross-sectional study design was employed to estimate the sero-prevalence of brucellosis in small ruminants in Tselemti districts.

The current study was conducted on small ruminants kept under extensive production system. Sheep and goats within the age of 6 months and above and both sexes in the selected flock with no previous history of vaccination against brucellosis were included in the study. Individual animals belonging to the study household flock were selected randomly for blood sampling to examine for brucellosis using serological tests. All data related to potential risk factors were also collected.

Sampling techniques

A combination of purposive and two stage random sampling were used to select district, PA, and individual animals. A two stage cluster sampling was employed to determine the sero-prevalence of brucellosis considering districts as primary clustering units and PA as the secondary clustering units. There are six districts, namely, Medebay Zana, Tahtay Koraro, Asgede Tsimbela, Tselemti, Lalay, and Tahtay Adiyabo in the Northwestern zone of Tigray. From the six districts, Tselemti was selected purposively based on high livestock population and ease of transportation service in order to synchronize the present study with research activities of the Shire-Maitsebri Agricultural Research Center. In Tselemti districts, there were 25 PA, of which 6 were selected purposively for the study based on proximity to the main roads and ease of transportation. Finally, individual households having sheep or goats or both species were selected randomly for blood sampling from the selected PA.

Sample size determination

The sample size for the study was determined according to the formula given by Thrusfield (2005) for random sampling method. A 5% absolute precision and 95% confidence interval was used to determine the sample size. An expected prevalence of 50% was taken to determine the maximum sample size. Accordingly, 384 animals were used during the study period. In order to increase the accuracy, the sample size was increased to 558 animals.

$n= 1.96^2 \times Pexp (1 - Pexp) / d^2$

Where, n=total sample size; d=absolute precision; and Pexp=expected prevalence.

Accordingly,

$n= 1.96^2 \times 0.5 (1 - 0.5) / (0.05)^2$

Sample collection

About 10 ml of blood sample was collected aseptically from the external jugular vein of each animal using plain vacutainer tubes after the animal was restrained properly. All samples were serially identified and labeled properly using permanent marker. The blood sample was allowed to clot in slant position at room temperature and transported using an ice box to the Maitsebri Veterinary Clinic. After 24 h of collection, serum was then separated gently by decanting into 2 ml cryo vials tubes following centrifugation at 3000 rpm for 3 min and stored at -20°C in Maitsebri Veterinary Clinic until tested. The sera were then transported to Mekelle University College of Veterinary Medicine and National Animal Health Diagnostic and Investigation Center (NAHDIC) for serological diagnosis.

Serological diagnosis

The Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) were used as screening and confirmatory tests for brucellosis (OIE, 2009).

Rose Bengal Plate Test (RBPT)

Initially, the entire serum sample was tested using Rose Bengal Plate Test (RBPT) by adding an equal volume of antigen (30 µl) and serum onto glass slides. The antigen and test serum were then mixed thoroughly by plastic applicator, shaken for 4 min and the degree of agglutination was observed visually and recorded immediately as positive for the presence of agglutination and negative for its absence of agglutination (OIE, 2009).

Agglutination was then recorded as 0, +, ++, +++ according to the degree of agglutination where 0 indicates absence of agglutination, + indicates barely visible agglutination, ++ indicates fine agglutination, and +++ indicates coarse clumping. The samples identified with no agglutination (0) were recorded as negative, while those with +, ++, and +++ were regarded and recorded as positive.

Complement fixation test (CFT)

All the sera tested positive to RBPT were confirmed using Complement Fixation Test (CFT). A known antigen was incubated at 37°C with test and control sera to form immune complexes. A defined amount of complement was added to reaction mixtures. An immune complex was then produced in positive antigen and antibody reaction which was suggestive of the complement was fixed or consumed. In negative sera, an immune complex was not produced.

An animal is considered positive if tested for sero-positive on both RBPT and CFT in serial interpretation. The use of RBPT/CFT combinations, the most widely used serial scheme, is generally recommended (Dohoo et al., 2003).

Data collection and statistical analysis

Data obtained on serological test was entered and stored on Microsoft excel sheet. Statistical analysis was performed using STATA version 11.1 statistical software. Chi-square and univariate logistic regression analysis were used to check the association between the outcome and explanatory variables and the degree of association was then expressed as odds ration and 95% confidence interval. Those independent variables that were statistically significant were again subjected to multivariate logistic regression. For all analysis, a cut-off point of $P<0.05$ was used for significance difference. The final model was developed using a step wise reaction. In the final model, all variables with a P value < 0.05 were considered statistically significant and retained in the model.

RESULTS

A total of 558 animals sera comprising 145 sheep and 413 goats were examined for the presence of *Brucella* antibodies. A total of 13 and 10 sera were found positive for RBPT and CFT tests, respectively. Accordingly, the overall sero-prevalence of brucellosis in the study area was 1.79% (10/558). The higher prevalence was recorded in goats (2.18%, 9/413) as compared to sheep (0.69%, 1/145). This observed difference was found to be statistically insignificant (P>0.05) (Table 1).

In the present study, explanatory variables such as PA,

Table 1. Sero-prevalence of small ruminant brucellosis in Tselemti district.

Test variable	Total sera examined	Serological tests	
		RBPT positive N (%)	CFT positive N (%)
Sheep	145	2 (1.38)	1 (0.69)
Goats	413	11 (2.66)	9 (2.18)
Total	558	13 (2.33)	10 (1.79)
P-value	-	0.37	0.24

sex, age, species, pregnancy status, lactation status, parity and herd size had no effect on being sero-positive to *Brucella* (P>0.05). However, animals with previous history of abortion and retained fetal membrane were found to be statistically associated with small ruminant brucellosis (P<0.05) (Table 2).

More importantly, the sero-positivity to brucellosis was higher in small ruminants with previous history of abortion (5.17%) as compared to animals with no previous history of abortion (1.08%). The odds ratio indicates that animals with previous history of abortion were found to be 4.05 (AOR=4.05; 95% CI: 1.01, 16.22) times more likely prone to the infection when compared with animals with no previous history of abortion. The difference observed was found to be statistically significant (P<0.05).

The prevalence of the disease was also found to be higher in those animals with previous history of retained fetal membrane (9.09%) as compared to animals with no previous history of retained fetal membrane (1.73%). Accordingly, the odds of being sero-positive to *Brucella* were found to be 5.68 (COR=5.68; 95% CI: 1.13, 28.53) times higher in sheep and goats with previous history of retained fetal membrane as compared to animal with no history of retained fetal membrane (P<0.05).

DISCUSSION

The present study indicates that the overall prevalence of small ruminant brucellosis was 1.79%. Several authors in Ethiopia have reported different sero-prevalence values of the infection in different parts of the country. Prevalence of 1.76% in Debrezeit and Modjo export abattoirs (Tsegay et al., 2015) and 1.9% in Somali (Teshale et al., 2006) was reported which is comparable to the current finding. However, higher prevalence of the disease was also recorded with 16% in Afar (Teshale et al., 2006), 13.6% in Afar (Adugna et al., 2013), and 3.5% in Southern part of Tigray (Teklue et al., 2013). Conversely, relatively low prevalence of 0.7% was also recorded in Kombolcha (Tewodros and Dawit, 2015). The difference in the prevalence rates in the current study and other studies might be due to differences in management practice and agro ecology.

Statistically, species of the animals had no significant effect on the sero-positivity in the current study. Sheep and goats were found equally susceptible to the infection but the magnitude of the disease was higher in goats (2.18%) as compared to sheep (0.69%). This finding is in agreement with those of Teklue et al. (2013) and Bekele et al. (2011). As opposed to the current study, there are significant differences in species susceptibility to the infection in which goats were found at higher risk of getting the infection than sheep as reported by Teshale et al. (2006), Ashenafi et al. (2007), Adugna et al. (2013), and Tegegn et al. (2016). This observed difference could be due to the fact that cattle and goats are the principal livestock species in the study area, while sheep is domesticated and raised in small pocket areas of the district which might not be the same in other study areas.

Additionally, statistically insignificant difference was also noted between sex of the animals and sero-positivity to *Brucella* in which higher prevalence was recorded in females as compared to males. Similar findings were also reported by Teshale et al. (2006), Ashenafi et al. (2007), Bekele et al. (2011), Debassa et al. (2013), Teklue et al. (2013), and Tsehay et al. (2014). Conversely, sex had an effect on the prevalence of the disease as reported by Tegegn et al. (2016). Lack of difference between the female and male animals on the prevalence of the disease observed in the current study and other study might be due to smaller samples of male animals used. Small ruminants especially male goats are considered as first candidates for marketing and serve as immediate source of income to satisfy house hold demands and purchase of agricultural inputs. Another reason for such variation in the present study and others might be small number of male animals kept as sires for the purpose of breeding in the study area.

Similarly, higher sero-positivity to *Brucella* was detected in adult animals as compared to young animals. The difference observed was found to be statistically insignificant (P>0.05). The present study was consistent with the work of Teklue et al. (2013), Tewodros and Dawit, (2015), and Tsehay et al. (2014). Contrary to the present study, there was significant association between age of the animal and sero-positivity to *Brucella* spp. as reported by Adugna et al. (2013), Ashenafi et al. (2007), and Bekele et al. (2011). The variation observed might be related to small sample size used in the present study. Additionally, there is high market and consumer preference for small ruminants especially when the age

Table 2. Logistic regression analysis of the effect of risk factors on prevalence of small ruminant brucellosis.

Variable	Total examined	Positive [N (%)]	COR (95% CI)	P-value	AOR (95% CI)
Peasant Association					
Maytsebri	98	1 (1.02)	-	0.53	-
Medhinealem	188	4 (2.13)	-	-	-
Wihdet	160	5 (3.13)	-	-	-
Mayteklit	59	0	-	-	-
May Ambesa	17	0	-	-	-
May Ayni	36	0	-	-	-
Sex					
Male	73	0	-	0.21	-
Female	485	10 (2.06)	-	-	-
Age					
Young	73	0	-	-	-
Adult	485	10 (2.06)	-	-	-
Species					
Sheep	145	1 (0.69)	-	0.24	-
Goats	413	9 (2.18)	-	-	
Lactation status					
Lactating	259	4 (1.54)	-	0.39	-
Non lactating	226	6 (2.65)	-	-	-
Pregnancy status					
Non pregnant	329	8 (2.43)	-	0.40	-
Pregnant	156	2 (1.28)	-	-	-
History of abortion					
No	369	4 (1.08)	1	-	1
Yes	116	6 (5.17)	4.97 (1.37, 17.95)	0.01	4.05 (1.01, 16.22)
History of retained fetal membrane					
No	463	8 (1.73)	1	-	1
Yes	22	2 (9.09)	5.68 (1.13, 28.53)	0.03	2.44 (0.42, 14.03)
Herd size					
1-20	111	1 (0.90)	-	0.42	-
>20	447	4 (2.01)	-	-	-
Parity					
No	56	0	-	0.29	-
1-3	269	7 (2.60)	-	-	-
>3	160	3 (1.88)	-	-	-

of the animals reaches 6 to 12 months in the districts.

Higher sero-positivity to brucellosis was found in animals with previous history of abortion (5.17%) than animals with no history of abortion (1.08%). Accordingly, the odds ratio (OR) indicates that sheep and goats with previous history of abortion were found to be 4.05

(AOR=4.05; 95% CI: 1.01, 16.22) times more likely prone to brucellosis as compared to animals with no previous history of abortion (P<0.05). The present finding was in agreement with reports of Teklu et al. (2013) and Tadeg et al. (2015). This is due to the fact that there exist tropism/preference of *Brucella* spp. to the key target cells called trophoblasts. Growth of *Brucella* inside trophoblasts is apparently enhanced synergistically in the presence of high concentration of steroid hormones and erythritol during the final gestation of ruminants. The capacity to replicate rapidly and extensively in trophoblasts can compromise the integrity of the placenta and infection of the fetus, resulting in abortion or birth of weak offspring (OIE, 2012; Xavier et al., 2009).

Likewise, the odds of being sero-positive to *Brucella* were 5.68 times higher in (COR=5.68; 95% CI: 1.13, 28.53) sheep and goats with previous history of retained fetal membrane as compared to goats and sheep with no history of retained fetal membrane. The observed difference was statistically found to be significant (P<0.05). The current study was found to be consistent with the work of Tadeg et al. (2015).

Conclusions

The current study revealed that the prevalence of small ruminant brucellosis is low in the study area as compared to previous works. Risk factor analysis also revealed that factors such as species, age, lactation and pregnancy status, parity, and herd size had no significant effect on the sero-positivity of *Brucella* spp. However, small ruminants with previous history of abortion and retained placenta had significant effect on sero-positivity to *Brucella* spp. More importantly, animals with history of abortion were identified as a risk factor in the final model. The existence of positive animals in the flock can serve as foci of infection to in contact animals and humans and responsible for the spread of the infection. Hence, implementation of better management practices like introducing brucellosis free animals, the use of maternity pen of separation for animals during parturition, use of personal protective equipments, proper disposal of fetal membranes and/or aborted fetus, culling of positive animals, and proper cleaning and disinfection activity. Moreover, extensive extension service including health education must be launched to make animal owners, animal attendants and the consumers aware of the public health significance of the disease.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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