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# Seroprevalence of Bovine Brucellosis under Extensive Production System in Wolaita Zone, Southern Ethiopia

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**Abstract:** A cross-sectional epidemiological study was carried out in Sodo Zuria and Humbo districts of Wolaita zone southern Ethiopia from November 2016 to April 2017 to determine the seroprevalence and potential risk factors for bovine brucellosis in cattle under extensive production systems. The study populations comprised both indigenous and cross breed cattle were kept with other species such as sheep and goats. Serum samples were collected from 462 extensively managed cattle at least one year of age by using multistage sampling technique. All serum screened for Brucella antibodies by the Rose Bengal Plate Test and reactor sera were further tested by the Complement Fixation Test. Moreover, information was gathered on individual animal and herd level risk factors by using a structured questionnaire survey. The overall seroprevalence of brucellosis was 1.3% (95% CI: 0.5-3) and 5.8% (95% CI: 2-12) at both animal and herd level respectively. The result indicated that there was a statistically significant increase in seroprevalence of brucellosis in cow with history of abortion and retained placenta. Nevertheless, in the multivariable logistic regression analysis, herd size (p = 0.02, OR=13.7, CI: 1. 4 -29.7) and abortion (p = 0.01, OR=9.8, CI: 1.5 - 64.4) were statistically significant risk factors for individual animal seroprevalence. Control measures such as culling of aborted animal, proper disposal of aborted fetus, pasteurization or boiling of milk before consumption should be carried out to reduce risk of infection and transmission of the disease in livestock and human in the study area.

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## 1. Introduction

Ethiopia maintains huge number of livestock population ranking first in Africa. A huge and diverse livestock species of Ethiopia is maintained under different agro-ecological zones, management, migration and animal health care system. Livestock represents a major national resource and form an integral part of the agricultural production system (IFPRI, 2006; Lobago *et al.*, 2006).

Comparatively huge livestock resources of the country and the economic return gained from this subsector do not coincide. The main technical limitations on livestock development and that determine the biological efficiency of production in Ethiopia are inadequate feeding, poor animal health, low potential of the genotypes used for yield traits and the traditional low input livestock management practices (Shiferaw *et al.*, 2003). Bovine brucellosis is one of these limiting factor and has been reported from several parts of the country (Bekele *et al.*, 2000; Tolosa *et al.*, 2008; Kebede *et al.*, 2008; Asmare *et al.*,2010; Megersa *et al.*,2011; Adugna *et al.*,2013, Alemu *et al.*, 2014, Bashitu *et al.*, 2015; Asegdom *et* 

al., 2016).

Brucellosis is an infectious contagious bacterial disease usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs. It is slow-growing, Gram negative, small cocobacilli and intracellular bacteria that is capable to survive and multiply within epithelial cells, placental trophoblasts, dendritic cells and macrophages (Gorvel, 2008).

The disease is an important zoonosis that exists worldwide and is more or less endemic in most African countries (John *et al.*, 2002). It causes significant reproductive losses in animals. Abortions, placentitis, stillbirth and birth of weak offspring in female and epididymitis and orchitis in male are the most common consequences (OIE, 2009). Bovine brucellosis is an infectious and contagious disease known for its impact on reproductive performance of cattle in Africa (McDermott and Arimi, 2002). The disease is primarily caused by *Brucella abortus* and occasionally by *Brucella melitensis* where cattleare

kept together with infected sheep or goats (OIE, 2009). Bovine brucellosis has been eradicated in most developed countries that have implemented a tight eradication program (Makita et al., 2008). Yet, it is prevalent in the Mediterranean basin, Middle East, Western and Central Asia, Latin America, Africa and India.

The disease has a considerable impact on animal and human health, as well as wide socio economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products (Roth et al., 2003). It poses a barrier to trade of animals and animal products, an impediment to free animal movement. The economic and public health impact of brucellosis remains of concern in developing countries (Roth et al., 2003).

The epidemiology of brucellosis in livestock and cost-effective prevention measures are not well understood and available data are limited particularly in sub-Saharan countries (McDermott and Arimi, 2002). Hence, brucellosis remains widespread in livestock population and presents enormous economic and public health problems. It also causes losses due to abortion or breeding failure in the affected animal population, diminished milk production and causing reduced work capacity through sickness of the affected human (FAO, 2003).

Brucellosis is endemic in Ethiopia since 1970. Since then, few fragmented studies have demonstrated thepresence of antibodies against Brucella in animals and humans in different parts of the country. The prevalenceof brucellosis has been found to range from 0.2% to 38% in cattle (Bekele et al., 2010; Ibrahim et al., 2011).

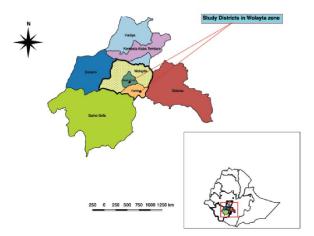
Though various prevalence studies of brucellosis were carried out in different agro-ecological zones of country, there is limited information on the status of bovine brucellosis in Wolaita zone of SNNP region.

In present study we determine the seroprevalence of bovine brucellosis and its associated risk factors in cattle under traditional extensive husbandry in Wolaita zone, Southern Ethiopia.

#### 2. **Materials and Methods**

The present study was conducted in Sodo Zuria and Humbo districts of Wolaita zone southern Ethiopia. The study areas situated at6°35' N 37° 50' E to 6°53 N 37° 49' E. Wolaita zone is one of the thirteen zones of the SNNPR region covering an area of 4471.3 km<sup>2</sup>. It is located at a distance of 332 km. south of Addis Ababa and 157 km away from Hawassa town. It is one of the Omotic speaking people inhabiting the basins of Omo River and Lake Abaya. For administrative purpose the zone is divided in to twelve woredas or districts. Topographically it lies on an elevation ranging from 1200 to 2950 meters

above sea level. The rainfall pattern is bimodal, a short rainy season runs from March to May and long rainy season runs from June to September. The mean annual temperature of the zone is about 19°C being maximum in February which is 29°C and minimum in August which is 15°C. Regarding the land utilization data, 261,000 hectares (ha) is used for cultivation, 5318 ha for grazing, 8261 ha for Bush- land and the remaining 35382.5 ha is a cultivable land. The farming system of the study area is largely characterized by mixed crop-livestock production system. Considerably, variable number and diversity of animal species are maintained under traditional extensive management system. Livestock production system is generally predominated by extensive system in which animals are allowed to forage freely during day time and kept in house during the night. (WZFEDD, 2013; CSA, 2008).



A cross-sectional epidemiological study was carried out on both indigenous and cross breed cattle to determine seroprevalance of brucellosis and their association with different risk factors using two serological tests Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) and structured questionnaire survey from November 2016 to April 2017 in Wolaita zone, Southern Ethiopia.

In order to determine the desired sample size. there were no previous reports of prevalence of brucellosis in the present study area. Therefore, the average expected prevalence rate was assumed to be 50% for the area within 95% confidence interval (CI) at 5% desired precision as stated by Thrusfield (2007). Hence, using the formula, calculated sample for the current study becomes 384 heads of cattle; however, a total of 462 serum samples (234 from Humbo and 228 from Sodo Zuria districts) of both sexes having different ages were sampled to increase the precision of the result.

$$N = \frac{(1.96)^2 P (1-P)}{d^2}$$

Where

N= Total calculated sample size

P= expected prevalence

d= absolute precision

Data was collected and stored in Microsoft (MS) Excel Spread Sheet program and Categorical variables were summarized as frequency and percentages while continuous variables were summarized as mean ± standard deviation (SD). Descriptive statistical analysis of various risk factors and dependent variables were done using (STATA software version 13). The Fisher's exact test was used to test Brucella seroprevalence association with incriminated categorical risk factors. The total prevalence was calculated by dividing the number of RBPT- and CFT-positive animals by the total number of animals tested. Herd prevalence was calculated by dividing the total number of herds with at least one reactor in RBPT and CFT by the number of all herds tested. In these study a herd, defined as the total number of cattle belonging to the same household. Univariate logistic regression was used to test the significance of the effect of different risk factors on sero-prevalence of brucellosis. Odds ratio (OR) was utilized to measure the degree of association between risk factors and Brucella seropositivity. All risk factors that hadnon-collinear effect and p-value < 0.25 in the

univariable logistic regression analysis were subjected to multivariable logistic regression analysis. Age of animals were categorized into <3, 3–6 and >6 years; herd size was categorized into<6, 6-10 and >10 heads of cattle and parity number 0.1 and >1 was categorized as nullparous, monoparous and multiparous.

#### 3. Results

A total of 462 animals, 105(22.7%) male and 357(77.3%) female animals above 1 year of age were sampled for B. abortus antibodies. Of which 10 (2.2%) (95% CI: 1-4) animals tested positive by RBT and 6 animals were confirmed positive by CFT, giving seroprevalence of 1.3% (95% CI: 0.5-3) (Table 1).

The prevalence of bovine brucellosis was significantly higher in animals included in herd size greater than 10 (p = 0.009). Seroprevalence rate of 2.03% was observed in older animals (>6 years) and 1.23% in animals within 3-6 years old. No animal less than 3 years old was found to be seroreactive.

All seropositive animals were females and were either pregnant or lactating. Except for the cow with history of abortion and RFM, other variables did not significantly associate with animal level seropositivity in female animals. The seroprevalences of brucellosis were significantly associated with aborting cows (p=0.035) and cows with a history of RFM (p=0.011) (Tables 2, 3, 4).

Table 1: Brucellosis seropositivity at individual animal level

Risk factors	No. Tested	RBPT positive (%)	CFT positive No. (%)	p-value
Study districts				0.216
Humbo	234	7 (2.99%)	5 (2.13%)	
Sodo Zuria	228	3(1.3%)	1 (0.44%)	
Age				0.438
<3 years	103	0 (0%)	0 (0%)	
3-6 years	162	2(1.23%)	2 (1.23%)	
>6years	197	8(4.06%)	4 (2.03%)	
Sex				0.345
Female	357	10(2.8%)	6 (1.68%)	
Male	105	0(0%)	0 (0%)	
Breed				1.00
Local	364	9(2.74%)	5 (1.37%)	
Cross	98	1(1.02%)	1 (1.02%)	
Herd size				0.009
<6	221	4(1.8%)	1 (0.45%)	
6-10	168	2 (1.19%)	1 (0.59%)	
>10	73	4(5.48%)	4 (5.48%)	
Repro. Status				0.530
Heifer	80	0(0%)	0 (0%)	
Pregnant	111	4(3.6%)	2 (1.8%)	
Lactation	140	5 (3.57%)	4 (2.85%)	



Dry	26	1(3.85%)	0(0%)	
Mating system				0.172
Non	79	0(0%)	0(0%)	
Natural	190	6(3.57%)	3(1.57%)	
AI	51	1(1.96%)	1(1.96%)	
Mixed	37	3(8.1%)	2(5.4%)	
Parity				0.061
Null parous	115	0(0%)	0(0%)	
Mono parous	70	0(0%)	0(0%)	
Multiparous	172	10(5.8%)	6(3.5%)	
Abortion				0.035
Absent	338	8(2.37%)	4(1.18%)	
Present	19	2(10.5%)	2(10.5%)	
RFM				0.011
Absent	325	5(1.54%)	3(0.9%)	
Present	32	5(15.6%)	3(9.37%)	

Table 2: Individual animal level seroprevalence of brucellosis and associated risk factors

Risk factors	No. Tested	CFT positive (%)	95%CI	OR	P-value
Study districts					
Humbo	234	5 (1.86%)			
Sodo Zuruia	228	1 (0.44%)	0.02, 1.74	0.20	0.14
Breed		, , ,			
Local	364	5(1.37%)			
Cross	98	1(1.02%)	0.1, 6.4	0.74	0.78
Herd size		, ,			
<6	221	1(0.45%)			
6-10	168	1(0.59%)	0.1, 21.2	1.32	0.85
>10	73	4 (5.48%)	1.4, 26.0	12.75	0.02
Abortion (n=357)		, , ,			
Absent	338	4(1.18%)			
Present	19	2(10.5%)	1.7, 57.4	9.82	0.01
RFM (n=357)					
Absent	325	3(0.9%)			
Present	32	3(9.37%)	2.14, 57.52	11.10	0.00

Table 3: Herd level seroprevalence of brucellosis and associated risk factors

Variables	No. herd tested	CFT Positive (%)	95% CI	OR	p- value
Study Districts					
Humbo	50	5 (10%)			
Sodo Zuria	54	1 (1.85%)	0.02, 1.5	0.17	0.11
Herd size					
<6	55	2 (3.6%)			
6-10	35	3 (8.6%)	0.4, 15.7	2.48	0.33
>10	14	1 (7.1%)	0.2, 24. 2	2.04	0.57
Abortion					
Present	19	3 (15.8%)	0.9, 27.7	5.12	0.06
Absent	85	3 (3.5%)			
RFM					
Present	29	5(17.2%)	1.7, 138.6	15.4	0.01
Absent	75	1(1.3%)			

Risk factors	No. Tested	CFT Positive No. (%)	95%CI	OR	P-value
Herd size					
<6	221	1 (0.45%)			
6-10	168	1 (0.59%)	3.9, 26.2	1.6	0.74
>10	73	4 (5.48%)	1.4, 29.7	13.7	0.02
Abortion					
Absent	338	4 (1.18%)			
Present	19	2 (10.5%)	1.5, 64.4	9.8	0.01

Table 4: Multivariable logistic regression analyses

#### 4. Discussion

Cross-sectional serological study, attempted to look the status of bovine brucellosis in two districts of Wolaita zone, southern Ethiopia. The study reveals that, the animal level prevalence of bovine brucellosis in extensive management system was found to be (1.3%). This relatively low prevalence might be attributable to extensive grazing conditions; these could reduce both animal to animal contact and the contamination of pastures under dry climatic conditions (Crawford et al., 1990; Adugna et al., 2013). Another explanation could be that, in the area studied, most of the farmers partly practice alternative farm products such as cash crops. Therefore, in the area small numbers of animals (on average five animals) are kept separately and free movement of animals were restricted and are tied around farmland specially during crop harvesting in order to feed on byproducts (post-harvest products) of the farms as reported by (Megersa et al., 2011). In the present study area, the majority of farmers replace their animals from their own stock instead of buying animals from markets.

Corresponding present study the low prevalence of bovine brucellosis has been reported in other studies on cattle under similar production systems in different parts of Ethiopia; 1.66% prevalence reported from Sidama zone (Asmare et al., 2010), 1% from Benshangul Gumuz (Adugna et al., 2013), 1.97% from East Wollega (Moti et al., 2012), 1.2% from Western Tigray (Haileselassie et al., 2010), 1.7% from Arsi Zone (Tsegaye et al., 2016) and 3.3% from Alage district (Asgedom et al., 2016). It also agrees with 2% from Sudan (Senein and Abdelgadir, 2012), 2.77% from Eritrea (Scacchia et al., 2013). Lower prevalence of brucellosis also has been reported in intensive farms (Bashitu et al., 2015; Asgedom et al., 2016). On the contrary higher prevalence has been reported from the highland areas of Ethiopia among cattle in smallholder production systems based on the same diagnostic tests (Kebede et al., 2008). This

variation is merely due to differences in cattle production systems (Mohan et al., 1996). Based on the same test, a higher prevalence was also reported in pastoral areas, compared with an extensive cattle production system (Dinka & Chala 2009: Tibesso et al., 2014).

The present study showed that there is nonsignificant difference in seroprevalence of brucellosis between the two districts (Sodo Zuria and Humbo). This finding is in agreement with the report of (Berhe et al., 2007; Ibrahim et al., 2009; Adugna et al., 2013). This could be due to similarity among traditional management systems in the two districts and where sedentary livestock raising is predominant. The study also revealed that all seropositive animals were females. This finding agrees with the reports by (Kebede et al., 2008; Tolosa et al., 2008; Dinka & Chala 2009; Adugna et al., 2013). However, Hailemelekot et al., (2007) reported 2.11% seroprevalence of brucellosis in male under extensive management system. Female animals are maintained in herds over extended time period thus, have ample time for exposure to the pathogen and being source of infection for other animals (Megersa et al., 2011; Adugna et al., 2013). Other explanation for this finding could be that the number of male animals in each herd was low and were mostly reared separately. thus the chance of exposure is lower for males.

Breed of animal was not significantly associated with brucellosis in this study. Breed differences in susceptibility have not been clearly documented in cattle, although genetically determined differences in susceptibility of individual animals have been demonstrated (Corbel, 2006). There is still argument among different authors on the issue of breed susceptibility to brucellosis. In this study, the seroprevalence was found to be higher in local breed animals (1.37%) than cross breed (1.02%). However, this difference was not statistically significant which is in agreement with the report of (Lidia, 2008) and (Moti et al., 2012) in central highland and East Wollega zone of Ethiopia respectively. This could be due to, limited number of cross breed animals in this study because of their low number in extensive production system. On the contrary, (Jergefa et al., 2009) in their study found that breed of cattle has significant effect on the sero prevalence of brucellosis and is higher in crossbreed than in indigenous ones. This is due to the compounded effect of management systems in cross-breed and also the farmers who follow owned cross-bred tend to intensive management.

The present study also revealed that the seroprevalence of bovine brucellosis was not significantly associated with the age of the cattle. Brucellosis appears to be more associated with sexual maturity (Radostits et al., 2007), and higher seroprevalence is repeatedly reported in sexually matured animals. In this study, seropositive to brucellosis were insignificantly higher in age greater than three years including males. This agrees with the report of (Asfaw et al., 1998; Bekele et al., 2000; Omer et al., 2000; Jergefa et al., 2009; Asmare et al., 2010; Adugna et al., 2013). In this study, seropositivity occurred only in cow having mono parity. Similarly, higher seropositivity has been reported in other studies in animals older than five years, when compared with younger animals (Berhe et al., 2007; Dinka & Chala, 2009; Adugna et al., 2013). Seroprevalence may increase with age as a result of acquired immunity in infected animals and prolonged exposure to pathogen.

Herdsize remained independently significantly associated with the animal level seropositivity to brucellosis in this study. This finding is in agreement with the reports (Asfaw et al., 1998; Tolosa et al., 2008; Asmare et al., 2010, Haileselassie et al., 2010; Ibrahim et al., 2010; Adugna et al., 2013;). An increase in herd size is usually accompanied by an increase in stocking density, as well as an increase in the risk of exposure to infection. Stocking density is an important determinant of the potential for transmission between susceptible and infected animals (Crawford 1990; Omer et al., 2000). In this study the number of animals per herd was generally low, with a maximum herd size of 18 animals, which is typical of mixed livestock and crop production. This would suggest that the risk of brucellosis increases with herd size. Similarly, the increased herd seropositivity has been reported in Zimbabwe (Matope et al., 2010). The result of present study indicates that bovine brucellosis should be considered in extensive production system as in intensive production in Ethiopia.

There was no seropositive reactor in nulliparous, monoparous as well as in animals less than 3 years of age. This finding correspondence with the report of 0.0% by (Ibrahim et al., 2009), 0.69% (Berhe et al., 2007), 1.4% (Kebede et al., 2008) for the same group of animals. This shows that brucellosis is highly related with age and sexual maturity of animals. The reproductive status did not significantly determine seropositivity in the present study. However, all seropositive animals were either pregnant or lactating. This agrees with the report of (Omer et al., 2000; Tolosa et al., 2008; Adugna et al., 2013). Sexually mature and pregnant cows are more susceptible to infection with Brucella than sexually immature cattle of either sex. This has been attributed to the affinity of these bacteria to the pregnant uterus and to erythritol in fetal tissue, possibly also to steroid hormones (Radostits et al., 2000).

Seroprevalence of brucellosis was significantly associated in cow with history of abortion and placenta retention in the current study. Thus the history of abortion and placenta retention were found to be 10 and 11times more likely to be seropositive when compared to no history of abortion and RFM Association between respectively. brucellosis seroprevalence and occurrence of abortion and placenta retention also reported (Berhe et al., 2007; Tolosa et al., 2008: Ibrahim et al., 2010: Adugna et al., 2013; Tsegaye et al., 2016). Due to its collinearity with history of abortion, history RFM was not included in the multivariable analysis. The reason is that, in most case the effects of abortion leads to placenta retention. This could be explained probably by the fact that abortion is the typical outcome of brucellosis infections (Schelling et al. 2003).

The overall herd level seroprevalence of bovine brucellosis was 5.8%, which is comparable to herd level seroprevalence report of 3.3% (Haileselassie et al., 2010), 4.9% (Adugna et al., 2013) and 7.3% (Tsegaye et al., 2016) under extensive management Nevertheless, higher systems. herd seroprevalences have been reported in other parts of Ethiopia in herds under extensive production systems (Berhe et al., 2007; Kebede et al., 2008; Tolosa et al., 2008; Dinka & Chala 2009; Jergefa et al., 2009; Asmare et al., 2010; Ibrahim et al., 2010; Asgedom et al., 2016). Similarly higher herd-level prevalence has also been reported in dairy cattle in other African countries (Matope et al., 2010). This inconsistency could be due to relatively larger herd sizes compared with herds in this study and different in management.

Cow with a history of RFM was significantly affects herd seropositivity. The herd seroprevalence of brucellosis was higher in herds that had a history of RFM (17.2%), compared with no history of RFM (1.3%). This could be explained by the fact that retained placenta is typical outcomes of brucellosis. On the contrary the presence of a cow with a history of abortion did not significantly affect herd seropositivity. However, the herd seroprevalence of brucellosis was higher in herds that had history of abortion (15.8%) compared with non-aborted (3.5%). This could be due to the presence of other causes of abortion in herd. This finding is in agreement with a previous reports (Kebede et al., 2008; Adugna et al., 2013).

A total of 80 cattle owners and attendants were interviewed to assess their awareness levels regarding animal management, brucellosis and occupational risks using structured questionnaire. Knowledge of diseases is a crucial step in the development of prevention and control measures (Prilutski, 2010). Despite huge efforts of the government and nongovernment institutions to improve animal production in the areas, general knowledge of brucellosis among the farmers was still poor. The educational status attained by majority of the respondents was low which falls between illiterate and lower grades. This low level of educational status may lead to reduced production of dairy farms because of low use of dairy innovations such as cultivation of improved forages, breeding techniques and use of modern dairy farming. In addition to this, personal hygiene, proper disposal of aborted materials and the use of a separate parturition pen were not under consideration. These could have led high risks of transmitting the disease within and between the herds and human. This is in agreement with previous studies in extensive livestock production system (Ragassa et al. 2009; Megersa et al. 2011; Adugna et al., 2013). Likewise, mixing of different animal species having its own economic importance also increases the chances of transmission of brucellosis to the cattle.

The occurrence of brucellosis in humans is associated with contact with domestic animals (Alballa, 1995), exposure to aborted animals and assisting animal parturition (Cooper 1992; Kozukeev et al. 2006). In this study, the majority of the farmers have the habit of drinking raw milk and assisting parturition. This implies that little attention has been given to preventing brucellosis and that this, in turn, contributes to the spread and transmission of the infection to human in the area.

The present study has established that the bovine brucellosis persists at a low seroprevalence in Wolaita zone southern Ethiopia. The seroprevalence of bovine brucellosis was found to be 1.3% and 5.8% at animal level and herd level respectively. The low awareness of livestock owners on zoonotic importance of brucellosis and custom of consumption of raw milk, assisting parturition and handling of aborted materials were to be risk factors for human brucellosis. Therefore, the low prevalence of brucellosis in the present study area could serve as source of infection

to other cattle of the different herd as there is free movement of animals between herds.

Community educational program should be carried out targeting brucellosis in the areas to aware livestock owners as well as general public in order to avoid direct or in direct contact with infected animals and their products.

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