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Seroprevalence and Risk Factors of Bovine Brucellosis in Arsi Zone, Oromia Regional State, Ethiopia

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Abstract

A total of 756 cattle sera from 74 herds of intensive (dairy) and extensive (mixed crop-livestock) production systems were collected and serially tested by Rose Bengal Plate Test (RBT) and by Complement Fixation Test (CFT) between November 2009 and March 2010 in Arsi Zone of Ethiopia with objective of determining seroprevalence and risk factors. The overall herd and animal level seroprevalences were 9.5% and 2.6%, respectively. Herd level seroprevalences were 40% (n=5) in intensive system and 7.3% (n=69) in extensive system. Animal level and within-herd range of brucellosis seroprevalence in cattle under intensive system were 4.4% (n=274) and 0.0 - 7.5%, while for those in extensive system were 1.7% (n=482) and 0.0 - 30%, respectively. Binary logistic regression showed both herd and animal level seroprevalence to be significantly higher ($p < 0.05$) in intensive than in extensive system. The statistical significant difference observed between production systems in this study implied different control strategies need to be addressed in Arsi zone. Thus, implementing a culling practice in the extensive system to eliminate the existing low risk of brucellosis and targeting calves in the intensive system for vaccination in addition to culling reactors could minimize the economic loss and reduce the potential occupational exposures in particular.

Keywords: Brucellosis; seroprevalence; risk factors; cattle; production systems; Arsi zone; Ethiopia.

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1. Introduction

Bovine brucellosis is usually caused by *Brucella abortus*, less frequently by *B. melitensis*, and by *B. suis* imposing economic lose and zoonoses. Infection in cattle is still widespread globally, despite several countries in Northern and central Europe, Canada, Japan, Australia and New Zealand are believed to be free from the agent [13].

As a result of compulsory pasteurization of milk products and strict control of the disease in dairy cattle, the incidence of brucellosis in human has steadily declined in most industrialized countries during the last 50 years [14]; unlike in Ethiopia where people still consume unpasteurized milk and milk products. Seroprevalence and rates of infection of brucellosis vary greatly from one country to another, within a country and production systems [10]. In Ethiopia, results of few seroprevalence studies on bovine brucellosis in various regions taking the extensive and semi-intensive cattle productions into consideration show not only some similarities but also varying figures [4, 5]. Yet, these studies on seroprevalence and distribution of brucellosis among different production systems of cattle are not sufficient and are underestimated-making priority setting and control programs difficult to implement in Ethiopia. An animal level seroprevalence of 7.2 % of bovine brucellosis in the extensive (mixed crop-livestock) cattle production system is recorded by author in [1] using RBT and SAT in Arsi zone. No any recent record is found in different production system at herd level and animal level in the present study area. Thus, the objective of this study was to estimate the present situation of herd- and animal-level seroprevalence of brucellosis in selected extensive (mixed crop-livestock farming) and intensive (dairy) cattle productions within Arsi Zone using RBT and CFT serological tests; and to identify potential risks and measure their strength of association with brucellosis.

2. Materials and Methods

2.1 Study area and study animals

This study was conducted in Asela town and Robe district of Arsi zone of Oromia regional state, Ethiopia (Fig 1). Asela, the capital of the zone, is located 175 km east of Addis Ababa, at an altitude of 2400 meter above sea level (masl) and at 07° 57’ 43.5’’N and 039° 07’ 49.0’’E. Robe district and its main town, is located 100 km north of the zonal capital and 225 km away from Addis Ababa, at an altitude 2420 masl and at 07° 52’ 05.7’’N and 039° 37’ 35.8’’E.

This study involved two cattle production systems: extensive (mixed-crop livestock production) and intensive (dairy) cattle production. The 2007 animal population data of the Zonal Livestock Development and Health Agency show that Arsi zone holds about 2,366,959 cattle and 35,500 (1.5%) of these are kept under urban and peri-urban semi-intensive or intensive livestock production system either for milk, dairy or beef production; and the rest 98.5% being kept in the rural areas for the purpose of mixed crop-livestock production. Intensive dairy farms exist in Asela town running small to medium sized herds with up to 100 milking cows, most of which are cross-bred Holstein-Friesian, few Jersey and local cattle introduced by the AI program and exotic breeds, since the establishment of CADU (Chilalo Agricultural Development Unit) of Arsi, Ethiopia, in the mid-1960s by the Swedish-funded integrated rural development in Africa.

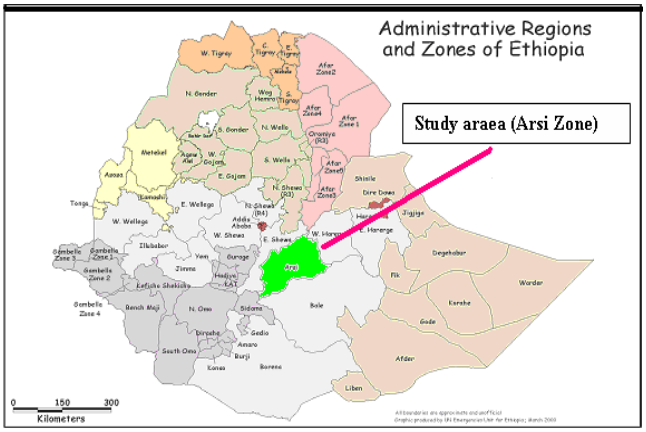

Figure 1 Map showing the study area (Arsi zone in Ethiopia)

2.2 Study design and sampling strategy

A cross-sectional study design was used to estimate the sero-prevalence of brucellosis in both production systems. Sera samples were collected and questionnaires were administered to each farm/herd owner between November 2009 and March 2010. Using a one stage cluster sampling technique [8], in which all cattle in each selected herd were sampled, was applied on both extensive (mixed crop-cattle) and intensive (dairy) production systems. Yet, due to biological effect cattle less than six months of age were not included. Accordingly, 69 households possessing the 69 herds (their 482 cattle) were randomly accessed in extensively managed cattle and 5 herds from the intensive (dairy) production system were sampled having 274 individual cattle with average number of 55 heads of cattle per herd.

2.3 Blood sample collection and serological test methods

None of the animals was vaccinated against *Brucella abortus*. About 5 - 10 ml of blood was collected in plain vaccutainer tube by the author and vet lab technician from vet laboratory. The sera were separated and stored at -20°C until analyses. Rose Bengal test (RBT) as screening test and Complement Fixation Test (CFT) as confirmatory test were used in detecting antibody against *Brucella* antigen. The tests were undertaken at the National Veterinary Institute, Debre Zeit, Ethiopia. The procedure and interpretation of results described by author in [13] were followed.

2.4 Questionnaire survey

Questionnaires designed to obtain information on the cluster/herd management practices together with individual animal related history for a survey of the potential risk factors at herd and individual animal level were administered to each farmer. Categories of the type of management system (intensive or extensive), herd size, previous abortion in the herd and occurrence of previous retained placenta in the herd were the risk factors considered at herd level. While categories of sex, age, breed, functional status, occurrence of previous abortion and retained placenta of the relevant animal were the risk and clinical factors considered at animal level. Age of animals was categorized into 6 month to 3 years and above 3 years, and herd size was categorized into ≤ 20 and

> 20 heads of cattle.

2.5 Data management and analysis

The questionnaires and serological data were transferred into MS spreadsheet. With regard to objectives of this study, the statistical analysis and calculation of specific prevalence data of brucellosis at a particular level were performed using SPSS software. Binary and multivariable logistic regression models were used to identify the potential risk factors associated with Brucella infection in animal and variables with a p-value lower than or equal to 0.05 were considered risk factor.

3. Results

3.1 Seroprevalence of bovine brucellosis using RBT and CFT

Herd level seroprevalence: Of the total 74 herds of both systems tested, 7 (9.5%) (95% CI: 2.8 - 16.2) were positive to antibody detection against *Brucella* anigen. Two herds (40%) (95% CI: 0.0 - 80.0) from intensive production system and five herds (7.3%) (95% CI: 1.1 - 13.3) from extensive production system out of the investigated cattle herds had at least one reactor animal to RBT and CFT serial test (Table 1).

Animal level seroprevalence of bovine brucellosis: Of 756 tested sera of both production systems, 20 (2.6%) (95% CI: 1.5 - 3.7) were positive for brucellosis (Table 1). Animal level seroprevalence of brucellosis of cattle in intensive production system (n = 274 heads) and in extensive production system (n = 482 heads) were 4.4 % (95% CI: 2.0 - 6.8) and 1.7 % (95% CI: 0.5 - 2.9), respectively. The within herd animal seroprevalence varied from 0% to 7.5% for animals kept under intensive production and 0% to 30% for animals kept under extensive production system.

Table 1: Summary of herd and animal level seroprevalence of bovine brucellosis in Arsi zone

Parameter	Intensive system		Extensive system		Both system	
	n	RBT>CFT positive (%)	n	Positive (%)	n	Positive (%)
Herd-level	5	2 (40.0)	69	5 (7.2)	74	7 (9.5)
Animal-level	274	12 (4.4)	482	8 (1.7)	756	20 (2.6)
Within-herd	5	(0.0 -7.5)	69	(0.00-30.00)	74	(0.00-30.00)

3.2 Analyses of risk and clinical factors

Herd level risk and clinical factors analyses: The herd level binary logistic regression analysis revealed four variables with p-value ≤ 0.05: cattle production system, herd size, herd with history of previous abortion in the second half of gestation and retained placentas were found to be strongly associated with herd seropositivity to *Brucella* (Table 2). The odds of brucellosis in intensively managed cattle herds was eight times (odds ratio <OR>

= 8.5) higher than the extensively managed cattle herds with statistical significance. The odds of brucellosis in larger herds was also eleven times (OR = 11.8) higher than those of the smaller herds and was also at significant level (p = 0.007). Herds with pervious abortion history showed more likely chance (OR = 11.8) of being seropositive to the disease than those herds without previous history of abortions with statistical (p = 0.007) significance level. The odds of brucellosis in herds with previous history of retained fetal membrane was also twenty-six times (OR= 26.4) higher than herds without previous history of retained fetal membranes with statistical significance (p = 0.012).

Table 2: Binary logistic regression analysis of RBT and CFT seropositive results of herd level risk factors

Factors/ variables	n	Positive herds (%)	OR	95% CI_{OR}	*p*-value
System					
Intensive	5	2 (40.0)	8.5	1.15 - 63.52	0.036
Extensive	69	5 (7.2)	1.00	-	-
Herd size					
> 20	7	3 (42.9)	11.8	1.9 – 71.9	0.007
1 – 20	67	4 (6.0)	1.00	-	-
Previous abortion in the herd/cluster *					
Yes	7	3 (42.9)	11.8	1.94 - 71.90	0.007
No	67	4 (6.0)	1.00	-	-
Previous retained placenta in the herd					
Yes	3	2 (66.7)	26.4	2.03 - 343.85	0.012
No	71	5 (7.0)	1.00	-	-

<OR> odds ratio - Reference category *abortion in the second half of gestation

Animal level risk and clinical factors analyses: The overall animal level binary logistic regression analysis revealed five variables with p-value ≤ 0.05: the type of production system cattle kept in, between cattle breeds, among functional status of the cattle, between cows with previous history of abortion and without previous history of abortion, and between cow with and without previous history of retained fetal membrane (Table 3). The odds of brucellosis in intensively managed cattle was at least two times (OR=2.71) higher than the extensively managed cattle and it was statistically at significant level (p=0.031).

The odds of brucellosis in cross-breed cattle was at least two times (OR = 2.6) more than those of local breeds and was found to be statistically (p =0.04) significant. Seroprevalence of brucellosis in cattle aged over 3 years were three times (OR = 2.65) higher than cattle aged under three years, though it was not statistically (p > 0.05) significant. The study also revealed the odds of brucellosis in dry open cows to be at least eight times (OR = 8.44) more than the reference bulls in the group with functional status such as pregnant cows, lactating cows, heifers and bulls as of the overall production systems and was found to be statistically (p < 0.05) significant. The odds of brucellosis in cows with previous history of abortion was almost six times (OR = 5.83) more than those of cows without previous history of abortion and was also found to be statistically (p = 0.014) significant. The odds of brucellosis in cows with previous history of retained fetal membranes was nine times (OR =9.26) more

than cows without previous history of retained fetal membranes and was also found to be statistically (p = 0.003) significant without considering the type of management in which the cows had been kept.

Table 3: Binary logistic regression analysis of RBT and CFT seropositive results of animal level risk factors in Arsi

Factors/ variables	n	Positive animals (%)	OR	95% CI_{OR}	*p*-value
System					
Intensive	274	12 (4.4)	2.71	1.09 - 6.72	0.031
Extensive	482	8 (1.7)	1.00	-	-
Sex					
Female	509	16 (3.1)	1.97	0.65 - 5.96	0.229
Male	247	4 (1.6)	1.00	-	-
Age					
> 3 years	459	16 (3.5)	2.65	0.88 - 7.99	0.085
6 month to 3 years	297	4 (1.3)	1.00	-	-
Breed					
Cross	316	13 (4.1)	2.65	1.05 - 6.73	0.040
Local	440	7 (1.6)	1.00	-	-
Functional status					
Pregnant cows	58	3 (5.2)	2.88	0.63 - 13.24	0.175
Lactating cows	205	7 (3.4)	1.87	0.52 - 6.47	0.326
Dry open cows	29	4 (13.8)	8.44	1.99 - 35.86	0.004
Heifers	170	2 (1.2)	0.63	0.11 - 3.47	0.594
Bulls	215	4 (1.9)	1.00	-	-
Previous abortion (n = 271)					
Yes	17	3 (17.6)	5.83	1.42 - 23.97	0.014
No	254	9 (3.5)	1.00	-	-
Previous retained placenta (n = 271)					
Yes	12	3 (25.0)	9.26	2.14 - 40.12	0.003
No	259	9 (3.5)	1.00	-	-

The multivariate logistic regression model revealed that production system (odds ratio <OR> = 1.92; 95%CI: 0.48 -102.03), herd size (OR = 5.11; 95%CI: 1.44 - 18.13, P = 0.01), sex (OR = 1.36; 95%CI: 0.39 -4.78) and age of animals (OR=1.04; 95%CI: 0.92 -8.73) were the risk factors for cattle seropositivity to *Brucella* antigens (Table 4).

Table 4: Multivariable logistic regression analysis of the variables associated with herd level and animals level sero-positivity

Variable*	β	SE	p-value	OR	95% CI for OR	
					Lower	Upper
Constant	-3.81	3.25	0.24	-	-	-
Production System	1.92	1.38	0.16	6.83	0.48	102.03
Herd size	1.63	0.65	0.01	5.11	1.44	18.13
Sex	0.31	0.64	0.63	1.36	0.39	4.78
Age	1.04	0.57	0.06	2.84	0.92	8.73
Breed	-0.29	1.01	0.77	0.75	0.10	5.44

*β: standard coefficient (that is affected by the positive "risk" or negative "protective" sense), SE: standard error,

4. Discussion

The overall herd level seroprevalence found in the current study was lower than the one reported 13.7% in Southern Ethiopia by authors in [6]. Comparison of herd level seroprevalence in the two production systems indicated the occurrence of the disease among herds to be much higher in herds managed under intensive (dairy) system than under extensive (mixed crop-livestock (MCL) system with statistically significant level.

The size of the herd, type of production, the housing methods and the population density are factors that may be considered in the progress of the disease [7]. All of the studied intensive herds were dairy farms which kept larger proportion of dairy female cattle (85.8%) than male (14.8%) cattle which was different proportion with that of extensive- (56.8%) female cattle; also maintaining smaller herd size. Dairy animals have a much greater chance not only of contracting brucellosis but also of spreading it faster than beef animals. The reason is far from being a genetic or physiological factor, but instead is due to husbandry. Animals that live concentrated in smaller areas come into close contact when they are grazing and when they are milked [7].

The animal level seroprevalence was comparable to 1.66% in an extensive cattle production system previously reported in Southern Ethiopia by authors in [6]. However, the 1.7% animal level seroprevalence for extensive system found in this study was found to be lower than studies carried out in other parts of the country in extensively managed cattle 3.86% by authors in [11] and 3.82% by author in [5], much lower than studies conducted to be 11% by authors in [16] and 7.2% in Arsi (same study area) by author in [1]. The difference between the present study and the previous study by author in [1] could be attributed to the enforcement of farmers under close settlement program-‘’Sefera’’ prior to the 1989 during previous regime which could have contributed to the more prevalence of disease by facilitating spread of disease among extensively managed cattle herds or animals grazed in communal land.

However, at present keeping animals under intensive production system was found to be a higher risk factor compared to impact of brucellosis in animals kept under extensive system. Authors in [15] also reported higher seroprevalence in animals kept under intensive management systems (4.8%) than those in semi-intensive (2.8%)

and extensive (2.4%) management systems. It could be due to larger number of animals per herd were being kept under intensive than in the extensive system observed in this study. Because, larger herd size was also identified as a risk factor in this study with statistical significance value. Our finding is also in accordance with the result of authors in [18] to which they found significant association between *Brucella* infection and large herd size. Contrary to this observation, however, authors in [16] reported risk of seropositivity is independent of herd size in smallholder farms from Wuchale –Jida district, in Ethiopia. Authors in [2] noted large herd size enhances the exposure potential, especially following abortions through increased contact at common feeding and watering points promoting transmission of *Brucella* organisms.

The study also showed herd level brucellosis seroprevalence significantly differed among herds which experienced abortion and retained foetal membrane with those which did not when observed in both systems. The proportion of herds that experienced abortion and retained fetal membrane in each system shown in table 3 has also corresponded with prevalence of brucellosis. Conversely, animal level seroprevalence in cows with previous history of abortions and retained placenta was significantly associated in overall systems. Herd seropositivity has also been reported to have a significant association with history of abortion and retained fetal membrane [4, 11]. As abortion and retained fetal membrane are clinical features of brucellosis, it is not uncommon to find such results [3]. Thus, maintaining aborting cows in a herd could be a risk factor serving as source of infection to other animals within the herd.

Even though, univariable logistic analysis showed no statistical significant difference in seroprevalence between sexes and between age groups; the odds of acquiring brucellosis in females than males and in cattle aged above three years than below were higher showing some biological significance irrespective of the production system. The increase of bovine brucellosis seropositivity with age and sex was also reported by authors in [4, 16, 17] in different parts of Ethiopia. According to authors in [12], sexually matured and pregnant cattle are more susceptible to infection with Brucella organisms than sexually immature animals of either sex. On the other hand, younger animals tend to be more resistant to infection and frequently clear infections; although latent infections could occur and such animals may present a hazard when mature [9]. 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 [12].

The largest seroprevalence among functional groups was observed among dry open cows in this study. Similar results were reported with significant effects by authors in [11] and without significant effects by author in [5] in extensive system in Ethiopia. The high risk of brucellosis observed among dry cows could be attributed to absence of regular testing and culling or lack of other brucellosis control measures in both production systems leading to development of chronic infection and infertility in individual cattle. Such cows are often associated with multiple abortions, hence acting as a source of infectious organisms to maintain transmission and constant presence of new infection.

In general, in this study, the multivariable logistic regression model revealed herd size to be the major risk factor with a significant level to the occurrence of brucellosis in a herd with positive influence of production system, sex and age of cattle on the occurrence of the disease though not statistically at significant level. And breed of

the cattle had no influence to brucellosis. Breed differences in susceptibility have not been clearly documented in cattle although genetically determined differences in susceptibility of individual animals have been demonstrated [9].

The limitations of this study are only small number of intensive cattle dairy farms in geographically confined area was included in the study. Due to financial constraints, wide area was not covered. Thus, all herds and all animals in each herd were included without random sampling among the intensively kept herds/animals, while of the extensive herds the reveres holds true. Apart from this, only serological studies were conducted. It was not supported with gold standard test: bacterial isolation and identification form the animal, milk and environment- again due to limitation of supply of consumables and level of laboratory; which would have been of greater importance in proposing better control strategy.

5. Conclusions and recommendations

The statistically significant difference between intensive and extensive cattle production systems shown in this serological *Brucella* study implied each requires some independent control strategies. Thus, implementing a culling practice in the extensive system to eliminate the existing low risk of brucellosis and targeting calves in the intensive systems for vaccination in addition to culling reactors could minimize the economic loss and reduce the potential occupational exposure in particular and the public health in general through production and distribution of safe or *Brucella* free animal products. For this, the country has to introduce or produce *Brucella* vaccine at least for the intensive dairy farms that supply dairy products to the vast majority of urban dwellers. In addition to serology, intensive bacteriological study for isolation and identification of the agent from the animal, animal products and its environment should be given priority to set determinant factors for the spread of the disease among herd and to propose possible all-round control strategy.

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