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Article

Seroprevalence of Sheep and Goat Brucellosis in Arero District of Borana Zone Southern Ethiopia

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Abstract: The cross-sectional study was conducted at four peasants' associations (PAs) in Arero district of Borana zone, namely Halona, Fuldowa, Silala and Renji. The seroprevallence and potential risk factors of Brucellosis in sheep and goats in study area were determined using serological tests. Serum samples were collected from 238 unvaccinated and apparently healthy sheep and goats. The samples were serologically examined by Rose Bengal Plate Test (RBPT) for first screening test and confirmed by Indirect-ELISA. Almost half of samples that screened positive with RBPT became negative after confirmation by an indirect ELISA test. Out of 238 sera extracted from sheep and goat in the study area, 18(7.56%) animals were found to be positive by RBPT screening. Of all serum samples tested positive by indirect ELISA and confirmed by retest, 8(3.7%) animals were found to be positive. By direct ELISA, of 183 sera from caprine, 6(2.5%) animals were positive, 177(74%) animals were negative; 2 (0.84%) of 55 sera from ovine brucellosis were positive. The seroprevalence of sheep and goats in the Arero district did not show significance among peasant associations ($\chi 2 = 2.070$, P value=0.558), age groups ($\chi 2 = 0.531$), P value=0.392) and between species ($\chi 2 = 0.017$, P value=0.897). The disease was prevalent in both female (4, 1.68%) and male (4, 1.7%), but was more prevalent in male (11% within male population) than female (2% within female population), showing a very high significance with p-value at 0.005 and Chi-Square at 7.842. These positive animals may pose a potential risk to both animals and humans in the area. Therefore, attention should be paid to the certification of breeding bucks or rams to reduce the spread of disease in animals and humans.

Keywords: Borana, Sheep, Goat, RBPT, I-ELISA, Brucellosis, Buck, Ram.

1. Introduction

Ethiopia is a developing country that has 102 million population and its GDP per capita is 767 USD. Agriculture is the backbone of the economy, contributing about 35 percent to GDP and 68.2 percent to employment, and 90 percent of export value. Livestock is an integral part of agriculture, accounting for about 45 percent of the total value of agricultural production and supporting the livelihoods of a large share of the population. More than 14 million households – or 70 percent of the population – keep livestock, including many poor. The national herd comprises 57 million cattle, 30 million sheep and 23 million goats, and 57 million chickens, as well as camels, equines, and a small number of pigs. Most animals are raised in the highlands, where also most of the populations live (FAO, 2019).

Sheep and goats are one of the most preferred livestock species towards improving smallholder livelihoods and considered as convenient in terms of financial asset as they can be sold or exchanged to fulfil immediate cash requirements; to meet basic needs such as foods, medicines, and school fees; are easy to raise with little space and minimum feed requirements; and are symbols of wealth and social wellbeing (Wodajoa et al., 2020; Dossa et al., 2008). Brucellosis is a zoonotic bacterial disease characterized by reproductive losses in animals and nonspecific illness or localized involvement of various organs in humans. Even though brucella abortus and brucella Suis have been found occasionally in small ruminants with rare clinical cases, Brucella melitensis mainly causes brucellosis in small ruminants (OIE, 2018).

The disease in sheep and goat can be transmitted by contact with organisms in vaginal discharges and birth products, venereal and through broken skin and mammary gland is usually colonized during a systemic infection; however, organisms can also enter it from the environment, via the teats. They can shed B. melitensis whether they abort or shed in milk, urine, and semen. Kids and lambs can be born infected or when they nurse from the dam and may pass B. melitensis in their feces (OIE, 2018). It is recognized that four free-ranging wildlife species are self-sustaining reservoirs of B. abortus or B. melitensis and are

potential sources of livestock infections (spill-back). Bison (Bison bison) and elk (Cervus Canadensis) in the Greater Yellowstone Ecosystem in the USA, and African buffalo (Syncerus Caffer) in South-East Africa sustain B. abortus (Tanner et al., 2015; Kamath et al., 2016), whereas the Alpine ibex (Capra ibex) in the French Alps, sustains B. melitensis (Mick et al., 2014).

Human brucellosis becomes the common worldwide zoonotic disease with more than 500 000 new cases annually. The disease is caused by various Brucella species, which mainly infect cattle, swine, goats, and sheep. Humans' infection can occur from ingesting organisms or via contaminated mucous membranes (including the conjunctiva and respiratory tract) and abraded skin. Routes of person-to-person transmission of brucellae like blood transfusion, bone marrow transplantation, exposure to contaminated material while assisting at a delivery, sexual intercourse, and nursing (infants) were concerned with rare incidences. The most rational approach for preventing human brucellosis is the control and elimination of the infection in animals (OIE, 2018; Godfroid et al., 2005). To control and mitigate disease transmission and decrease economic losses that may occur from the disease's impact on livestock, launching epidemiological studies is very important. Therefore, this study concerns seroprevalence of brucellosis in sheep and goats of Arero district of Borana zone southern Ethiopia.

2. Materials and Methods

2.1 Descriptions of study area

The study was conducted in Arero district of Borana zone, Oromia regional state. The center of the district, Matagafarsacity, was found 770 km south of Addis Ababa. The district is bordered by Dubluq district in southwest, Yabelo district in the west, Guji zone in the northeast, and Gomole district the northwest, Somali region in the east and Dhas district in the south and Wachile district in the southeast (Figure 1). The mean average temperature and the average annual rain fall is 18-34 degree Celsius and 1050-1750mm respectively. The number of livestock in the district is estimated at 134 370 cattle, 68 023 goats, 37 321 sheep, 32 272 camels, 23 horses, 1 053 mules and 4 594 donkeys (Fenetahun and Fentahun, 2020).

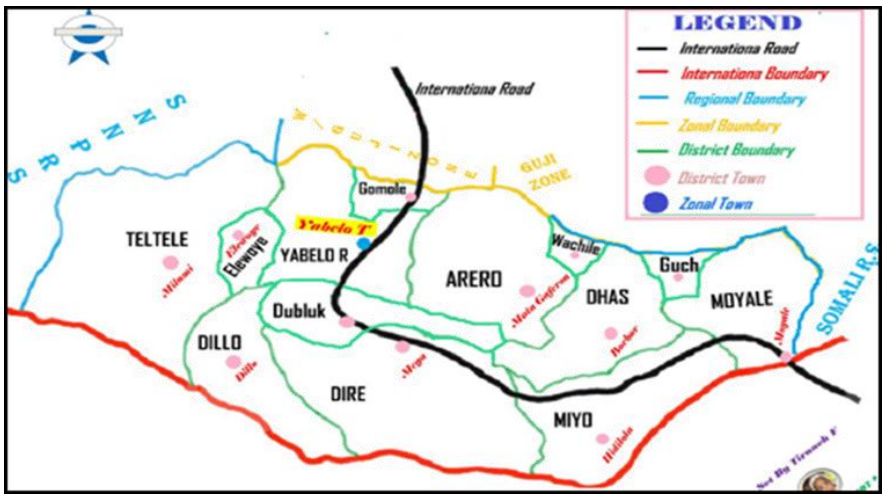

Figure 1. Administrative districts of Borana zone (Fenetahun and Fentahun, 2020).

2.2 Study population

Goat and sheep of different age group and sex groups in the area reared under pastoral production system are our study population. They are multi-functioning since the community has habits of consuming goats and sheep milk. The animals were extensively managed with no extra feed supplement, they are adapted to harsh environments. Since there is inadequate work done on the species regarding the disease, the brucellosis status in sheep and goats of this area is rarely known.

2.3 Study design

The study was conducted to determine the seroprevalence of brucellosis in pastoral goats and sheep. Serological tests are used as a tool to determine the prevalence and assess the associated risk factors. The selection of the peasant association was based on random sampling. From each of the PAs, the household was again randomly selected. Peasant association, species, sex, and age were taken as possible risk factors for the prevalence of brucellosis in goats and sheep which were randomly selected from the study population.

2.4 Sample size determination and sample size

To calculate the total sample size, the following parameters were used: 95% level of Confidence Interval (CI), 5% desired level of precision and with the assumption of 8.11% expected prevalence of brucellosis in sheep and goats in the study area. The sample sizes were determined using the formula given by Thrust field (2005). Therefore, using the formula Hence with 8.1 expected prevalence 114 serum samples were calculated. But to increase representativeness of the samples to the sheep and goat

population, the calculated number made two folds such that 228 samples were needed for the district. And three PA were randomly selected, and 60 blood samples were collected randomly from each PA. Samples were decanted after 24 hours and packed and put in an ice box for cold chain maintenance.

2.5 Sample collection

From all selected sheep and goat in the PA of study area serum was collected from jugular vein using disposable needles and vacutainer tubes aseptically. About 8 ml of blood sample was collected and allowed to clot at room temperature. Finally, serum was separated from clotted blood by decanting it into plastic cru vials. Serums were labeled properly packed, transported to the laboratory, and submitted and stored at -20 °C for serological test at Yabello regional veterinary laboratory.

2.6 Serological tests

2.6.1 *Rose Bengal plate test (RBPT)*

All serum samples are initially screened using RBPT. The test uses Rose Bengal Antigen, which constitutes a suspension of B. abortus. RBPT was performed as per standard procedure (Alton et al., 1975). Exactly, by using a single channel micropipette 75ul of serum was taken on a Rose Bengal white plate. Then the Rose Bengal Colored Antigen bottle was shaken well to ensure homogenous suspension and 25ul of the Rose Bengal Colored Antigen was added to the serum. The antigen and serum were mixed thoroughly and left to stand for 4 minutes. The result was observed immediately after 4 minutes. Definite clumping /agglutination was considered as a positive reaction, whereas no clumping/agglutination were regarded as a negative one.

2.6.2 Indirect ELISA test

All sera that become positive by the RBPT were retested for confirmation by an indirect ELISA (ID.vet) kit provided by IDvet (310 rue louis Pasteur, Grabels, France). By using the manual that was provided by IDvet kit producer, all RBPT-positive sera were tested for antigen detection as follows. First, all important reagents and sera to be tested were brought to room temperature. After that 190ul of dilution buffer 2 was added to all wells and 10ul of negative, positive and test sera were added and incubated for 45 minutes at room temperature.

After incubation, all wells were emptied and washed 3 times with 300ul of freshly made wash solution and then 100ul of prepared conjugate solution was added and incubated for 30 minutes at room temperature. After 30 minutes of incubation, each well was emptied and washed 3 times with 300 l of wash solution and 100ul of substrate solution was added and incubated for 15 minutes in a dark place. And finally, a stop solution was added to each well and immediately color (OD) forms were read and recorded by ELISA reader at 450nm. And then each sample percentage S/P% were calculated using formula (1).

S/P%= (OD sample –OD negative control)/ (OD positive control –OD negative control) x100 (1)

If S/P% was less than or equal to 110, the samples are considered negative; if it is greater than 120, the samples are considered positive; otherwise, it is suspicious.

3. Results

3.1 Rose Bengal Plate Test

Among 238 sera extracted from sheep and goat in the study area, 18 (7.56%) animals were found to be positive by RBPT screening. The disease was prevalent in both goat and sheep, but the prevalence was higher in caprine (6.72%) than in ovine (0.84%), including both overall seroprevalence and intra species prevalence. The prevalence within the peasant association was there. This means the disease present in each of the PAs, namely Fuldow (2.5%), Hallona (0.4%), Silala (0.8%) and Renji (3.78%). Within different PAs, the highest prevalence was recorded in Rengi (3.78%), followed by Fuldow (2.5%), and Silala (0.8%) and list prevalence was recorded in Hallona (0.4%). The disease has occurrence in both age (young and adult) and sex groups (male and female), but the disease was common in male, with 4(11%) from 36 male and 14 (6.9%) from 202 female, become positive with RBPT. Furthermore, when we compared across age ranges, high prevalence was more common in adults (16, 6.7%) than in young animals (2, 0.8%) (Table 1).

Table 1. Arero seroprevalence of sheep and goat brucellosis with RBPT.

	Factors									
	Peasant association				Sex		Age		Species	
	fuldow	Hallona	Renji	Silala	Female	Male	Adult	Young	Caprine	Ovine
No.tested	60	59	59	60	202	36	187	51	183	55
Positive	6 (2.5%)	1 (0.4%)	9 (3.78%)	2 (0.8%)	14 (5.8%)	4 (1.7%)	16 (6.7%)	2 (0.8%)	16 (6.72%)	2 (0.84%)
Negative	54 (22.6%)	58 (24.3%)	50 (21%)	58 (24.3%)	188 (78.9%)	32 (13.4%)	171 (71.8%)	49 (20.5%)	167 (70%)	53 (22%)
Chi-Square	9.943				0.764				1.578	
P value	0.019				0.382				0.209	

3.2 Indirect ELISA result

Almost half of samples that were screened positive with RBPT become negative after confirmation by indirect ELISA test. The percentage of sera which tested positive for anti-Brucella antibodies by RBPT were higher (7.7%) than tested positive by I-ELISA (3.7%). This could be due to cross-reactions between Brucella and other bacteria which share similar epitopes 31. Of all serum samples that became positive by RBPT and retested for confirmation by indirect ELISA test, 8(3.7%) animals were found to be positive. Of 183 sera extracted from caprine 6(2.5%) animals were found positive and 177(74%) animals became negative and of 55 sera taken from ovine 2(0.84%) animals became positive for brucellosis by Indirect ELISA.

The disease was prevalent in both female (4, 1.68%) and male (4, 1.7%) but was more prevalent in male (11% within male population) than in female (2% within female population) showing very high significance with 0.005 p value and 7.842 Chi-Square (see table 2 and figure 3). The seroprevalence of sheep and goat in Arero district did not showed significance across PA ($\chi2$ = 2.070, P value=0.558), age group ($\chi2$ = 0.531), P value=0.392) and between species ($\chi2$ = 0.017, P value=0.897) (Table 2).

Table 2. Arero sheep and goat brucellosis prevalence with ID -ELISA.

	Risk Factors									
	Peasant association				Sex		Age		Species	
	Fuldow	Hallona	Renji	Silala	Female	Male	adult	Young	Caprine	Ovine
No.tested	60	59	59	60	202	36	187	51	183	55
Positive	3 (1.26%)	1 (0.4%)	3 (1.26%)	1 (0.4%)	4 (1.68%)	4 (1.7%)	7 (2.94%)	1 (0.4%)	6 (2.5%)	2 (0.84%)
Negative	57 (??%)	58 (24.3%)	56 (21%)	59 (24.7%)	188 (78.9%)	32 (3.4%)	180 (71.8%)	50 (21.4%)	177 (74%)	53 (22%)
Chi-Square	2.070				7.842		0.531		0.017	
P value	0.558				0.005		0.392		0.897	

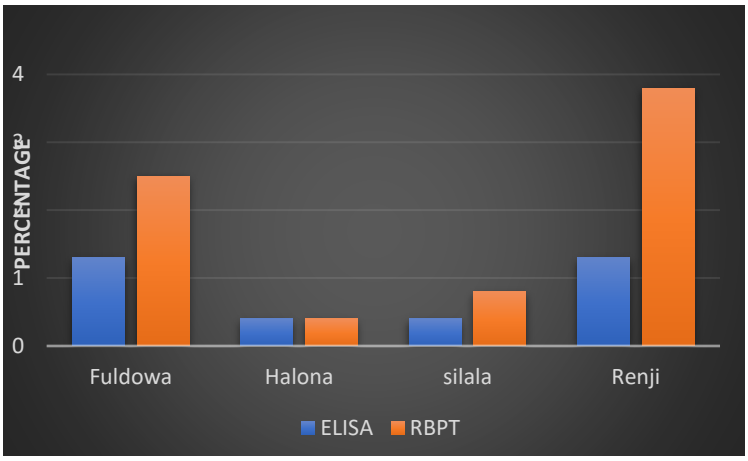

Figure 2. Arero seroprevallence of sheep and goat brucellosis across PA.

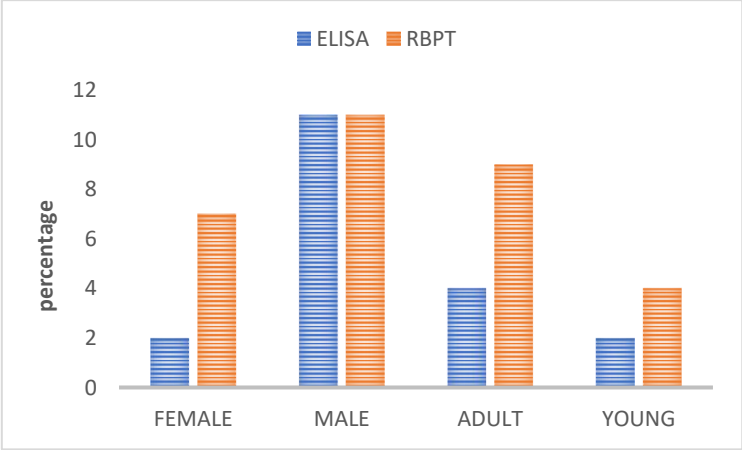

Figure 3. Arero seroprevallence of sheep and goat brucellosis across with in sex and age group.

4. Discussion

The overall seroprevalence for brucellosis in sheep and goats in the Arero district of Borana zone was 3.7%. The study was conducted in four PAs in the Arero district, namely Halona, Fuldow, Silala and Renji. The study found that sheep and goat brucellosis is a prevalent disease in the area, as the disease was recorded in every PA sampled. Almost half of samples that were screened positive with RBPT became negative after confirmation by an indirect ELISA test. The percentage of seropositive serotypes detected by RBPT was higher (7.7%) than that detected by I-ELISA (3.7%). This may be due to cross-reactions between Brucella and other bacteria with similar epitopes. The overall seroprevalence of sheep and goat (3.4%) agreed with 3.3% prevalence reported by Sintayehu et al. (2015) in the Borena zone and 3.2% reported by Edao et al. (2020). The prevalence of this study was also significantly higher than that reported (1.56%) by Dabasa et al. (2013) in the Borena zone of the Oromia region and by that (0.0%) by Tesfaye et al. (2020) in small ruminant brucellosis. The prevalence was also lower than that reported (8.1%) by Zewdie et al. (2018) in Yabelo district of Borena zone and (8.8%) by Zewdie (2020) in Yabelo and Dire district of Borana zone. This difference between the two studies conducted in the same area could be explained as the variation in the serological tests used. In addition, 6(2.5%) animals were positive from 183 goat samples, while 2(0.84%) became positive from 55 samples collected from sheep. The higher seroprevalence in goats was documented than that in sheep, but the difference between prevalence was not significant. The seropositivity of goats in this study was higher than that of sheep, which is consistent with studies conducted by other research groups (Dabasa et al. (2013); Tsehay et al. (2014); Bezabih and Bulto (2015); Wedajo et al. (2015); Zewdie et al. (2017). Another interesting finding of this study is that the prevalence was higher in male (11% from the male population) than in female (2%) sampled, which is inconsistent with the findings of other authors (Reference???). One possible reason for the findings of the current study is the sharing of male animals between villages, which could have led to an increased likelihood of infection and may explain the higher prevalence.

5. Conclusion and recommendations

The study found that brucellosis is a widespread disease in Arero district. It was a well-established infection among goats and sheep in the study area. The seroprevalence of brucellosis was higher in goats than sheep. Also, males have a higher prevalence than females. It could be determined that the positive animal may pose a potential risk for both animals and humans in the area. Specifically male animals, if they remain in the herd, can spread the disease in a short period of time, and infections can grow exponentially. The incidence of brucellosis in sheep and goats in the district will cause numbers of problems in the production and productivity of sheep and goats. Traditional husbandry and poor management practices, if not properly controlled, mix and share breeding bucks or rams with other animals. Close contact with animals can spread disease from animal to animal and from animal to human. Therefore, based on the above conclusion, several points are suggested.

- Further investigations should be performed to elaborate more incidence of the disease to the district.
- Certification of breeding buck or ram should be of concern to reduce the spread of the disease.
- Public health education on improved animal management and risk of zoonotic transmission to humans should inform the herding communities.
- Owners should take precautions during restocking not to buy aborted animals and, where possible, require confirmatory testing of the animal before deciding to buy.
- They should avoid handling aborted materials of sheep and goat with their bard hands and should safely dispose them safely in toxic areas out of reach of dogs and other carnivores.
- They should avoid the habit of drinking raw sheep and goat milk and eating raw meat to control zoonotic transmission.

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Conflicts of Interest: The authors declare that there is no conflict of interest.

References

1. FAO (Food and Agriculture Organization of the United Nations) Rome, 2019; the future of livestock in Ethiopia. Opportunities and challenges in the face of uncertainty.Pp.14-16.
2. Wodajoa HD, Gemedaa BA, Kinatib W, Mulema AA, Eerdewijkc A, Wielanda B. 2020. Contribution of small ruminants to food security for Ethiopian smallholder farmers.Sma. Rum. Res.Pp.7-8.
3. Dossa, LH, Rischkowsky, B, Birner, R, Wollny, C, 2008. Socio-economic determinants of keeping goats and sheep by rural people in southern Benin. Agric. Human Values.Pp.25, 581.
4. OIE, Institute for International Cooperation in Animal Biologics. 2018. Iowa State University;Brucellosis: Brucella melitensis
5. Godfroid J, Cloeckaert A, Liautard JP, Kohler S, Fretin D, Walravens K, et al.From the discovery of the Malta fever′s agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet Res. 2005; 36:313–26.
6. Tanner M, Inlameia O, Michel A, Maxlhuza G, Pondja A, Fafetine J et al.: Bovinetuberculosis and brucellosis in cattle and African buffalo in the Limpopo National Park, Mozambique. TransboundEmerg Dis 2015, 62: 632-638.

7. Kamath PL, Foster JT, Drees KP, Luikart G, Quance C, Anderson NJ, et al.Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. Nat Commun. 2016; 7:11448.
8. Mick V, Le CG, Corde Y, Game Y, Jay M, Garin-Bastuji B. Brucella melitensisinFrance: persistence in wildlife and probable spillover from Alpine ibex todomestic animals. PLoS One. 2014; 9: e94168.
9. Fenetahun Y, Fentahun T. Socio-economic profile of arid and semi-arid agropastoral region of Borana rangeland Southern, Ethiopia. MOJ Eco Environ Sci. 2020; 5(3):113–122. DOI: 10.15406/mojes.2020.05.00183
10. Alton, G.G., L.M. Jones and D.E. Pietz, 1975. Laboratory Techniques in Brucellosis. 2nd Edn., WHO Monograph Series, No. 454. World Health Organization, Geneva.
11. Sintayehu G., Melesse B., Abayneh A., Sintayehu A., Melaku S., Alehegne W., Mesfin S., De Blas I., Casal J., Allepuz A., Martin-Valls G., Africa T. and Abera J. (2015). Epidemiological survey of brucellosis in sheep and goats in selected pastoral and agro-pastoral low-lands of Ethiopia, Revue scientifique et technique (International Office of Epizootics), 34 (3), pp. 881-893.
12. Edao BM, Ameni G, Assefa Z, Berg S, Whatmore AM, Wood JLN (2020) Brucellosis in ruminants and pastoralists in Borena, Southern Ethiopia. PLoSNegl Trop Dis 14(7): e0008461.doi:10.1371/journal.pntd.0008461.
13. Dabasa G., Tefera M. and Addis M. (2013). Small Ruminant Brucellosis: Serological Survey in Yabello District, Ethiopia. Asian Journal of Animal Science, 7(1), pp. 14-21
14. Tesfaye A, Sahele M, Sori T, Guyassa C, and Garoma A. (2020). Seroprevalence and associated risk factors for chlamydiosis, coxiellosis and brucellosis in sheep and goats in Borana pastoral area, southern Ethiopia.BMCVetRes.2020; 16: 145.
15. Wubishet Z, Sadik K, Abdala B, Mokonin B, Getachew T, Getachew K. Small Ruminant Brucellosis and Awareness of Pastoralists Community about Zoonotic Importance of the Disease in Yabello districts of Borena Zone Oromia Regional State, Southern Ethiopia. Curr Trends Biomedical Eng&Biosci. 2018; 12(1):
16. Zewdie, W (2020). *American Journal of Animal and Veterinary Sciences 2020*, 15 (1): 26.31.
17. Tsehay H, Getachew G, Morka A, Tadesse B, Eyob H, et al., 2014. Seroprevalence of brucellosis in small ruminants in pastoral areas of Oromia and Somali regional states, Ethiopia. *Journal of Veterinary Medicine and Animal Health* 6(11): 289-294.
18. Bezabih MK, Bulto WC, 2015. Sero prevalence of small ruminant brucellosis in wer agricultural research afar region, Northern Ethiopia. *Academia Journal of Microbiology Research* 3(2): 031-035.
19. Wedajo MT, Fekadu RG, Tefera YM, Yalew TA, Alemayehu LB, et al., 2015. Seroprevalence of small ruminant brucellos is and its effect on reproduction at Tellalak District of Afar region, Ethiopia. *Journal of Veterinary Medicine and Animal Health* 7(4): 111-116.
20. Zewdie W, Kasim S, Ahmed A, Abdallah B, Liban H, Bulcha A, Belete M, Abichu G, Kinfe G, 2017. Small ruminant brucellosis and awareness of pastoralist community about zoonotic importance of the disease in Yabello districts of Borena Zone Oromia regional state, southern Ethiopia. *JZD* 2 (2): 27-36.