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## Research Article

# Case-control Study on Risk Factors Associated with Brucellosis in Aborted Breeding Goats in Jimma Zone, Ethiopia

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## Abstract

**Background and Objective:** Brucellosis is a bacterial disease of major socioeconomic and public health importance and it is one of the major causes of abortion in goats worldwide. The objective of the study is to evaluate the risk factors associated with brucellosis in aborted breeding goats. **Materials and Methods:** A case-control study was conducted to investigate brucellosis as a cause of abortion and associated risk factors in breeding goats of the Jimma zone, Ethiopia. During the study period, 134 cases and 268 controls were selected to evaluate and compare the presence of *Brucella* antibody between cases and controls. The existence of *Brucella* antibodies in serum samples first tested by the Rose Bengal Plate test, then all positive samples confirmed using a complement fixation test. **Results:** An overall of 6.47% seroprevalence of brucellosis was recorded in the study areas. Antibody against *Brucella* organism was higher in cases (10.45%) than controls (4.48%) with statistically significant variation ( $p < 0.05$ ). This study showed that goats from a mixed species (goat with cattle and/ sheep) were more likely to be *Brucella* seropositivity than those with no contact with cattle and/sheep ( $p < 0.05$ , OR = 2.3). Similarly, breeding goat from large flock size ( $p < 0.05$ , OR = 2.8) and lowland ( $p < 0.05$ , OR = 2.7) were also found to be at higher risk of harboring *Brucella* infection. **Conclusion:** Hence, it is important to conduct applicable control methods, creating public awareness on the transmission of brucellosis and further study to identify the specific cause of abortion in goats was suggested.

**Key words:** Brucellosis, risk factors, case-control, breeding goat, seroprevalence

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Brucellosis is a bacterial disease of major socioeconomic and public health importance worldwide<sup>1</sup>. Currently, brucellosis has been considered as the commonest re-emerging zoonotic disease global and cause considerable human morbidity in endemic areas<sup>2-4</sup>. This disease is especially important in developing countries where control programs are either not existent or inadequate. The occurrence of brucellosis has long been reported from several African countries<sup>5,6</sup>. In Africa, brucellosis in animals was first recorded in Zimbabwe (1906), Kenya (1914) and South Africa in 1915 and then spread to other East African countries including Ethiopia<sup>7</sup>.

Abortion implies the expulsion of a fetus before full terms and viability outside of the uterus<sup>8,9</sup>. It remains a significant issue to the goat industry worldwide and the largest contributor to economic losses in goat production<sup>10,11</sup>. Goat abortion is caused by infectious and non-infectious agents. Infectious agents are the most cause of abortion in goats compared to non-infectious causes<sup>12,13</sup>. The most important infectious causes of abortion in goats are bacterial diseases such as chlamydiosis, listeriosis, leptospirosis, coxiellosis, and brucellosis. Moreover, toxoplasmosis and neosporosis from protozoa diseases and Border virus disease are common pathogens associated with pregnancy loss<sup>10,14-16</sup>.

Among the infectious causes of abortion, brucellosis is one of the major bacteria agents causing tremendous economic losses due to abortion and other reproduction problems in goat flock<sup>17</sup>. The disease is characterized by causing abortion, stillbirth, infertility and weak offspring in goats<sup>18</sup>. *Brucella melitensis* is the primary cause of brucellosis in goats and the pathogen is zoonotic<sup>18,19</sup>. In goats, brucellosis causes severe economic losses as a result of abortion storm or reproductive disorder, infertility and reduced milk production. Moreover, brucellosis in goats reduced the foreign exchange earnings by denying exportation of goats to international markets<sup>20,21</sup>.

In Ethiopia, brucellosis is endemic and it was first reported<sup>22</sup> in cattle in 1970 and remains a major neglected zoonotic disease by World Health Organization (WHO) and World Organization for Animal Health (OIE) in the country<sup>23,24</sup>. Since then, several studies have demonstrated varying brucellosis prevalence levels in key different livestock species parts of the country. Generally, the prevalence of brucellosis has been found to range from 1.6-16% in goats<sup>25,26</sup>. The previous study conducted elsewhere identified several risk factors for *Brucella* infectious which related to the host,

environment, and management. The report of these studies was the lack of consistency cross-study sites and species of animal studied<sup>18</sup>.

Besides, those studies did not give sufficient epidemiological information about the disease in the country. In particular, information on the status of brucellosis in breeding goats and it associated with abortion has been not well investigated in the Jimma zone, one of the areas where there are large numbers of goats in Ethiopia. A limited number of the studies were conducted on brucellosis with the case-control approach. Moreover, almost all of the surveys were limited to the study of goat brucellosis based on the cross-sectional study. A case-control study is paramount for *Brucella* organism assessment as a cause of abortion and to identify associated risk factors of brucellosis in aborted breeding goats<sup>27</sup>. Therefore, this study aimed to determine the associated risk factor of brucellosis and it related to abortion in breeding goats in Jimma zone, Ethiopia.

## MATERIALS AND METHODS

**Study area:** The study was carried out in Limu Seka and Chora Boter district of Jimma zone. Limu Seka district was located about 463 km from Addis Ababa, the capital of Ethiopia and 109 km from Jimma town, the capital of Jimma zone from October 2017-October 2018. The district covers an area of approximately 1,694 km<sup>2</sup> and divided into 38 kebeles (the smallest administrative units). The agro-ecology of the district is characterized by highland (13%), mid-high land (55%) and low land (32%). The altitude of the district is between 1,400-2,200 meters above sea level. Chora Boter district is located 466 km away from Addis Ababa, and 112 km from Jimma town, zonal capital. This district has 19 kebeles and agro-ecologically it is characterized by highland, mid-highland and lowland. The altitude of the district is between 1,100-2,200 meters above sea level and has an average temperature of 22°C. Chora Boter has 228,846 cattle, 47,854 sheep and 68,037 goats. Both districts have two distinct seasons. The rainy season is starting in late March and ending in October and the dry season is occurring from November to early March. The map of the study area<sup>28</sup> is shown in Fig. 1.

**Study design:** The target population of this study comprises breeding goats in study districts and study populations are female goats at risk of abortion in selected kebeles of Limu Seka and Chora Boter districts. The case-control study design was carried out on goats by grouping as case and control in the districts. The case is defined as those goats which had at

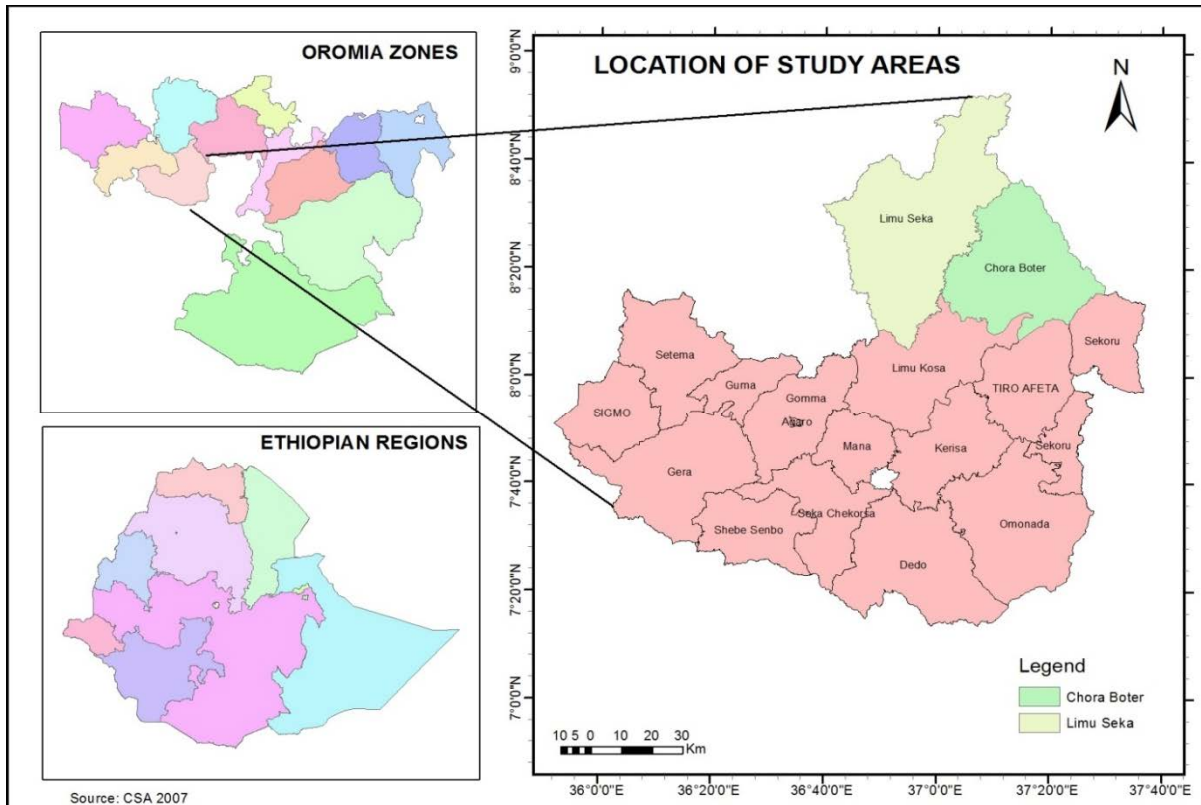


Fig. 1: Map of the study areas

least one abortion history within the previous one year while controls were those goats that had no history of abortion. The list of cases recorded in the last one year in the district veterinary clinic case book and owner's information was used as a sampling frame. Before the selection of flocks with the history of abortion, the availability of cases goats was checked. In each flock was one to three cases were available those cases were selected and control groups were selected randomly by lottery method from that flock. In flocks where large numbers of cases and controls were found, individual animals were picked randomly using a lottery method after registration of goats and given a name in their respective category.

**Sampling procedures and sample size determination:** Two study districts were selected purposively based on the reports of abortion cases. A total of 10 peasant associations were included from these selected two districts using a random sampling technique where six peasant associations were from Limu Seka and four of them from Chora Boter districts. Sample size was calculated using (Ausvet) Epi tool epidemiological sample size calculation for case-control study with CI = 95%, power = 80, ratio of cases to controls = 2.0, OR = 2.5, expected

proportion exposed among controls = 0.5. To correct for a difference in design, the calculated sample size was multiplied by a design effect (D) which was calculated using this formula:

$$D = \rho(n-1) + 1$$

where, n is average number of goats in a cluster (9), the intracluster correlation coefficient of  $\rho = 0.09$  was reported<sup>29</sup> for *Brucella* and the design effect was 1.72. The sample size per group was  $78 \times 1.72 = 134$ . Thus, a total of 402 (cases n = 134, controls n = 268) goats were involved in this study.

**Blood sample collection procedure:** Blood samples were collected from the jugular vein, (3-5 mL) aseptically using sterile plain vacutainer tubes and needle, were kept in a slanting position overnight at room temperature to separate the serum. Parallel to blood sample collection relevant information such as agro-ecology, management system, flock size, species composition, introducing new animals, management of aborted material, age (years), parity, body condition and reproduction status were collected using the separate format. Then sera were gently decanted into sterile

screw capped tubes, labeled and transported in ice packs to Jimma University, Immunology Laboratory and stored at  $-20^{\circ}\text{C}$  until screened and tested for antibodies against natural *Brucella* exposure analysis using Rose Bengal Plate Test (RBPT). This test was performed following the procedure described by Blasco *et al.*<sup>30</sup> mixing 75  $\mu\text{L}$  of sera and 25  $\mu\text{L}$  of the antigen. The antigen and test serum were mixed thoroughly by a plastic applicator, shaken for 4 minutes and the occurrence of agglutination was recorded immediately. The test was repeated when test results were ambiguous. Positive sera were further subjected to the Complement Fixation Test (CFT). The CFT has a specificity of up to 100% and sensitivity<sup>31</sup> of up to 96% and is a prescribed diagnostic method by the OIE<sup>32</sup>. As there has never been a history of vaccination for brucellosis, all positive results were attributed to natural infection.

**Data management and analysis:** Data obtained from this study were recorded, coded and stored in Microsoft Excel for Windows 2010 and transferred to SPSS version 20. Associations between brucellosis and risk factors for all the units of analysis were investigated by multiple logistic regression models described in terms of adjusted odds ratio (OR). The variables with p-value less than or equal to 0.25 in univariable logistic regression, after checking for multicollinearity were taken forward for multivariable modeling. The model fit was observed using the Hosmer-Lemeshow test. Subsequently, the predictive ability of the model was validated using the receiver operating characteristic (ROC) curve. For all statistical analysis, a 95% confidence intervals (95% CI) and a critical value of 0.05 was used.

## RESULTS

From a total of 402 breeding goats examined, 7.21% of them were tested positive on screening using RBPT test. Further, confirmation using CFT identified 6.47% of breeding goats were seropositive to *Brucella* antibodies. Higher seropositivity to *Brucella* antibodies was recorded in cases (10.45%) than controls (4.48%) groups. This difference was statistically significant ( $p < 0.05$ ) (Table 1).

Higher seroprevalence of *Brucella* antibody (11.71%) was recorded in mid-altitude than lowland (4.76%). There was a statistically significant variation ( $p < 0.05$ ) between seroprevalence of *Brucella* antibody and agro-ecology. Goats originated from lowland was almost three (OR = 2.8) times more likely to be infected with *Brucella* organism than goat

originated from mid-altitude under univariate logistic regression analysis. However, localities, age group, parity, body condition, flock size, species composition, management system, the introduction of a new animal, reproductive status and management of aborted materials were not able to explain seroprevalence of brucellosis in breeding goats ( $p > 0.05$ ) (Table 2).

In multivariable logistic regression analysis, explanatory variables with a p-value of less than or equal to 0.25 in univariate logistic regression analysis were included. This model showed that breeding goats from a mixed (goat with cattle and/sheep) species were more likely to be *Brucella* antibody seropositivity than those no contact with cattle and/sheep (OR = 2.3). Similarly, a breeding goat from large flock size was also found to be at higher risk of *Brucella* infection, than those from small flock size (OR = 2.8). This result also showed that agro-ecology was a statistically significant difference in *Brucella* antibody seropositivity with a breeding goat from lowland was 2.7 times more (OR = 2.7) likely to harboring *Brucella* infection than those from mid-altitude (Table 3). No significant interactions ( $p > 0.05$ ) and multicollinearity between variables were detected. Hosmer-Lemeshow test ( $X^2 = 3.21$ ;  $p = 0.865$ ) indicated that the model was fit data well. ROC curve (0.765) showed that the model was good predicting ability in Fig. 2.

Table 1: *Brucella* antibody determined from cases and controls breeding goats in study areas

Brucellosis status	Cases	Controls	Total (%)	p-value
Positive	14	12	26 (53.85)	
Negative	120	256	376 (31.91)	0.026
Total (%)	134 (10.45)	268 (4.48)	402 (6.47)	

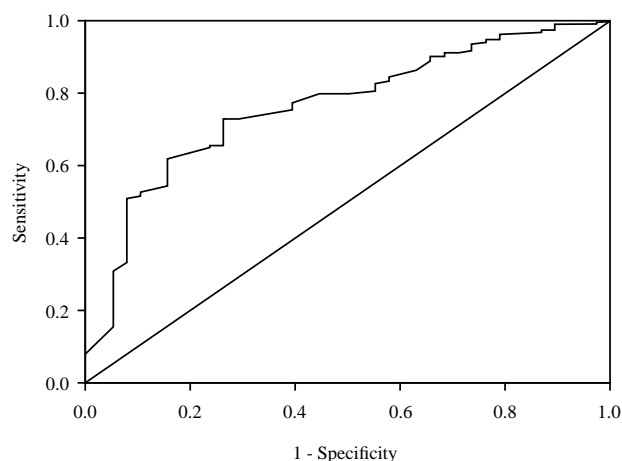


Fig. 2: Diagonal segments are produced by ties. Graph of ROC curve

Table 2: Univariate logistic regression analysis of potential risk factors of breeding goats in study areas

Variables	Category	Cases	Controls	Total animals positive (%)	OR (CI, 95%)	p-value
Localities	Limu Seka	92	184	20 (7.25)		
	Chora Boter	42	84	6 (4.76)	1.6 (0.61-3.99)	0.351
Agro-ecology	Mid-altitude	37	74	13 (11.71)		
	Lowland	97	194	13 (4.47)	2.8 (1.27-6.33)	0.011
Age	1-2 years	17	100	7 (5.98)		0.373
	2-3 years	46	48	9 (9.57)	0.6 (0.22-1.68)	0.331
	>3 years	71	120	10 (5.24)	1.2 (0.43-3.11)	0.781
Parity	Null	6	89	7 (7.37)		0.597
	1-2	36	48	7 (8.33)	0.9 (0.29-2.61)	0.810
	>3	92	131	12 (5.38)	1.4 (0.53-3.67)	0.495
Body condition	Good	31	54	3 (3.53)	1.7 (0.41-6.90)	0.365
	Medium	74	139	17 (7.98)	0.7 (0.27-1.85)	0.476
	Poor	29	75	6 (5.77)		0.478
Flock size						0.068
	3-7	44	94	7 (9.93)		
	8-12	45	87	5 (3.79)	1.4 (0.42-4.39)	0.610
	13-18	45	87	14 (10.61)	0.5 (0.18-1.15)	0.097
Species composition	Only goat	83	157	20 (8.33)		
	Mixed	51	111	6 (3.70)	2.4 (0.93-6.02)	0.071
Management system	Sem-intensive	17	34	5 (9.80)		
	Extensive	117	234	21 (5.98)	1.7 (0.61-4.75)	0.305
Introduction of new animals	No	78	147	18 (8.00)		
	Yes	56	121	8 (4.52)	1.8 (0.78-4.33)	0.164
Reproduction status	Non-pregnancy	79	120	15 (7.54)		
	Pregnancy	55	148	11 (5.42)	1.4 (0.64-3.18)	0.390
Management of aborted materials	No	84	170	18 (7.09)		
	Yes	50	98	8 (5.41)	1.3 (0.57-3.15)	0.510

OR: Odds ratio, CI: Confidence interval, BCS: Body condition score

Table 3: Final multivariable logistic regression model for *Brucella* antibody seropositivity in breeding goats in study areas

Variables	Category	Cases	Controls	Total animals positive (%)	OR(CI, 95%)	P-value
Agro-ecology	Mid-altitude	37	74	13 (11.71)		
	Lowland	97	194	13 (4.47)	2.7 (1.19-6.28)	0.018
Flock size						0.031
	Medium (8-12)	45	87	5 (3.79)	2.6 (1.00-6.86)	0.021
	Large (13-18)	45	87	14 (10.61)	2.8 (1.95-8.00)	0.031
Species composition	Only goat	83	157	20 (8.33)		
	Mixed	51	111	6 (3.70)	2.3 (1.87-6.28)	0.025

OR: Odds ratio, CI: Confidence interval

## DISCUSSION

This study showed that overall of 6.47% seroprevalence of *Brucella* antibody was recorded in goats in the study areas. This finding is comparable with the result of Gumi *et al.*<sup>33</sup>, Ashenafi *et al.*<sup>7</sup> and Ashagrie *et al.*<sup>34</sup>, who reported *Brucella* seroprevalence of 7.8, 5.8 and 4.2%, respectively in a different part of the country. However, the present result is higher than the finding of Bekele *et al.*<sup>26</sup> and Dabassa *et al.*<sup>35</sup>, who reported a seroprevalence of 1.9% in South Omo and 1.2% in Borana zone, respectively in Ethiopia. This result is lower than the values 13.2% reported in Afar and Somali regions<sup>25</sup> and 9.39% in Dire Dawa<sup>36</sup>. These differences could be mainly due to the

current study was conducted on animals that had a history of abortion and this maybe shows the strong associated of abortion and brucellosis<sup>18,32</sup>. It also may be due to variation in agro-ecology, management and production system in different study areas.

A case-control study was conducted to identify brucellosis as a cause of abortion in aborted breeding goats in study areas. A statistically significant variation ( $p < 0.05$ ) was observed between cases and controls. This indicated that brucellosis may associate with abortion in breeding goat in the study areas. It is important to identify the actual causes in aborting goat and aborted fetuses or placental tissue to confirm the pathogen is responsible for abortion<sup>27,37</sup>. This

result is in line with the finding of Asmare *et al.*<sup>38</sup> and Tassew and Kassahun<sup>39</sup>, who reported the risk of *Brucella* seropositivity is associated with abortion in goat. However, the present result is different from the finding of Gebremedhi<sup>40</sup> and Wubishet *et al.*<sup>41</sup>, who state that *Brucella* seropositivity is not associated with abortion in goat. This difference may be due to variation agro-ecology, management and husbandry conditions in various areas. This can also difference between study areas regarding conditions that favor the transmission of several causes of abortion in goats<sup>18</sup>. The epidemiologic approach based on case-control comparison could be elucidated more about the contribution of brucellosis to abortion in goats<sup>42</sup>. Limited study about the estimation of calculated odds ratio which is a strong indication of an association between brucellosis and abortion in goats in Africa including Ethiopia<sup>9,27</sup>.

Species mix is associated with *Brucella* seropositivity where goat kept together with cattle and/sheep is two times (OR = 2.3,  $p < 0.05$ ) more likely to be positive for *Brucella* antibody than a single species flock. Cross-species infection with other *Brucella* species, especially *B. abortus* has been documented in goat as a cause of *Brucella* infection<sup>43</sup>. Multiple livestock species herding together, especially keeping goat along cattle has been reported as an important determinant risk factor of *Brucella* seropositivity<sup>44</sup>. Keeping goats in contact with *Brucella* infected sheep, also a potential risk factor for brucellosis spread among goat flock<sup>18</sup>. However, this result is different from the report of Coelho *et al.*<sup>45</sup>, who stated herding of goats with cattle and/ sheep is not a risk factor for brucellosis. This variation may be due to differences in agro-ecology, management system and breed of goat in study areas.

Flock size also associated with *Brucella* seropositivity in goat with large flock size was almost three times (OR = 2.8;  $p < 0.05$ ) more odds of brucellosis than small flock size. Flock size has previously been reported as an important determinant for transmission of *Brucella* organism between susceptible and infected animals<sup>46</sup> and because one positive animal at least available in large flock size compared to small size flock<sup>47</sup>. This result also related to a higher density of animals per flock. Keeping a large flock allows greater contact among animals. This makes a higher bacteria load in the environment and hence the probability of brucellosis transmission will be increased. Moreover, grazing in the communal pasture may facilitate the contact between infected and non-infected flock<sup>45,48</sup>. The association of flock size with the *Brucella* seropositivity in the present finding is confirmed with previous results<sup>45,47-49</sup>.

The present study also indicated that agro-ecology was significantly affected the occurrence of *Brucella* infection in

goat flock ( $p < 0.05$ ). The goat originated from lowland was almost three times (OR = 2.7) more probability of brucellosis than those from mid-altitude. The influence of the agro-ecological has been noted as a brucellosis risk issue, having a higher prevalence in dry areas<sup>50</sup>. Since pasture areas are scarce in a dry area, animals should obtain pasture over large areas implying an unrestricted animal to animal contact with the potential transmission<sup>51</sup>.

## CONCLUSION

The current study showed that higher *Brucella* seropositive status in cases than controls. This indicated that brucellosis was associated with abortion in a breeding goat in study areas. The seroprevalence of brucellosis in the goat was moderately high recorded in the areas. Breeding goats from large flock size and lowland agro-ecology and goats kept mixed with cattle and/sheep species are at increased risk of acquiring of *Brucella* organism. Therefore, it is important to carry out applicable control methods, increasing public awareness on the zoonotic transmission of brucellosis. Also, further study should be conducted to identify specific causes of abortion in goats.

## SIGNIFICANCE STATEMENT

Currently, brucellosis is a serious problem in both animals and humans. It is a widespread disease in the country, resulting in huge economic losses in goats' production due to abortion. However, there is limited information on the epidemiology of brucellosis and its association with abortion in goats. This study is therefore required to provide evidence on epidemiology of brucellosis and its association with goat abortion in the country. This study can notify interventions aimed at reducing the effects of the brucellosis both in goats and human in Ethiopia.

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