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ORIGINAL RESEARCH

Seroprevalence of Bovine Brucellosis and Associated Risk Factors in Western Ethiopia

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¹Wollega University, College of Veterinary Medicine, Nekemte, Ethiopia; ²West Wollega Zone, Haru District Livestock and Fishery Development Office, Gimbi, Ethiopia **Background:** Brucellosis is a contagious bacterial disease, which affects domestic animals, humans, and wildlife in Ethiopia and other countries.

Methods: A cross-sectional study design using a simple random sampling technique was conducted in Diga, Guto, Gida, and Sibu Sire districts of East Wollega Zone, Western Oromia, from November 2019 to July 2020 to determine the prevalence of bovine brucellosis and identify associated risk factors. A lottery method was used during serum collection and risk factors identified during interview.

Results: A total of 1152 serum samples were collected from cattle of 6 months age and above. Serum samples were tested by enzyme linked immunosorbent assay (I-ELISA). An overall seroprevalence of brucellosis was 1.82% (21/1152). Statically significant variation (P<0.05) in seroprevalence of bovine brucellosis was recorded among potential risk factors such as breed [P≤0.05; OR:8.905; CI:1.568–50.573], parity [P≤0.05; OR:0.017; CI:0.042–5.195], retained fetal membrane [P≤0.001; OR:0.018; CI:0.00–2.169], and abortion history [P≤0.001; OR:0.030; CI:0.004–0.212]. Conversely, seroprevalence variations in sex, district, villages, body condition score, and age were statistically insignificant (P>0.05).

Conclusion: The current study revealed bovine brucellosis is posing a threat to livestock with no strict control and prevention scheme in place. Therefore, public awareness creation about the zoonotic importance of the disease is crucial and the Government should establish legislation, support, control, and prevention of the disease recommended.

Keywords: bovine brucellosis, *Brucella*, districts, ELISA, risk factors, seroprevalence, serum

Introduction

Brucellosis is a highly contagious, zoonotic, and economically important bacterial disease of wide variety of terrestrial and aquatic animals. This disease is considered one the most widespread world's zoonosis.¹ Domestic animals (cattle, sheep, goats, camels, and pigs), humans, and wildlife are affected by this disease.² There are usually three main causes of bovine brucellosis namely *Brucella abortus*, *B. melites* and *B. suis.*³ *Brucella abortus* serovar-1 is the common serovar.^{4,5} Third trimester abortion, weak calves, stillbirth, endometritis, infertility, and reduced milk production are common signs in female animals. In bulls the disease is characterized by orchitic, epidydimitis, seminal vasculitis, and sterility.^{6,7} Since brucellosis is an important cause of abortion, especially in first calf heifer, the disease can also cause economic losses in developing countries.³ Ingestions of contaminated pasture, feed, fodder, water and contact with aborted fetus, urine discharge, and new born calves which contains a large dose of infectious organisms constitute a very important

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source of infection.⁴⁴ The risk factors that influence transmission and maintenance of brucellosis were age, sex, number of parity, and number of service; preconception and reproductive status were associated with seropositivity of *Brucella* species.¹

Despite the contribution of the livestock sector in Ethiopia to the national economy, the productivity per animal is very low, primarily due to technical constraints and diseases like brucellosis.8 A large number studies on cattle have reported seroprevalence of brucellosis ranging from 0-50% in intensive management systems9,10 and 0.05–15.2 in extensive management systems.^{11,12} Human and animal brucellosis were reported from different localities of Ethiopia in particular which is associated with cattle in different agro-ecology and production systems.¹³ The prevalence studies in animals and humans are largely confined to serological surveys and commonly targeted bovine brucellosis, occasionally sheep and goat, and rarely camel. Therefore, so far an attempt to identify Brucella species in the country was unsuccessful; the distribution and proportion of their natural host have also not been exhaustively studied.¹⁴ The prevalence of bovine brucellosis is established in different parts of the country, but little information is available on its status in the study area. Further assessment on the status of the disease and associated risk factors has paramount importance for control and prevention of brucellosis in Ethiopia in general and western Ethiopia in particular. Hence the current study aimed to determine seroprevalence of bovine brucellosis in the study area, as well as the risk factors involved.

Materials and Methods Description of the Study Area

The study was conducted in three purposively districts of East Wollega Zone Western Oromia, Ethiopia, namely: Diga, Guto Gida, and Sibu Sire districts based on accessibility to the road, feasibility of sample collection, and number of cattle they possess. Diga district is located at a distance of 343 km from Addis Ababa and 12 km from Nekemte. There are about 69,965 cattle, 16,424 ovine, 11,857 caprine, 39,730 poultry, and 80,099 people. Its potential land area coverage is 59,545.43 hectares. It is bounded by East Guto Gida, West Wollega Zone and Benishangul Gumuz, North Sasiga district, and South Leka Dulecha, as reported by Diga Livestock and Fishery Office.¹⁶ Guto Gida districts are located at a distance of 320 km south west of Addis Ababa, the

capital city of Ethiopia. There are about 13,6005 cattle, 22,004 ovine, 23,349 caprine, 837,095 poultry, and 101,189 human population. It is bounded by East Wayu Tuka, West Diga and Sasiga districts, North Gida Ayana district, and South Leka Dulecha.¹⁷ Sibu-Sire district is located at a distance of 273 km west of Addis Ababa and 58 km from Nekemte. There are about 323,954 cattle, 45,723 ovine, 32,258 caprine, 186,215 poultry, and 92,099 human populations. A map of the study area is indicated in Figure 1.

Study Population

The inclusion criteria of the study population were cattle with the age of 6 months or above and both indigenous breeds of cattle kept under extensive husbandry and crossbreeds in the selected sites of Diga Guto Gida, and Sibu Sire districts were the study subject. Accordingly, by guidance of development workers 384 heads of cattle of age greater than 6 months and both sex were sampled from three selected PAs of each district. The animals were sampled from each PA by including different households and proportionally selecting from their cattle by lottery method. During sampling most of the animals included in the study were female animals since they were kept for milk and production where males sent for draught and slaughtering purposes.^{54,55}

Study Design

A cross-sectional study was used to screen for bovine brucellosis between November 2019 to July 2020.

Sampling Technique and Sample Size Determination

The total number of animals required was determined using the formula given by Thrusfield.¹⁸ Sample size was determined by using the 95% confidence interval at 50% expected prevalence and 0.05 desired absolute precision.

Therefore,

 $n = \frac{((1.96)2(Pexp)(1-pexp))}{d^2} = 384$. To increase the precision, three fold of the sample were included from different agroecology.

Where *n*=required sample size; *pexp*=expected prevalence, *d*=desired absolute precision.

Studied animals were selected by simple random sampling technique. Accordingly, 1152 heads of cattle of both sexes and different age groups were included in this study. Numbers of samples for each district and village are indicated in Figure 2.



Figure I Map of study area.

Blood Sample Collection and Transportation

Blood sample (9 mL) was collected from the jugular vein of each animal using sterile needles and plain vacutainer tube and allowed to clot in a slant position at room temperature. The serum was decanted into a labeled vial and stored at -20 °C until transported.¹⁹ The sera were transported to Bedele regional laboratory center (BRLC), bacteriology/serology unit, by using an ice box and stored at -20 °C for further processing.²⁰ Breed, sex, age, body condition, and peasant associations with other risk factors were recorded along with each animal blood sample.

Indirect Enzyme Linked Immunosorbent Assay

All collected sera were screened by indirect enzyme linked immunosorbent assay (I-ELISA) according to the World Organization for Animal Health²⁰ procedures and instructions. Indirect enzyme linked immunosorbent assay was

performed using a commercial I-ELISA kit (from ID.vet, BRUS-MS-5P, C35, 1014GB, Grabels, France). It detects antibody directed against B. abortus. The protocol provided by developers was followed.²⁰ Before performing the test the serum and all reagents were taken out of the refrigerator and left half an hour at room temperature. The serum and controls were added in to the microwells, which were coated with B. abortus LPS at 1/20 dilution rate. Following the addition of multispecies horseradish peroxidase (HRP) conjugate and substrate solution (TMB) at a recommended strength, the plate strength, the plate incubated, and examined for the intensity of reaction on automated ELISA reader at 450 nm. Yellow color developed within a well after dilution of stop dilution indicates that the tested serum has antibodies to Brucella. The test was valid if the mean odds ratio value of positive and the ratio of positive to negative control were calculated as ODPC>1.0 and ODPC/ODNC>0.1 respectively. The result harvested was interpreted as seropositivity percentage (S/P %) of $\geq 60\%$ as positive and < 60% as negative.²⁰



Figure 2 Sample size of each district and each village.

Data Management and Analysis

Data entered on Microsoft Excel spreadsheet were analyzed using STATA version 14. Multivariate logistic regression was used to test the significance effect of risk factors. Odds ratio (OR) was utilized to measure the degree of association between risk factors and brucellosis. For statistical inference P-value<0.05 at 95% confidence level and 5% precision was considered as statistically significance.

Ethical Approval and Consent to Participate

All participants were informed about the purpose of this study and signed written legal consent for participation prior to the commencement of the study. Study design involves animals for blood sample and human participants for interview. The Survey protocol and animal handling ethics was approved by Wollega University School of Veterinary Medicine Ethical Review Board. Support letters were also, granted from East Wollega Zone, Livestock and Fishery resource office, and the administration Office of East Wollega Zone.

Results

SeroPrevalence of Bovine Brucellosis

From the total of 1152 bovine sera collected, overall seroprevalence of brucellosis 1.82% (21/1152) was recorded. An almost similar finding was recorded in Guto Gida and Sibu Sire districts, 2.08% (8/384) and 2.34% (9/384), respectively. Lower 1.04% (4/384) seroprevalence reported in Diga District, specifically Jirata village, was reported zero free of brucellosis. However, the prevalence variations among districts were statistically insignificant (*P*>0.05) (Table 1).

Association of Potential Risk Factors with Seroprevalence of Bovine Brucellosis

According to the present study, higher anti-*Brucella* antibody was detected in female than male studied animals, which was 2.3% and 0.6%, respectively. However, the variation was statistically not significant (p>0.05).

District	Village	Tested	Negative	Positive	Village Level Prevalence	District Level Prevalence	P-value	Odds Ratio [95% CI]
Diga	Firomsa	126	123	3	2.4	1.04	0.669	
	Gemechis	128	127	1	0.8			
	Jirata	130	130	0	0			
Guto Gida	Gari	126	122	4	3.2	2.08	0.996	0.000 [0.000–10.00]
	Abdeta	131	130	I	0.8			
	Fayinera	127	124	3	2.36			
Sibu Sire	Burka Talo	129	126	3	2.3	2.34	0.370	3.009 [0.271-33.393]
	Chari Jarso	130	125	5	3.8			
	Ada Buke	125	124	Ι	0.8			
Total prevalence		1152	3	21		1.82		

Table I Seroprevalence of Bovine Brucellosis in Three Different Districts of East Wollega Zone

Factors	Variables	Tested	Positive	Seropositivity	P-value	Odds ratio [95% CI]
		Animai				
Breed	Cross	27	2	7.4		
	Local	1125	19	1.7	0.014	8.905 [1.568–50.573]
Sex	Male	336	2	0.6		
	Female	816	19	2.3	0.166	2.966 [0.638–13.794]
Age	6	240	2	0.8		
	months-3 years					
	3–6 years	622	12	1.9	0.358	0.307 [0.056–1.668]
	6 years	290	7	2.4	0.171	0.523 [0.147–1.862]
BCS	Poor	375	15	4		
	Medium	635	4	0.6	0.118	3.550 [0.725–17.375]
	Good	140	2	1.4	0.348	0.428 [0.073–2.518]
Retention of placenta (only	Present	60	13	21.66		
female)	Absent	756	6	0.79	≤0.001	0.018 [0.002 -0.169]
Abortion history (only	Absent	745	7	0.94		
female)	Present	71	12	8.7	≤0.001	0.030 [0.004 -0.212]
Parity (only female)	No parity	359	5	1.39		
	Single parity	92	2	2.17	0.040	27.372 [1.158 -646.908]
	Multiple parity	365	12	3.29	0.045	0.017 [0.042 -5.195]
Pregnant status (only female)	Not Pregnant	542	6	1.11		
	Pregnant	274	13	4.66	0.633	0.566 [0.055–5.831]

 Table 2 Step Wise Association of Risk Factors with Seropositivity of Bovine Brucellosis

Similarly, seroprevalence variations in district, villages, breed, age, and body condition score were statistically non-significant (p>0.05) (Table 2). On the other side, other variables, breed, pregnancy status, retained fetal membrane, and abortion history were significantly associated with the disease (P<0.05) (Table 2).

The results of univariable analysis indicated that there is a significant association between the local and crossbreeds of female cattle and *Brucella* seroprevalence. The odds of bovine brucellosis in the crossbreed are 8.905 (95% CI:1.568–50.573) times higher than those of the local breed. Similarly, history of abortion and placental retention in females was found to be significantly associated with bovine brucellosis. Univariable logistic regression revealed that cattle with a history of abortion had 0.03 times (95% CI:0.004–0.212) odds than female cattle with no history of abortion. The odds of cattle with single parity were 27.372 times higher than no parity whereas cattle with multiple parity were 0.017 times higher than that of no parity (Table 2).

Discussion

Bovine brucellosis remains largely underdiagnosed and uncontrolled amongst many pastoral peoples and small scale livestock holders. Scarcity of resources combined with problem in diagnosis of the disease, limited knowledge of regional epidemiology, and little understanding of the social factors contributing to the disease increases the persistence of the disease in bovine population in Diga, Guto Gida and Sibu Sire Districts of East Wollega Zone.¹⁴ The overall prevalence of brucellosis in the western part of Ethiopia was 1.82%. This finding was comparable with previous recorded reports from different parts of Ethiopia,²¹ such as 1.3% in Eastern Ethiopia,²² 1.38 in Somali Regional state, Jijjiga Zone,^{4,15} 1.4% in Bushoftu and Asella,²³ 1.49% in North West Ethiopia and⁵⁶ 1.04% seroprevalence of bovine brucellosis recorded by I- ELISA technique in in Becho District, South West Shewa, Oromia Regional State, Ethiopia.

On the other side, lower seroprevalence was recorded in different parts of Ethiopia,¹¹ with 0.73% in Jimma Zone,²⁴ 0.49% in Western Shewa,²⁵ 0.4% in urban dairy farms of Northern Ethiopia,²⁶ 0.2% in Debrebirhan and Ambo Towns,¹² and with 0.05% in Arsi Zone. There are reports of a higher seroprevalence rate of bovine brucellosis in other parts of the country,²⁷ 3.1% in Jimma Zone of Oromia region,²⁸ 2.77% in the Addis Ababa dairy cattle,²⁹ 4.3% in Adami Tulu, central Ethiopia,³⁰ 4.9% in Western Tigray,³ 8.0% in pastoral region of the country,³¹ 9.87% in Asella organized dairy farm,³² 10.6% in Borana,³³ 11.0% in Wuchale-Jida district, central Ethiopia,³⁴ and with 11.2% in pastoral and agro-pastoral areas of East Shewa Zone. Similarly, higher seroprevalence was reported in other African countries³⁵ with 5.3% in Tanzania,³⁶ 24.0% in Nigeria,³⁷ 21.9% in Ghana³⁸ and 24.5% in Sudan. The difference in total prevalence may be due to the difference in study season, type of production system, differences in sample size, laboratory techniques employed, and ecology.

Prevalence in cross-bred (7.4%) animals was higher than in local (1.7%) cattle. This difference was statistically significant (p<0.05).^{31,39–42} There was similarly reported significant variation in the serological prevalence of brucellosis with the prevalence in cross-bred than indigenous ones. The higher seroprevalence in crossbreeds might be due to the lower sample size, the smaller number of the crossbreeds examined compared to local breeds.

In the present study, sex was one of the risk factors affecting the susceptibility of cattle to the *Brucella* infection. Seropositivity in females (2.32%) was indicated in the study area whereas (0.6%) seropositive in males. However, the finding was statistically insignificant which disagrees with the previous report.^{11,43} The lower seroprevalence in males could be due to smaller number of males examined when compared to females and it was also reported that the serological response of male animals to *Brucella* infection is limited.^{44,45} In addition to this, it has also been reported that infected male animals are usually found to show low antibody titers.⁴⁶

It is obvious that sexually matured animals are more susceptible to *B. abortus* infection,^{11,45} which was explained by the fact that susceptibility increases during sexual maturity and gestation period due to the influence of sex hormones and elevation of fetal fluid and erythritol sugar in the placenta. This stimulates the growth and multiplication of bacteria in the reproductive organs.^{1,30,47} However, in the current finding, seroprevalence variations among groups of ages are statistically insignificant.

In present study, the association of parity and *Brucella* infection was statistically significant. Different findings were reported.^{45,49} The existence of abortion history and retained fetal membrane were significantly associated (p<0.05) with the infection in this study. This finding was consistent with the Tolosa and Geresu.^{48,49} This could be explained by the fact that these two factors are the typical outcome of the brucellosis.^{31,41,49} Other studies

have also shown a significant association between the disease and abortion and retained fetal.^{27,41,43,50} Similarly, studies from different African countries show that brucellosis in bovine directly correlates with the presence of abortion.^{51–53}

Conclusion and Recommendations

The present study revealed that the overall prevalence of bovine brucellosis in the study area was 1.82. Sex, BCS, breed, retained fetal membrane, and abortion history were potential risk factors for the studied disease. But, there is no control and prevention scheme in place because of lack of information on the disease status and the cost of elimination of the disease from the country is far beyond the economic status of the farmers and the country at large. Therefore;

- Awareness creation about the impact of the disease, the way of its transmission, risk factors, methods of prevention of the disease, and culling of the positive animals should be made.
- Proper hygienic and sanitation practices to reduce the risk of exposure to brucellosis.
- Furthermore, detailed study to investigate the link of the disease with human in the study area was recommended.

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Disclosure

The authors report no conflicts of interest in this work.

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