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Seroprevalence and Associated Risk Factor of Brucellosis in Bovine in and around Bekoji Districts of Arsi Zone South Eastern Ethiopia

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Abstract

Brucellosis is a major public and animal health problem in many parts of the world, particularly in pastoral settings where livestock is a major livelihood and food sources. Effective prevention and control of brucellosis depends on knowledge, attitude and practices of the community. The Disease affects both animals and human being resulting in a serious economic loss in animal production sector and deterioration of public health. A cross-sectional study was conducted between December 2021 and April 2022 in Bekoji District, Arsi zone, with the objectives of investigating the prevalence and associated risk factors of bovine brucellosis. A collection of blood samples (n=186) were carried out. The sera samples were screened using Rose Bengal Plate Test (RBPT) and positive ones were further confirmed by using i-ELISA. Potential Risk Factors for seropositivity were analyzed using Chi-square test and p- value. Results showed that 5 (2.68%) and 2 (1.07%) of the 186 animals were positive for RBPT and i- ELISA respectively. The present study revealed an overall 2.68% and 1.07% seroprevalence of bovine brucellosis in the study area in which difference in breed and parity were Found to be potential risk factors. The seroprevalence was higher in animals above two years than younger Animals. History of abortion and retained fetal membrane were found to be significantly ($P < 0.05$) associated with occurrence of bovine brucellosis. A statistically significant difference ($P < 0.05$) was observed in cross Breeds than local dairy cattle. The results of the present study showed that prevalence of bovine brucellosis in the study area was low and hence, test and use dairy product policy can be used in order to control the disease in the study area.

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Bovine, Brucellosis, Bekoji seroprevalence, zoonotic diseases, cattle, swine, sheep.

Introduction

Zoonoses are diseases that can be transmitted from wild and domestic animals to humans and are public health threats worldwide (Kahn and L.H., 2006). Their impact on health and socioeconomic state are increasingly being felt by many countries and most particularly, although not exclusively by developing countries (Seimenis and Tabbaa, 2014). At the individual health level, zoonotic diseases are a concern for all who live or work with animals (Elchos *et al.*, 2008).

Brucellosis is an infectious bacterial zoonotic caused by member of genus Brucella. The disease is caused by various bacteria of the family Brucella, Brucella organisms are gram negative, small cocobacilli, non-motile, non-capsulated concerning losses in animal production, and non-spore forming (Elchos *et al.*, 2008)

which tend to infect a specific animal species. However, most species of Brucella are able to infect other animal species as well. It affects cattle, swine, sheep and goats, camels, equines, and dogs. It may also infect other ruminants, some marine mammals and humans (Bonfini *et al.,* 2010).

Brucellosis in cattle (*B. abortus*)in sheep and goats (*B.melitensis*) and in swine (*B.suis*) are diseases listed in the World Organization for Animal Health (Ragan *et al.,* 2013). The disease is primary reproductive disease clinically characterized by abortion in the last trimester and retained placenta in the female whereas orchitis and epididymitis with frequent sterility occur in male (Singh *et al.,* 2018)

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and by *B. suis* imposing economic lose and zoonoses (Alemu *et al.,* 2014). Brucellosis has a worldwide distribution and is one of the most important zoonoses in Africa, Asia, Mediterranean region and Middle East (De Massis *et al.,* 2019). Where Africa is one of the endemic areas. In several areas of East African region; it significantly reduces animal productivity through abortion and weak offspring's; causing a major threat in national and international livestock trade (Ntirandekura *et al.,* 2018).

In Ethiopia, brucellosis is found to be one of the endemic diseases of livestock associated with reproductive problem all across the country (Abebe *et al.,* 2017; Megersa *et al.,* 2011). According to Muma *et al.,* (2007) and Schelling *et al.,* (2003) report, cows infected with Brucella are three to four times more likely to abort than unexposed cows.

The mode of transmission of the bacteria varies with the epidemiological area, the animal reservoir and the occupational exposed groups (Seleem *et al.,* 2010). Sources of infection for the transmission of the bovine brucellosis are aborted fetuses, the fetal membranes after birth, and vaginal discharges and milk from infected animals (Coelho *et al.,* 2014; Tolosa *et al.,* 2010).

The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water, and after birth, fetuses, and newborn calves, all of which may contain a large number of the organisms and constitute a very important source of infection. The bacteria can be transmitted to humans through direct contact with infected tissue via breaks in skin, ingestion of contaminated tissues or milk products, and inhalation or mucosal exposure to aerosolized bacteria (Fasil *et al.,* 2015; Radostits *et al.,* 2007). The prevalence of brucellosis is influenced by a number of risk factors related to production systems, biology of the individual host and environmental factors. These include age; breed sex, herd size and composition, hygienic status of the farm, rate of contact between infected and susceptible animals, farm biosecurity and climate (Radostits *et al.,* 2007; McDermott and Arimi, 2002). Brucellosis is perhaps one of the most widespread and economically important diseases in tropical and subtropical regions.

The direct loss of meat (because of abortion, infertility and weight loss) in infected herds of cattle was estimated to be 15% and for milk (reduction in milk production) 20% per infected (Nicoletti, 2010; Nielsen and Yu, 2010)

In Ethiopia, so far higher seroprevalence reports are 39% in western Ethiopia (Meyer, 1980), 22% in a dairy farm in northeastern Ethiopia (Tariku Sintaro, 1994), 11%-15% in dairy farms and ranches in southwestern Ethiopia (Tekleye Bekele *et al.,* 2000), 8.2% in Arsi area (Bayleyegne Molla, 1989) but there is no more information of Brucellosis in the study area and there is no information about brucellosis in the society.

Therefore, the objective of this study that include to determine the current seroprevalence of bovine brucellosis in cattle with extensive, intensive and Sami intensive management system in and around bekoji town. Also to identify the risk factors associated with the seroprevalence bovine brucellosis in and around bekoji town, Arsi Zone Oromia Region of Ethiopia.

Materials and Methods

Study area

The study was conducted in Bekoji Town of Lemu Bilbilo district of Oromia National Regional State. The town is located at a geographic coordinate of 070 32'North latitudes and. 039015' East longitudes.

The city is found at 205km away from Addis Ababa to the south eastern parts of the country at an elevation of 2817 meter above sea level. It receives biannual rainfalls with the average temperature ranges from 6 to 19 °C [9-11]. In this area mixed crop and livestock farming system was practiced. (LemuBilbilo district, 2021).

Study Animal

The study animals were indigenous and exogenous cattle breeds kept under intensive semi-intensive and extensive management system in the area. All cattle in the study area with the age of 6 months or above were considered as the study animals.

Study design

The study design was a cross-sectional study carried out in indigenous and exogenous cattle breed using serological tests where Rose Bengal Plate Test (RBPT) and I-ELISA test was used for confirmation. The study was undertaken from December 2021 to April 2022.

Sampling method

The study animals were selected using simple random sampling method. A total of 186 blood samples were collected from jugular vein of each selected animal after proper restraining. The individual animals in the study were identified by their respective identification numbers or names. About 5-10ml of blood was collected in plain vacutainer tubes. The blood samples were allowed to clot at room temperature. Serum was separated from clotted blood by decanting to cryogenic vial and was kept in a deep freezer (-20°C) till tested serologically using the Rose Bengal Plate Test. Sera testing positive, were tested further by i-ELISA test

Sample size

The sample size was determined by the formula recommended by Thrusfield (2018) as indicated below:

$n = 1.96^2 x\ PQ/D^2$

Where n is required sample size, P is expected prevalence based on previous preliminary surveys, Q is 1-P and D is the level of precision (5%). Since there was previous study carried out 14.1% in Asella district by (Desalegn and Gangwar, 2011) expected prevalence as used in the formula. Accordingly, the total number of animals to be bled from district was 186, which makes a total sample size of 186 for this district.

Questionnaire

A standard questionnaire format was used to collect information relevant to the epidemiological investigation such as species, age and sex. The study subjects included Local and cross bred cattle reared under intensive, semi intensive and extensive management system abortion history retain placenta history.

Serological Tests

Rose Bengal Plate Test (RBPT)

Rose Bengal Plate Test was performed according to the standard procedure described by Alton *et al.,* (1975). The test was carried out at Asella Regional Veterinary Laboratory. The antigen of RBPT, consisted of a suspension of *Brucella abortus*, was obtained from InstitutPurquier 326 (Rue de la Galera, 34097 MONTPELLIER CEDEX 5, France). The results were read by examining the degree of agglutination in good light source and when necessary using magnifying glass deemed. Any visible agglutination was considered positive (OIE, 2004)

Enzyme-linked Immunosorbent Assay (ELISA)

ELISA test was performed according to standard procedure. The test was carried out at Asella regional veterinary laboratory. The ELISA kit for the detection of brucellosis is commercially available. It is fast, convenient and can detect both IgG and IgM to bacteria Antigen than read and record the O.D at 450 nm and thus can be potentially used as a confirmatory test to detect brucellosis for this research.

Data Analysis

Data was stored in the Microsoft Excel spread sheet program and analyzed using STATA 14.1 (2016). The seroprevalence was calculated by dividing the number of ELISA positive animals by the total number of animals tested. Chi-square test was utilized to measure the association between the seroprevalence with categorical selective districts.

Results and Discussion

In dairy cows and bulls of above 6 month age investigated the overall seroprevalence of bovine brucellosis in the study areas was 1.07% which was recorded on the bases of both RBPT and i- ELISA (Table 1). Out of the 186 serum samples, 5 (2.68%) were positive for brucellosis using RBPT and only 2 (1.07%) sera samples further were confirmed positive using I-ELISA. In addition, 68 (36.5%) were local breed whereas 118 (63.44%) were cross-breeds of indigenous

zebu and Holstein Friesian dairy cows. In addition, 112 (60.21%) of the animals were not pregnant, 74 (39.78%) were pregnant. In 11 (5.91%) of the studied animals, there was a history of retained fetal membrane and 16 (8.6%) were with a history of abortion.

Generally, the frequency distribution of breed, parity, age group, sex, retained fetal membrane; breeding system and pregnancy status were summarized in the following table (Table 2). The difference in the seroprevalence of bovine brucellosis among the different age groups was not statistically significant (P>0.05). The recorded seroprevalence of the disease in the young and adults was found to be 0.00% and 2.1%, respectively as listed in Table 3.

The sexes of the tested animals didn't seem to have a significant association (P>0.05) with the seroprevalence of bovine brucellosis. The recorded seroprevalence of the disease in the current study was 1.51% and 0.00% in female and male dairy cattle, respectively (Table 3). Similarly, there was no significant association between the breed of animals and the seroprevalence of bovine brucellosis (P>0.05). The seroprevalence of bovine brucellosis in local and cross dairy cattle was 0.00% and 1.69%, respectively as stated in Table 3.

The seroprevalence of bovine brucellosis in different dairy cattle that use natural mating, artificial insemination (AI), and both methods of breeding were found to be 0.00%, 3.22%, and 0.00%, respectively. Thus, the method of breeding didn't have a significant association with the seroprevalence of bovine brucellosis (P>0.05) as illustrated in Table 3. Moreover, the history of abortion in the dairy cattle had a significant association with the prevalence of bovine brucellosis (P>0.05). The seroprevalence of bovine brucellosis in dairy cattle with a history of abortion and not aborted were found to be 12.5% and 0.00%, respectively (Table 3).

The present study attempted to look into the existence of any association between seropositivity and cows those had retained membrane. Thus, the prevalence of retained membrane and not retained membrane was compared in Table 3.

The sera prevalence of retained membrane and not retained membrane cattle was calculated as 18.18% and 0.00% having X^2 32.1640 and P-value of 0.000. The association of brucellosis with abortion and retained fetal membrane was tested using Chi-square. It was found that brucellosis was significantly associated with abortion and retained fetal membrane with Chi- square value of 21.4810 and 32.1640 and P-value of 0.000 and 0.0020 respectively (Table 4).

The present study revealed that the overall seroprevalence of bovine brucellosis Brucella with RBPT and ELISA in and around Bekoji District Arsi Zone oromia region were 2.68% and 1.07% respectively. This low sero-prevalence is comparable with previous 1.92% in Sidama Zoneby Kassahun Asmare *et al.*, (2007), 1.49% in Tigray Region by Gebretsadik Berhe (2005), 1.13% from cattle slaughtered at Addis Ababa and Sululata Abattoirs by Mulugeta Tefera (2006) and 2.3% in AdaaLiban Dairy Cooperatives by Abrham Abebe *et al.,* (2008). Slightly higher Seroprevalence was recorded in agro pastoral areas of East Shoa Zone (4.1%) by Hunduma and Regassa (2009), Arsisero prevalence 4.9% by Abay (1999) and other reports in different parts of Ethiopia; 4.2% in Ghibe and Gobe by Tekleye Bekele *et al.,* (1989), 2.15% in central highlands of Ethiopia by Assegid Bogale (1987), and in Bahir Dar milk shed (4.63%) by Mussie Hailemelekot *et al.,* (2007). In addition to this, seroprevalence as high as 38.7% was reported in cattle Owned by the Institute of Agriculture Research (IAR) by Muktar Reshid (1993), 22% in Chafa State Dairy Farm by Tariku Sintaro (1994), 19.5% in Abernosa Cattle Breeding Ranch by Taye Yirgu (1991), 16.65 % in and around Bahirdar by Abeje Shiferaw (1994) and 15.8% in Sidamo by Endrias Zewdu (1989). Similarly, moderate seroprevalence rates were reported by Bayleyegn Molla (1989) in Arsi (8.2%), by Gebreyesus Mekonnen (2001) (unpublished). In North Western Amhara on local indigenous zebu (8.2 %) and by Yilkal Asfaw *et al.,* (1998) in urban and peri-urban areas around Addis Ababa (8.11%). and higher sero-prevalence than0.61% in Jimma by Tadele Tolosa (2004), no positive reactors in Selale and Addis Ababa by Kelay Belihu (2002). The different in seroprevalence of bovine brucellosis reported from different parts of Ethiopia might be due to difference in management and grazing system, husbandry conditions, breeding system, different serological tests and epidemiological status..

In the current study, there was no positive reactor among male animals, although the difference in seroprevalence between the two sexes was not statistically significant. This finding is in agreement with the work done by Tesfaye Abebe (2003) in Tigray region, Taddess Yayeh (2003) in North Gondar Zone, and Tadale Tolosa (2004) in Jimma Zone who reported only female positive reactors.

Table.1 The overall seroprevalence of bovine brucellosis in the study area.

Serological tests	Total no. of animal tested	Total no. (%) of positive animals
RBPT	186	5(2.68)
ELISA	5	2(1.07)
Total	186	5(2.68)

Table.2 Frequency distribution of variables and percent

Variables	Category	Frequency	Percent
Breed	local	68	36.55%
	Cross	118	63.44%
Sex	Female	132	70.96%
	Male	54	29.03%
Age	(6month-1.8 years)	91	48.92%
	(above 1.8 years)	95	51.07%
Body condition	(poor)	44	23.65%
	(medium)	97	52.15%
	(good)	45	24.19%
Abortion	(no)	170	91.39%
	(yes)	16	8.6%
Parital	(no)	87	46.77%
	(one)	33	17.74%
	(2-4)	47	25.26%
	(above 4)	19	10.21%
Breeding system	(natural)	101	54.30%
	(artificial)	62	33.33%
	(both)	23	12.36%
Pregnancy status	(no)	112	60.21%
	(yes)	74	39.78%
Retained placenta	(no)	175	94.08%
	(yes)	11	5.91%
RBPT result	Negative	181	97.31%
	Positive	5	2.68%
I-ELISA test results	Negative	184	98.92%
	Positive	2	1.07%

Table.3 Sero-prevalence of bovine brucellosis among different risk factors.

Variables	No. of tested animals	No. of positive (%)	χ2	P-value
Breed		1.1651	0.280	
Local	68	0 (0.0)		
Cross	118	2 (1.69)		
Sex		0.8271	0.363	
Female	132	2 (1.51)		
Male	54	0 (0.0)		
Age		1.9366	0.164	
Young	91	2 (2.1)		
Adult	95	0 (0.0)		
Body condition		1.0366	0.596	
Poor	44	0 (0.0)		
medium	97	1 (1.03)		
good	45	1 (2.22)		
Abortion		21.4810	0.000	
no	170	0 (0.0)		
yes	16	2 (12.5)		
Parity		1.0591	0.787	
no parital	87	1 (1.14)		
one parital	33	0 (0.0)		
two-four parital	47	1 (2.12)		
>four	19	0 (0.0)		
Breeding system		4.0435	0.132	
natural	101	0 (0.0)		
AI	62	2 (3.22)		
Both	23	0 (0.0)		
Pregnancy status		3.0599	0.080	
no	112	0 (0.0)		
yes	74	2 (2.70)		
Retained placenta		32.1640	0.000	
no	175	0 (0.0)		
yes	11	2 (18.18)		
Overall	**186**	**2(1.07)**		

Table.4 Association of brucellosis with abortion and retained fetal membrane

ELISA	Test Result	History of abortion		History of Retained placenta		
Abortion	Not abortion	Total	Present	Not	Present	Total
Negative	14	170	184	9	175	184
Positive	2(12.5)	0(0.00)	2(1.07)	2(18.18)	0(0.00)	2(1.07)
Total	16	170	186	11	175	186
X2 =21.4810, p-value=0.000, X2 = 32.1640, p-value=0.000						

On the other hand, Yilkal Asfaw *et al.,* (1998) reported a 0.11%seroprevalence among male animals while Mussie Hailemelekot *et al.,* (2007) reported 2.11% seroprevalence in extensive management system. Although no controlled study has been conducted on the relative susceptibility of female and male cattle to brucellosis, based on reactor rates it is probable that bullsare more resistant than sexually mature heifers and cows, however, are less resistant than sexually immature heifers (Nicoletti, 1980). It is important to note that serological data may underestimate *Brucella abortus* infection in males as infected bulls tested might be generally non-reactors or only had low antibody levels (Crawford *et al.*, 1990).

In this study, higher seroprevalence of bovine brucellosis was observed in older age category (>2 years of age) (2.1%) than younger age category (6 months to 2years) (0%), although the difference was statistically insignificant. This observation is in agreement with that of Yilkal Asfaw *et al.*, (1998) where seroprevalence of bovine brucellosis in older cattle (4%) was higher than in younger ones (1.9%). Tariku Sintaro (1994) had also found higher proportion of old cattle being affected, but the difference among age groups was not statistically significant. Kassahun Asmare *et al.,* (2007) reported that the majority (97.87%) of sero-reactors were detected in the animals older than 2years in both the extensive and intensive management systems. Tadele Tolosa (2004) and Mussie Hailemelekot *et al.,* (2007) too reported significant variation among age groups in extensive production systems with higher seroprevalence rates in older animals. Similar result was also reported by Abraham Abebe *et al.,* (2008) in east Showa. It is evident that susceptibility of cattle to *Brucella abortus* infection is influenced by age of individual animals. Young and sexually immature animals tend to be more resistant to infection and frequently clear infection this may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of Brucella organisms, tend to increase in concentration with age and sexual maturity.

In this study, brucellosis seropositivity differs between management system which experienced abortion and retained fetal membrane are significant to this study. As abortion and retained foetal membrane are features of brucellosis, 2(12.5%) and 2 (18.18%) from (186) total herd this means from total cattle 2cows that show both sign brucellosis seropositivity in both test and other are give false negative. The reason could be inappropriate time of testing as serological tests of brucellosis could give false negative result immediately after parturition and few weeks before parturition. The other possible explanation could be that the observed abortion and retained foetal membrane were due to other factors such as malnutrition, deficiencies and other infections.

Conclusion and Recommendation

In conclusion, the result of this study showed that the seroprevalence of bovine brucellosis in Lemu Bilbilo district Arsi Zone is found to be low. However, it is highly likely that the disease spreads to the unaffected animals and herds given the intensive semi-intensive and extensive production system prevailing in the area which may allow contact of animals during grazing, breeding system and at watering points. Thus, there is a need to design and implement control measures aiming at preventing further spread of the disease in the Region through the use of better management practices. In addition, the public in general and high risk group in particular should be made aware of the zoonotic potential of brucellosis.

Conflict of Interests

The authors have not declared any conflicts of interests

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