# SERO-PREVALENCE OF SMALL RUMINANT BRUCELLOSIS IN KOMBOLCHA DISTRICT, NORTHEAST ETHIOPIA

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#### ABSTRACT

Cross-sectional study was conducted to determine the sero-prevalence of brucellosis and identify the potential risk factors for the occurrence of the infection in small ruminants. A total of 714 sheep and goats above six months of age and with no previous history of vaccination against brucellosis were sampled for the study. Serum samples were screened using the Rose Bengal Plate Test (RBPT) and positive sera further subjected to the Complete Fixation Test (CFT). All the subsequent test analysis was based on the sera that were positive to both RBPT and CFT and accordingly 0.7 % (5 of 714) overall sero-prevalence was found in small ruminants in the study area. Statistically no significant differences were observed between sheep and goats (p=0.354), the two sex cluster (p=0.708), young (<2 years) and adult ( $\geq$ 2 years) age group (p=0.592) and small ruminants under extensive and semi-intensive management systems (p=1.000). Although the overall sero-prevalence observed in this study is seem to be small still influential to indicate the occurrence of Brucella infection in the study area and the possible risk of the spread of the infection within small ruminants and human. The need for screening test, elimination of positive reactors and isolation and characterization of the circulating Brucella species are optional to provide considerable success in the control and prevention of brucellosis in small ruminants and its public health hazard in the area.

Keywords: Brucellosis; CFT, RBPT; Sero-Prevalence; Small Ruminant

#### 1. INTRODUCTION

Small ruminants are important domestic animals in tropical livestock production systems<sup>1,2</sup>. Ethiopia hosts over 24 million heads of sheep and 18 million heads of goats<sup>3</sup>. However, in spite of large population of small ruminants the country possesses, the economic return gained from this sub-sector is not satisfactory. The facts attributed are under nutrition, malnutrition, low productivity, age-old traditional management system and disease. Among the diseases that hampered small ruminant production and productivity in Ethiopia is brucellosis<sup>4</sup>.

Brucellosis is an infectious bacterial zoonotic disease caused by member of the genus Brucella. It is a disease of worldwide importance and affects a number of animal species<sup>5</sup>. Brucellosis in small ruminants is mainly caused by Brucella (B.) melitensis and B. ovis<sup>6</sup> and clinically the disease characterized by abortion and retained placenta in the female whereas orchitis and epididymitis with frequent sterility occur in male<sup>5</sup>. Brucellosis in general creates serious economic and public health problems in the tropics<sup>7, 8</sup>. The economic losses of brucellosis in livestock industry stems from breeding efficiency, loss of offspring, reduced meat and milk production as well as impedes export of live animal and their products<sup>9</sup>. The greater prevalence of brucellosis in human is also found in countries with the highest incidence of B. melitensis infection among goats and/or sheep<sup>10</sup>. Despite the presence of large population and brucellosis is said to be endemic in Ethiopia, very limited studies have been conducted on small ruminant brucellosis in the country in general



in the study area in particular. This study therefore conducted to determine the sero-prevalence of small ruminant brucellosis in and around Kombolcha district, and to identify risk factors that are likely to influence the occurrence of the disease in sheep and goats.

## 2. MATERIAL AND METHODS

#### 2.1. Study area

The study was conducted in Kombolcha district, northeast Ethiopia. Kombolcha is located 376 kms from Addis Ababa, the capital city of Ethiopia. It is situated 1,880 meters above sea level (m.a.s.l) and a latitude and longitude of 11°4'37"N, 39°44'42"E, respectively. The rainfall in the district is bimodal and the minimum and maximum mean annual rainfall is 750-900 mm. The daily temperature can reach 11.7°C to 23.9°C with relative humidity of 23.9%-79%. The total numbers of small ruminant population in the district are 44018 from which 12975 are sheep and 31043 are goats<sup>11</sup>. The type of management system of small ruminants is mostly extensive, mixed with other animal species but there are farmers who are keeping their sheep and goats under semi-intensive system.

#### 2.2. Study population

The study animals were sheep and goats kept by individual farmers in and around Kombolcha town. Small ruminants of both sex and above the age of six months were included in the study, while those with the history of vaccination against brucellosis were excluded.

### 2.3. Study methodology

The study was cross sectional and conducted from October 2009 to March 2010. The sample size was determined based on Thru field <sup>12</sup> and simple random sampling technique was employed to select the sample units. Accordingly, 714 small ruminants (210 sheep and 504 goats, based on their population proportion) were sampled and examined for the presence of Brucella infection. The study animals were grouped in to two age groups according to Gatenby<sup>13</sup>. Accordingly, those animals which are below 2 years of age were group as young and those two years and above clustered as adult. Animal associated information (such as sex, age, origin, management and species type) was also taken during examination.

#### 2.4. Serology

About 10ml of blood was collected from the jugular vein of each study animal using plain vacutainer tube and allowed to clot over night in a slant position at room temperature. The serum were separately taken for serological examination and stored at -20<sup>o</sup>C until tested for Brucella. Serum samples were screened using the Rose Bengal Plate Test (RBPT) and carried out according to the method recommended by OIE (2004). The antigen used for RBPT, considered of a suspension of Brucella (Br.) abortus (obtained from Institute Pourquer 326, Rue de la Galera 34097 MONTPELLIER CEDEX 5, France), inactivated by heat and phenol, adjusted to pH 3.65 and coloured with Rose Bengal. Sera that are positive to RBPT were also further tested by Complete Fixation Test (CFT) for confirmation. Preparation of the reagents was performed according to the protocols recommended and standard Br. abortus antigen S 99 (CVL, New Haw Weybridg,



and Surry KT15 3NB, UK) was used. Antigen, control sera and complement were obtained from the BgVV, Berlin, Germany. 2% sheep red blood cells suspension was prepared before the beginning of the test.

#### 2.5. Statistical analysis

The data obtained were classified, filtered, coded using Microsoft Excel sheet. Intercooled STATA 7.0 version software was used to analyze variables using appropriate statistical methods. 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 evaluate the association and P values < 0.05 at 5% significance level considered statistically significant.

#### 3. RESULTS AND DISCUSSIONS

In this study the overall sero-prevalence of small ruminant brucellosis in Kombolcha district was recorded 0.7% and found to be smaller than sero-prevalence reported within and the neighbouring countries of Ethiopia. In Ethiopia for instance Shimeles<sup>15</sup> has reported 4.89% in sheep in Eastern Amhara Regional State. Ashenafi et al.<sup>16</sup> from Afar Regional state, Mengistu<sup>17</sup> from Oromia and Southern Nations and Nationality Regional States, and Teshale et al.<sup>18</sup> from Somalia Regional State have also stated 4.8, 1.65 and 1.6, respectively in sheep and goats. Brucella sero-prevalence of 6.01% in sheep in Kenya<sup>19</sup>, 1.4% in sheep and 3.8% in goats in Eritrea<sup>20</sup> and 7.2% in sheep and 5.29% in goats in Somali<sup>21</sup> were also reported from bordering countries of Ethiopia. The difference in the over sero-prevalence reported between this and other studies mentioned so far could be due to differences in small ruminant population and management system. There is a positive association between population density and disease prevalence, which is attributed to increase contact between susceptible and infected animals<sup>22</sup>.

Comparisons of Brucella sero-prevalence were done between the two species type (Table 1), sex (Table 2), age (Table 3) groups and between the two management systems (Table 4). The differences in sero-prevalence observed within categories of each variable were found statistically non significant. However slight variations in the frequency were observed between categories each study variables and accordingly the frequency of sero-positivity were found higher in goats, female, adults ( $\geq$ 2 yrs) and small ruminants managed under extensive system than sheep, male, young animals and those under intensive system.

The higher sero-prevalence recorded in goats might be attributed to their greater susceptibility to Brucella infection<sup>23</sup> and larger sampling number. The excretion of Brucella organism for longer period in sheep reduce the potential of the spread of the disease among the sheep population<sup>5</sup> and this might also be partially contributed for the higher Brucella sero-prevalence in goats as compared to sheep population.

The variation observed between the two age groups might be influenced by the stage of sexual maturity because brucellosis is essentially a disease of sexually mature animals<sup>6</sup>. Therefore sexually matured and pregnant animals are more prone to Bruclla infection than sexually immature animals of both sexes and non pregnant animals<sup>5</sup>. Although latent infection could occur<sup>24</sup> younger animals are more resistant to Brucella infection and frequently clear the infection before it is established. This might be related to the fact that sex hormones and meso-erythritol (in male testicles and seminal vesicles) and erythritol in female



allantoic fluid stimulate the growth and multiplication of Brucella organisms and tend to increase in concentration with age and sexual maturity<sup>5</sup>.

The higher Brucella sero-prevalence reported in female as compared to male sheep and goats in this study is found in agreement with the idea of Gyles et al.<sup>25</sup> stated that females are more susceptible than male sheep and goats. Hirsh and  $Zee^{26}$  have described that since erythritol is absent in male sheep and goats, they are less susceptible to Brucella infection. The small sample size of male (n=296) as compared to females (n=418) sheep and goats could also credited for the variation of brucella sero-prevalence observed between the two sex groups.

The difference in sero-positivity observed between the two management systems might be due to the variation in the proportion of sheep and goats sampled at each management system. Moreover extensive management system allows unrestricted contact between animals and might also contribute for the spread of Brucella infection among small ruminants. Better management practices observed in semi-intensive management system (like introducing animals after testing for brucellosis and the habit of animal house cleaning) might have also impact for smaller Brucella sero-prevalence recorded in sheep and goats held under semi-intensive management system.

#### 4. CONCLUSION

The sero-prevalence found in this study has indicated the occurrence of brucellosis in sheep and goat, even though it is smaller than the sero-prevalence reported by similar studies conducted so far in different areas of Ethiopia. Moreover Brucella sero-positive animals can be potential sources for non-infected small ruminant and has also public health hazard in the area. Screening test before selection for breeding purpose, elimination of positive reactors, and isolation and characterization of the circulating Brucella species are suggested to provide considerable success in the control and prevention of brucellosis in small ruminants and its public health hazard in the area.

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#### 7. FIGURES AND TABLES

Species	No of animals tested	Positive for RBPT	Positive for CFT	Fisher's exact
Sheep	210	6(2.86%)	1(0.48%)	0.354
Goats	504	9(1.79%)	4(0.79%)	
Total	714	15(2.1%)	5(0.7%)	

**Table 1 S**ero-prevalence of brucellosis based on small ruminant species type

Table 2 Sero-prevalence of brucellosis in relation to sex type

Sex	No of animals tested	Positive for RBPT	Positive for CFT	Fisher's exact
Male	296	5(1.69%)	1(0.34%)	0.708
Female	418	10(2.39%)	4(0.96%)	
Total	714	15 (2.1%)	5(0.7%)	

**Table 3** Sero-prevalence of brucellosis in relation to age groups

Age group	No of animals tested	Positive for RBPT	Positive for CFT	Fisher's exact
Young (6m- 2 yrs)	41	-	-	0.592
Adult ( <u>&gt;</u> 2 yrs)	673	15(2.23%)	5(0.74%)	
Total	714	15(2.1%)	5(0.7%)	_

m = month; yrs = years

# Table 4 Sero-prevalence of brucellosis based on the management system

Management system	No	of	animals	Positive for RBPT	Positive for CFT	Fisher's exact
	teste	d				
Semi-intensive	48			-	-	1.000
Extensive	666			15(2.25%)	5(0.75%)	
Total	714			15(2.1%)	5(0.7%)	-

