

## **Small Ruminant Brucellosis: Serological Survey in Yabello District, Ethiopia**

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### **ABSTRACT**

Brucellosis is an infectious disease characterized by abortions in females and epididymitis and orchitis in males. There is paucity of information on the occurrence of brucellosis among small ruminants in Yabello district. Therefore, this study was designed with the aims to determine the prevalence and assess risk factors associated with the occurrence of brucellosis among small ruminants in the district. Seroprevalence study of small ruminant brucellosis in Yabello district was conducted from November 2009 to April 2010. Sera from 384 sheep and goat were tested by Rose Bengal Plate Test. Of 384 sera examined 9 (2.34%) were positive to RBPT. The sera screened positive by RBPT were retested using complement fixation test and among 9 sera samples tested 6 (1.56%) were positive for *Brucella* antibodies. The prevalence of brucellosis in this study among sheep and goats was found to be 1.17 and 1.88%, respectively. Small ruminant brucellosis among male and female animals was known to be 0.68 and 2.10%, respectively. Prevalence of small ruminant brucellosis in young animals was 0% while that of adult was 2.30%. Statistical analysis of the data showed that there was no significant difference in seroprevalence to *Brucella* antibodies and species, sex and age of the animals examined ( $p>0.05$ ). The occurrence of brucellosis among small ruminants in Yabello district might pose productivity and reproductivity problem and will be a public health risk. Therefore, best control and prevention methods brucellosis among small ruminants need to be implemented.

**Key words:** Small ruminant, brucellosis, seroprevalence, Yabello district, rose bengal plate test, complement fixation test

### **INTRODUCTION**

Brucellosis is an infectious bacterial disease caused by members of the genus *Brucellae*. It is a disease of worldwide importance and affects a number of animal species. The disease usually affects cattle, buffalo, pigs, sheep, goat, elk, dogs and occasionally horses (Hirsh and Zee, 1990). Brucellosis is characterized by abortion, retained fetal membrane and to a lesser extent, orchitis and infection of the accessory sex gland in males (Dwight and Yuen, 1999). Brucellosis causes considerable economic losses in livestock production due to abortion, infertility and reduction in milk production; in addition, the zoonotic nature of the disease has a serious impact on public health (Mustofa and Nicoletti, 1993).

Brucellosis is usually caused by *Brucella abortus* in cattle, *Brucella melitensis* or *Brucella ovis* in small ruminant, *Brucella suis* in pigs and *Brucella canis* in dogs. The distribution of *B. melitensis* is more restricted than that of *B. abortus* and its primary area of occurrence is in the Mediterranean region including southern Europe. Infection is also present in West and Central Asia, Mexico and countries in South America and countries in Africa. Northern Europe is free of infection, except for periodic incursion from South. Brucellosis of sheep caused by *B. ovis* has been reported in most of the major sheep producing regions of the world and is present in Australia, New Zealand, North and South America, Central Asia, South Africa and Europe (Radostitis *et al.*, 2000).

Brucellosis is a common problem of sheep and goats in pastoral areas of the Ethiopia such as Afar, Borana, Omo Valley and in the lowlands of Tigray where there is large population of goats (Tshale *et al.*, 2006). In spite of the aforementioned prevailing situation and the presence of a number of problems due to brucellosis in small ruminants there is dearth of well-documented information on the occurrence of the disease among small ruminants in Yabello district, Ethiopia. Therefore, this study was designed with the aims to determine the prevalence of small ruminant brucellosis, assess risk factors associated with the occurrence of brucellosis in small ruminants and evaluate the perception of the pastoral community about small ruminant brucellosis in the district.

## MATERIAL AND METHODS

**Descriptions of study area:** The study was conducted from November 2009 to April 2010 in Yabello district of Borana zone, Oromia regional state about 570 km from Addis Ababa. The numbers of livestock of the zone are, 1,496,652 cattle, 452,177 goats, 173,021 sheep, 106,366 camels, 355,700 chicken, 13,945 mules and 61,699 donkeys (CSA, 2010).

**Study population:** Animals that were used for this study belong to goat and sheep in the area managed under pastoral production system featured by communal grazing land. They are of multi function since the community has habits of consuming goats and sheep milk. There is no extra feed supplement, they are adapted to even harsh environment. Since there is inadequate work done on the species regarding the particular disease, the brucellosis status in small ruminants of this area is not known.

**Study design:** A cross sectional study was conducted to determine the occurrence of brucellosis in pastoral goat and sheep. Serological tests and questionnaire surveys was used as a tool to determine the prevalence and assess the associated risk factors. A cross sectional multi-stage sampling technique was used from randomly selected Peasant Associations (PAs) of Yebello District, the selection was based on simple random by lottery. Selection of the PAs again was also based on random sample. From each of the PAs, household was again randomly selected. Species, sex and age were considered as potential risk factors for the occurrence of brucellosis in goats and sheep which were randomly selected from the study population.

**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 50% expected prevalence of brucellosis in small ruminants in the study area the sample sizes were determined using the formula given by Thrustfield (2005):

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

where, n is required sample size,  $P_{exp}$  is expected prevalence,  $d^2$  is desired absolute precision.

Therefore, based on the above formula, the total sample size was calculated to be 384. Thus, a total of 384 blood samples were collected from small ruminants for further laboratory investigation.

**Blood collection:** About 8 mL of blood sample was collected from the jugular vein of each animals using plain vacutainer tube. Blood samples were allowed to clot at room temperature overnight; the sera harvested by separating serum and Red Blood Cells (RBC). Harvested serum was stored at  $-20^{\circ}\text{C}$  after proper labeling at Yabello regional veterinary laboratory.

**Questionnaire survey:** Questionnaire survey was used to assess the perception of the community about small ruminants brucellosis. During data collection history of abortion, fetal membrane, period of abortion, consumption habit of milk and history of presence of orchitis in ram and buck were recorded.

#### Laboratory serological tests

**Rose bengal plate test:** Serum samples were first screened using RBPT. The antigens that were used for RBPT consist of a suspension of *Brucella abortus*, inactivated by heat and phenol adjusted to pH 3.65 and colored with Rose Bengal Test sera and antigen were left at room temperature for half an hour before test. Exactly 30  $\mu\text{L}$  of each test serum was taken and placed in a clean plate, 30  $\mu\text{L}$  of RBPT antigen was added to the side of each test serum using a droppers then the antigen and the test serum was mixed thoroughly by an applicator and the plate was shaken by hand for 4 min. The result was appreciated by examining the degree of agglutination, any visible agglutination was considered as positive.

**Complement fixation test:** Sera tested positive to the RBPT were subjected to CFT for confirmation. The CFT test was carried out at the National Animal Health Diagnostic and Investigation Center (NAHDIC) at Sebeta, Ethiopia.

**Data analysis:** The data collected were entered in to a Microsoft Excel spreadsheet. Laboratory investigation results were analyzed using descriptive statistics by Fisher's exact test. In all the analysis, confidence level was at 95% and  $p < 0.05$  was taken for significance.

## RESULTS

**Overall prevalence:** In this study total of 384 small ruminant sera samples (213 goats and 171 sheep) were subjected to RBPT for screening and RBPT positive sera were subjected to CFT for confirmation. Among the total 384 sera sample tested, 9 (2.34%) were positive for brucellosis by RBPT. The sera screened positive by RBPT were retested using CFT and 6 sera were found positive for *Brucella* antibodies. Therefore, the overall prevalence of brucellosis in small ruminants this study was proved to be 1.56%.

**Potential risk factors of small ruminant brucellosis:** The prevalence of brucellosis in this study in sheep and goat was found to be 1.17 and 1.88%, respectively. Statistical analysis revealed that there was no significant difference ( $p>0.05$ ) in the seroprevalence of *Brucella* antibodies between the two species (Table 1).

Small ruminant brucellosis among male and female animals was known to be 0.68 and 2.10%, respectively. Statistical analysis revealed that there was no significant different ( $p>0.05$ ) in the seroprevalence to *Brucella* antibodies between male and female animals (Table 2).

Prevalence of small ruminant brucellosis in young animals was 0% while that of adult was 2.30%. Statistical analysis of the data showed that there was no significant difference ( $p>0.05$ ) in the seroprevalence of *Brucella* antibodies and age group (Table 3).

**Questionnaire survey:** In this survey, 89 household heads were interviewed and their responses were summarized as follows.

**History of occurrence and stage of abortion:** Out of the 89 household heads interviewed 80 (90%) proved the occurrence of abortion to be associated with the disease in the sheep and goats in their flock and 34 (42.5%) reacted that abortion occurs more frequently in sheep while 46 (57.5%) responded it happens more frequently in goats (Table 4). Out of 80 interviewed, 42 (52.8%) responded it happens at late stage of pregnancy. Again out of 80 interview 57 (71.6%) responded that abortion occurs in animals kidding/lambing for first time (Table 4).

**History of retention of fetal membranes and method of discarding:** Out of 89 respondents, 33 (37%) of respondent have awareness of retention of fetal membranes (RFM) due to the disease in sheep and goats in their flock. The respondent also asked about method of handling and discarding of the fetal membranes, aborted fetus, uterine fluid and of these interviewees, 82 (92.1%) replied that they threw to open environment. About 88 (98.88%) respondents replied that they handled aborted fetus and fetal membranes without protective gloves (Table 5).

Table 1: Seroprevalence of small ruminant brucellosis between species

Species	No. of animals tested	RBPT result		CFT result	
		Positive	Prevalence (%)	Positive	Prevalence (%)
Sheep	171	3	1.75	2	1.17
Goats	213	6	2.80	4	1.88
Total	384	9	2.34	6	1.56

Fisher's exact = 0.696

Table 2: Seroprevalence of small ruminant brucellosis between sexes

Sex	No. of animal tested	RBPT result		CFT result	
		Positive	Prevalence (%)	Positive	Prevalence (%)
Male	147	1	0.68	1	0.68
Female	237	8	3.38	5	2.10
Total	384	9	2.34	6	1.56

Fisher's exact = 0.413

Table 3: Seroprevalence of small ruminant brucellosis between ages

Age	No. of animal tested	RBPT result		CFT result	
		Positive	Prevalence (%)	Positive	Prevalence (%)
Adult	261	9	3.43	6	2.30
Young	123	0	0.00	0	0.00
Total	384	9	2.34	6	1.56

Fisher's exact = 0.183

F: Frequency

Table 4: Abortion-Occurrence, stage of pregnancy and parity number

Occurrence (n = 89)		Pregnancy stage						Parity number									
Sheep		Goats		Early		Mid		Late		1		2		3		≥4	
F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
34	42.5	46	57.5	18	22.6	20	24.5	42	52.8	57	71.6	9	11.3	8	9.4	6	7.5

F: Frequency

Table 5: Occurrence, handling and discarding method of aborted fetuses and fetal membranes

Occurrence of RFM				Method of discarding						Handling			
Yes		No		Thrown to environment		Burying		Other		With glove		Without glove	
F	%	F	%	F	%	F	%	F	%	F	%	F	%
33	37	56	63	82	92.1	1	1.1	6	6.74	1	1.12	88	98.88

F: Frequency

Table 6: Milk consumption habit and method of consumption

Consumption habit				Method of consumption							
Yes		No		Boiled				Raw			
F	%	F	%	F	%	F	%	F	%	F	%
88	98.88	1	1.12	63	71.6	25	28.4				

F: Frequency

**Milk consumption habit and method of consumption:** Out of 89 members of the interview 88 (98.88%) replied that they consume sheep and goats milk and only 1(1.12%) answered do not consume sheep and goats milk. With regard to the method of consumption, 63 (71.6%) replied that they consume boiled and 25 (28.4%) replied they consumed raw (Table 6).

## DISCUSSION

The overall seroprevalence of small ruminant brucellosis in Yabello district of Borana zone was proved to be 1.56% (6/384). The species specific prevalence of the disease was confirmed to be 1.88 and 1.17% in goats and sheep, respectively. The finding of the current study was in agreement with the finding of Bekele *et al.* (2011), around Jijiga, who showed prevalence of 1.61 and 2.0% in sheep and goats, respectively and Tshale *et al.* (2006) in Somali region who proved prevalence of 1.6 and 1.7% of brucellosis in sheep and goats, respectively.

The prevalence of brucellosis in small ruminants in this study was relatively lower than the finding by Ashenafi *et al.* (2007), who reported the prevalence of 3.2% in sheep and 5.8% in goats in pastoral regions of Afar. The difference might be due to differences in the sample size or the variation in herding practices. In the Afar region, mixing of animals from the various areas is common at communal grazing and watering areas. In Borana pastoralist there is cultural indigenous range and water holes management knowledge governing pasture and water holes and thus settlement is based in some predetermined scenario which allow some group of the community or clan to use the resource (Oba, 1998).

The prevalence of brucellosis in this study in sheep and goat was found to be 1.17 and 1.88%, respectively. Statistical analysis revealed that there was no significant difference in the seroprevalence to *Brucella* antibodies between the two species ( $p>0.05$ ). This could be attributed to the fact that sheep are at higher risk of acquiring *Brucella* infection than goats (Radostitis *et al.*, 2000).

Small ruminant brucellosis among male and female animals was known to be 0.68 and 2.10%, respectively. Statistical analysis revealed that there was no significant different ( $p>0.05$ ) in seroprevalence to *Brucella* antibodies between male and female animals. This could be best justified by the fact that male animals are less susceptible to *Brucella* infection, due to the absence of Erythritol (Dwight and Yuen, 1999).

The prevalence of small ruminant brucellosis in young animals was 0% while that of adult was 2.30%. Statistical analysis of the data showed that there was no significant difference in seroprevalence to *Brucella* antibodies and age group ( $p>0.05$ ). This fact might be explained by the fact that brucellosis is essentially a disease of sexually mature animals. Younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Quinn *et al.*, 1994; Walker, 1999).

Awareness of the community about the occurrence of abortion in relation to the stage of pregnancy and parity number has been assessed through interview. Accordingly greater proportion of the respondents replied that it takes place in later stages of pregnancy and in animals kidding/lambing for first time.

The respondents were asked about method of handling and discarding of the fetal membrane, aborted fetus, uterine fluid and greater proportion of the respondents replied that they throw to open environment and they handle without protective glove. Throwing to the environment might also expose the materials to the dog which act as mechanical vector of brucellosis, handling and contact of such material plays important role in transmitting the disease in small ruminant and man.

Milk consumption habit and method of consumption were also assessed and almost all replied that they consume sheep and goats milk, method of consumption asked and majority replies they consume boiled. Since there is close contact between humans and their livestock, which sometimes share the same housing enclosures, brucellosis is a significant health risk for the entire community.

Awareness of the community about transmission of small ruminant brucellosis to human was tried to assess by open-ended question and majority of the interviews explain that, if pregnant woman consumes milk from aborted animals, she might also abort. In addition, this idea in the community might reduce risk to the women in some amount. This idea also agree with scientific actuality that, the virulence of *B. melitensis* partly measured by their capacity to cause brucellosis in human being and cattle, that are not considered natural or preferred host, even though in this species the disease sporadically lead to abortion (Davidson *et al.*, 1990).

## **CONCLUSION**

This study indicated that brucellosis was known to occur among small ruminant in Yabello district of Borana zone, Ethiopia. Species, sex and age of the animals were proved to influence the occurrence of brucellosis among small ruminants. The occurrence of brucellosis among small ruminants in the study area might poses a huge productivity and reproductivity problem among small ruminants and will be a public health risk to the human population in the area. Therefore, attention towards best control and prevention methods brucellosis among small ruminants is essential to mitigate the economic losses in the sheep and goat industry in the study area in particular and the country as a whole. Thus, the results warrant further investigations to elucidate the animal and human health significance small ruminant brucellosis in the area.

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