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Sero Epidemiology of Cattle *Brucellosis* and Associated Risk Factors in Amibara District of Afar Region, Ethiopia

Fekadu Gutema[1*] and Juhar Tesfaye[2]

[1]*Holeta Agricultural Research Center, Holeta, Ethiopia*

[2]*Werer Agricultural Research Center, Werer, Ethiopia*

Abstract

Bovine brucellosis is the most common but under reported bacterial diseases known to create a serious socio-economic problem in both intensive and extensive livestock production systems. A cross-sectional study was conducted to determine seroprevalence and associated risk factors of cattle *brucellosis* in Amibara district of Afar region, Ethiopia from October 2019 to May 2020. A total of 181 cattle sera were collected and screened using Rose Bengal Plate Test (RBPT) and reactive samples were further confirmed by Complement Fixation Test (CFT). Risk factors associated with cattle *brucellosis* were assessed during serum sample collection using data collection format. As a result, the overall seroprevalence of cattle *brucellosis* was 10.5% and 2.2% by RBPT and CFT respectively. Assessment of potential risk factors showed that, Age (x^2=6.77, p=0.021), number of parity (x^2=9.433, p=0.004), abortion history (x^2=16, p=0.002) and history of placental retention (x^2=19.1, p=0.003) showed statistically significant association with *brucellosis* seropositivity in cattle. Based on firth's bias reduced logistic regression analysis, only multiparous animal (OR=10.68, P=0.0042, 95% CI=-1.19-7.595) and animals with placental retention (OR=72.72, P=0.0026, 95% CI=1.46-9.272) showed statistically significantly association with *brucella* infection in cattle. In conclusion, the results of the current study indicate the presence of *brucellosis* in cattle in Amibara district of Afar region, Ethiopia. Hence, implementing preventive measures such as developing vaccination strategy, regular screening and culling of the reactive animal is important to create diseases free herd.

Keywords

Vaccination • Culling • *Brucellosis* • Cattle • Seroprevalence

Introduction

Ethiopia stands first in Africa based on cattle populations despite gaining minimum return from this resource because of managements, policy and different infectious diseases [1]. Bovine *brucellosis* is the most common but under reported bacterial diseases which is known to create a serious socio-economic problem in both intensive and extensive livestock production systems [2]. It is caused by *Brucella* spp. and manifests itself as abortion and infertility in domestic and wild animal species and reduced milk production [3]. In cattle the disease is mainly caused by *B. abortus* and characterized by inflammation of the genitals and foetal membranes, abortions, sterility and lesions in the lymphatic system and joints [4].

Developing countries with limited resources have not yet fully launched programs featuring any aspects of *brucellosis* intervention, since they are facing other priority diseases that are more spectacular [5]. Risk factors associated with *brucellosis* can be categorized into management, animal, and environmental factors [6]. Sources of infection for the transmission of the bovine *brucellosis* are aborted fetuses, retained fetal membranes, and vaginal discharges and milk from infected animals [1]. In human, *brucellosis* is one of the most common bacterial zoonotic infections, but remains under reported disease in Ethiopia due to the absence of diagnostic facility in public hospitals. *B. melitensis, B. abortus*, and *B. suis* are known to induce significant public health problems [7].

The risks of zoonotic transmission of this disease from animals to human are associated to climate change, unprotective husbandry practices, eating habits and social behavior of the concerned population. To control and eradicated this disease, different measures have to be taken including vaccination, maintaining farm hygiene, public education and environmental protection. The elimination of sero-reactors, development of control strategies, and education programs regarding the prevention and control of this zoonotic disease are highly needed in developing countries [5]. There is no documented information on how and when bovine *brucellosis* was introduced into Ethiopia. However, several serological studies have been conducted in the last two decades and showed that it is endemic and widespread [8]. Pastoralists with high livestock population are known for their seasonal migration habit to search for feed and water during the dry period which results in intermingling of different herd groups and sometimes with wild animals at watering point and on the field. This results in transmission of disease like *brucellosis* from one herd to another and from domestic to wild animals. Because of this, periodic investigations need to be implemented to enhance understanding about *Brucella* epidemiology which in turn is very important to refine control strategy [9]. Therefore, this study was conducted to determine seroprevalence and to investigate potential risk factors of cattle *brucellosis* in Amibara district of Afar region.

Materials and Methods

Description of the study area

This study was conducted in Amibara district of Gabirasu zone (Zone 3 of Afar region) located in the Middle Awash Valley of Ethiopia (Figure 1). Amibara district is about 270 km to the North East of Addis Ababa and has 19 kebeles with total population of ~63,378, of which 35,374 were men and 28,004 women. The altitude of Amibara district is 740 m above sea level. Fourteen years climatic data on monthly basis showed that the average maximum and minimum temperature of the area is 34°C and 19°C, respectively, and its annual total rainfall is about 571 mm [10]. The livestock population of the Amibara district is composed of 103, 959 cattle, 122, 526 goats, 48,043 sheep, 3,888 donkeys and 39,995 camels [11].

Study population

In the present study the target study population was cattle owned by pastoralists. Only indigenous local breed of cattle with no history of vaccination and older than six months of age were recruited into the study. During sampling, cattle's were classified into three age groups (<2 years,

***Address for Correspondence:** Fekadu Gutema, Holeta Agricultural Research Center, Holeta, Ethiopia; Tel: +251932153584; Email: fikadu881@gmail.com*

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2-5 years and >5 years) as young, adult and old respectively [12].

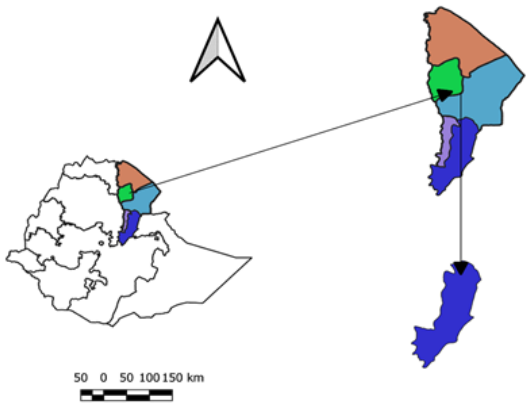

Figure 1. Map showing the Afar regional state and proposed study district.
Note: Afar region (Zone1, (Zone 2, (Zone 3, (Zone 4, (Zone 5.

Study design and sampling techniques

A cross-sectional study design was employed from October 2019 to May 2020 in order to determine seroprevalence and associated risk factors of cattle *brucellosis*. Study kebeles were selected by simple random sampling technique. To select cattle herds in the proposed kebeles, purposive sampling technique was employed base accessibility and willingness of the herd owners to cooperate. Then each herd were stratified into subgroup based on age and sex to ensure equal representation of all subgroup. From each subgroup, individual animals were selected by systematic sampling technique and information related to environmental and study animals were also accessed. The sample size for serological study was calculated by Thrusfield formula using previous study result by who reported 2.4% in Alage district. However, in order to increase precision and reduce standard error, the minimum sample size obtained by calculation was increased by four-fold and 181 cattle were sampled [13].

Blood sample collection

After disinfecting the site of jugular vein, about 8 ml of blood sample was collected into sterile plain vacutainer tube and labeled with code describing each study animal. Then; the samples were taken to Werer Agricultural Research center, animal health research laboratory and kept overnight at room temperature to separate the serum and the clotted red blood cells according to [14]. Then the serum was gently decanted into sterile cryovials (1.8 ml), labeled and stored at -20˚C

Laboratory diagnosis

Rose Bengal Plate Test (RBPT): All serum samples were screened using RBPT at National Veterinary Institute according to the procedure described by the World Organization for Animal Health [14]. In the laboratory, serum samples were kept at room temperature for 30 minutes and then, screened for anti-*Brucella* antibodies using commercial kits of the standard Rose Bengal Plate Test (RBPT). *B. abortus* antigens (Lillidale Diagnostics, Holt wimborne, Dorset, BH21 7DG, United Kingdom) and their positive and negative control sera were used. The detailed procedures for the RBPT were that, 30 µl of cattle serum and 30 µl of antigen was mixed on a test plate and rocked for 4 minutes. After four minutes of rocking, visible agglutination was considered as positive. Agglutinations were recorded as 0, +, ++ and +++, according to the degree of agglutination [15]. A score of 0 indicates the absence of agglutination; + indicates barely visible agglutination; ++ indicates fine agglutination, and +++ indicates coarse clumping. The presence of agglutination at any degree was considered as positive reaction while the absence of agglutination was considered as negative.

Complement Fixation Test (CFT) genomic: Serum samples that reacted positively to RBPT were further retested by CFT for confirmation using standard *B. abortus* antigen S99 (New Haw, Addlestone, Surrey, KTI5, 3NB-UK). Preparation of the reagent is evaluated by titration and was performed according to protocols recommended by World Organization for Animal Health [14]. Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above was considered as positive and lack of fixation/complete hemolysis was considered as negative result. Only samples that gave signals for both RBPT and CFT were considered positive since no single test is appropriate in all epidemiological situations due to problems of sensitivity and or specificity of the tests as recommended by OIE and other reports [16].

Data analysis

Risk factors and serological results were recorded into Microsoft Excel® Spread Sheet and analysis was done using R-Software version 4.0.3. Prevalence was calculated by dividing the number of positive animals to the total number of animals tested. Fisher's exact test was used to calculate associations of risk factors with *brucella* seropositivity. Since the number of outcomes of interest is less than 10%, firth's bias reduced logistic regression model was used to measure the association of potential risk factors with *brucella* seropositivity [17]. Odds Ratio (OR) was used to indicate the relationship between risk factors with animal level seroprevalence of *brucellosis*. P-value less than 0.05 were considered statistically significant in all analysis.

Ethical consideration

Ethical approval for collection and analysis of animal materials was offered by animal research ethical review committee of the College of Veterinary Medicine and Agriculture (CVMA) with certificate ref. number of VM/ERC/03/01/12/2020.

Results

Result of *brucellosis* diagnosis in cattle and associated risk factors

In this study, the sero-prevalence of *brucella* infection at individual animal level was computed by RBPT and CFT. Among 181 serum samples tested, 10.5% (95% CI:0.06-0.15) were found reactive for *brucella* infection by RBPT whereas 2.2% (95% CI:0.00-0.04) of them were confirmed to be *brucella* seropositive by CFT (Table 1).

Table 1. Joint animal and herd level prevalence of cattle *brucellosis* in Amibara district of Afar region.

Herd size	Individual animals			Herd level		
	No tested	RBPT positive (%)	CFT positive (%)	No tested	RBPT positive (%)	CFT positive (%)
Small	45	3(6.67)	1(2.22)	8	3(37.5)	1(12.5)
Medium	46	2(4.35)	0(0.00)	4	2(50)	0(0.00)
Large	90	14(15.56)	3(3.33)	7	6(85.7)	2(28.57)

Based on fisher exact test, different host risk factors were considered and age of the animals (2^b=6.77, P ≤ 0.02), number of parity (x^{2b}=9.433, P ≤ 0.004), abortion history (x^{2b}=16.02, P ≤ 0.002) and placental retention (x^{2b}=19.1, P ≤ 0.01) were found significantly associated with *brucellosis* seropositivity in cattle (Table 2).

Table 2. Seroprevalence and potential risk factors of *brucellosis* in cattle.

Variables	No tested	Seropositive	Prevalence (%)	Fisher exact test value	P-value
Sex				1.948[a]	0.259
Male	13	1	7.7		
Female	168	3	1.78		
Age				6.77[b]	0.021*
Young	30	0	0		
Adult	123	2	1.62		
Old	28	2	7.14		
Body condition				1.462[b]	0.481
Poor	46	0	0		
Medium	107	3	2.8		
Good	28	1	3.57		
Herd size				0.364[b]	1
<20	44	1	2.27		
20-50	90	2	2.22		
>50	47	1	2.13		
Number of parity**				9.433[b]	0.004*
0	25	0	0%		
01-May	117	1	0.85		
>5	26	2	7.69		
Abortion history**				16.02[b]	0.002*
Recent abortion	21	3	14.3		
No abortion	147	0	0%		
P. retention**				19.1[b]	0.003
Yes	4	2	50		
No	162	1	0.167		

In the present study, abortion was considered as an important risk factor and showed statistically significant association with *brucella* seropositivity in cattle $p \leq 0.002$*; No of test: Total number of animals tested; b-Fishers exact test value, x^2: Chi-square, a- Chi- square value, *Significant; **Male animals were excluded from the total number of cattle.

However, sex and body condition of the study animals did not show statistically significant association *brucella* seropositivity (P>0.05).

Firth bias reduced logistic regression analysis of factors associated with *brucella* seropositivity cattle

After computing firth's bias reduced logistic regression, multiparous animals (>5) and placental retention showed statistically significant association with *brucella* infection in cattle. It was also indicated that, adult animals were 16.78 times more at risk of contracting *brucellosis* when compared with young one even though it doesn't stand statistically significant. In addition, multiparous animals were 10.68 times and animals with history of placental retention were 72.72 times more due *brucella* infection when compared with bovine having zero parity and those with no history of placental retention respectively (Table 3).

Table 3. Firth bias reduced logistic regression analysis of factors associated with *Brucellosis* seropositivity in cattle by CFT.

Variables	Number tested	Seropositive (N,%)	OR	(95% CI)	P-value
Age					
Young	25	0	1	1	-
Adult	117	1	16.78	(-0.235,7.86)	0.0695
Old	26	2	0.122	(-8.586,3.93)	0.446
Parity number					
Null	25	0	1	1	-
01-May	116	0	0.007	(-10.8, -1.65)	0.181
Greater than 5	27	3	10.68	(-1.19,7.595)	0.0042
Placental retention					
Yes	6	2	72.72	(1.46,9.272)	0.0026
No	173	2	1	1	-

Discussion

In the present study, 10.5% of tested cattle were found reactive for *brucella* infection by RBPT among which 2.2% were confirmed to be *brucella* seropositive by CFT. The present study finding is in close agreement with the earlier finding in Amibara, in Sidama, in East shoa zone, in Sidama and in central Oromia who reported 2.4%, 2.46%, 2.0%, 2.5% and 2.9% respectively. However, it is lower when compared to the findings of who reported 10.6% in Borana zone, who reported 4.9% in Western Tigray, who reported 4.8% in pastoral community of Afar and Oromia region adjacent to Awash national park and who reported 15.2% in East Showa zone of Oromia region [18-26].

The seroprevalence result of the current study is also lower than some reports in other African countries. For instance, a prevalence of 3.4% in Cameroon, 24.5% in Sudan, 12-15.8% in Uganda by Miller and 26.50 % in India were relatively higher than the present finding [27-29]. The difference in *brucellosis* seroprevalence of the current and previous study results might be due to variation in sample size, husbandry and management practices, virulence of the organisms and diagnostic test employed. In addition to estimation of seroprevalence, this study was also carried out aiming to assess the risk factors associated with disease occurrence in cattle. Therefore; sex, age, body condition, number of parity and herd size of the cattle and history of placental retention were accessed. As a result, some of the considered risk factors were not significantly associated with *brucellosis* seropositivity in cattle except age, number of parities, abortion history and placental retention.

The insignificant association of sex with *brucellosis* seropositivity disagreed with findings of that who reported higher cases of *brucellosis* in female animals than in males. This might be due to the incomparable number of male cattle with female animals in the present study. Concerned with body condition score, 3.57% of animals with good body condition were found positive whereas no reactor animals encountered from those with poor body condition [26,30].

This finding is in agreement with the report, who stated insignificant association of body condition score with susceptibility to *brucellosis*. Herd size of the cattle was also considered as risk factors since *brucellosis* is a disease of herd importance. Accordingly, the herd level seroprevalence was 18.18% which is in close agreement with the finding [27,31]. Even though large herd sizes are more prone to *brucella* infection, no significant association of herd size with *brucellosis* seropositivity was observed in the current study (P>0.05). Consequently, this finding disagrees with findings of [32]. After computing firth bias reduced logistic regression analysis, only multiparous and animals with history of placental retention showed statistically significant association with *brucella* infection (p<0.05).

This finding is in close agreement with who reported significant association of parity and placental retention with *brucella* infection respectively. It was also appreciated that; the owners usually keep their cattle in national park to look for water and pasture which allow close contact of domestic animals with jungle beast. Due to this, *brucellosis* can spread in either direction from contaminated pasture and water point or by direct contact of aborted fetal materials. Study conducted in Tanzania indicated, the interactions between wildlife and livestock as potential risk for *brucellosis* transmission to humans and livestock [22,30].

In human, *brucellosis* is transmitted through consumption of unpasteurized dairy products or through direct contact with infected animals, placentas or aborted fetuses [33,34]. Evidences shown that, the social habit of raw milk and meat consumption, unsafe handling of aborted fetuses and placenta, assisting parturition, and occupations related to animal contacts have been reported to be some important epidemiological factors for human *brucellosis* [26,34]. Study conducts indicated, *brucellosis* were 5.11 times more in those who had assist animals during parturition compared to those who did not. This stands true for pastoral society whom believed to lead mobile lifestyle with few accesses to veterinary service. So, this condition creates a favorable condition for widespread and permanent occurrence of *brucellosis* in the area [35-37].

Conclusion

Brucellosis is a chronic bacterial disease of domestic and wild mammals having worldwide distribution. Cattle contract *brucellosis* through direct and indirect contact with infected animals and their excreta, ingestion of infected materials and sometimes aerosol transmission is expected. Based on the nature of the disease and ease of transmission, *brucellosis* is common in the pastoral society of Afar region due to their habit of mobile life style which allows intermingling of livestock and consumption of raw milk in human. Generally, the current study provides baseline data on cattle's *brucellosis* in the current study district of Afar region, Ethiopia. Hence, working to enhance the awareness level of the pastoral society about the public health and economic significance of *brucellosis* through training and subsequent isolation and characterization of circulating strain and biotype is very important.

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