RESEARCH ARTICLE

Case-control study on risk factors associated with brucellosis in aborted cattle of Jimma zone, Ethiopia

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ABSTRACT

Brucellosis is one of the most important causes of abortion in cattle resulting in significant economic losses and public health concerns in the developing countries. A case-control study was conducted from October 2016 to October 2017 to investigate risk factors of brucellosis in aborted cattle in Jimma zone. During the study period, 141 cases and 282 controls were selected to assess and compare the presence of anti-Brucella antibodies between cases and controls. Cattle that had experienced abortion were defined as cases, whereas controls were cattle that had no record of abortion. Sera samples were collected from both cases and control cattle groups for laboratory tests (serological test). The existence of the anti-Brucella antibodes in serum samples was first tested by the Rose Bengal Plate test, and the all positive samples were confirmed using the complement fixation test. An overall of 4.02% seroprevalence of brucellosis was recorded in the study areas. Antibody against *Brucella* organism was higher among cases (6.38%) than controls (2.84%). Multivariable logistic regression analysis identified age (OR 14.16, CI= 2.91-28.84), breed (OR 5.36, CI= 1.76-11.33), herd size (OR 11.82, CI= 1.31-16.17) and species composition (OR 5.10, CI=1.49-13.43) as risk factors ($p < 0.05$) for *Brucella* seropositivity. This study documented the occurrence of cattle brucellosis in study areas. Thus, applicable control methods and creating public awareness on the zoonotic transmission of brucellosis should be conducted. Moreover, further study considering more causes should be carried out to identify the specific causes of abortion in cattle for the preparation of the appropriate vaccine.

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Introduction

Abortion in cattle is defined as loss of pregnancy within the day 42 to day 260 of gestation [1]. Termination of pregnancies before day 42 is typically referred to as early embryonic death, whereas a calf that is born dead between day 260 and the full term is mentioned as stillbirth [2]. Abortion results from infectious agents (bacteria, viruses, protozoa, and fungi) and non-infection causes such as heat stress, nutritional deficiencies, trauma, toxic substances, etc [3,4]. The infectious agents result in extensive economic losses, showing the requisite for control measures to prevent infection cause of abortion [5].

Among infectious causes, brucellosis is one of the most important causes of abortion in cattle and challenges to dairy industry [6]. It results in huge economic losses and public health concerns worldwide [7, 8]. Brucellosis in cattle is primarily caused by *Brucella abortus*, and *B. melitensis* and *B. suis* are seldom cause the disease in cattle. This disease is characterized in cattle by causing abortion, retained fetal membranes and infertility [9]. Contact with aborted cow, aborted fetus, or the contaminated fomites are the major routes of *Brucella* transmission [10]. Risk factors such as increased herd sizes, increased age, sex of cattle and husbandry practices are identified as precipitating factors for the occurrence of diseases in cattle [11, 12, 13, 14].

Brucellosis has higher incidence in a mixed livestock production system. Where people live with their livestock, they are also at higher risk of acquiring the disease [15]. The evidence for *Brucella* infections in cattle has been serologically evaluated by different authors [15, 16, 17, 18, 19]. According to some reports, *Brucella* seroprevalence is higher in the intensive farming systems than in extensive cattle rearing systems.

Recent reports by different reports [6, 20, 21, 22, 23] indicate that brucellosis is still a widespread disease, resulting in huge economic losses due to abortions. A limited number of studies have been conducted on brucellosis in a case-control approach. Moreover, almost all of the surveys were limited to the study of bovine brucellosis based on the cross-sectional study. A case-control study is paramount for *Brucella* organism assessment as a cause of abortion in cattle [24]. Hence, this study was carried out with the aim of investigating the risk factors of brucellosis in aborted cattle of Jimma zone, Ethiopia.

Results

From a total of 423 (141 cases and 282 controls) tested cattle 4.02% were positive for the anti-Brucel-

la antibody by using CFT. A higher seroprevalence of brucellosis was observed in cases (6.38%) than controls (2.84%). Statistically significant ($p < 0.05$) differences in serostatus of the anti-Brucella antibody was observed among age categories. Relatively older animals were found to be more likely to be seropositive than their younger counterparts. Besides, variation in the seroprevalence of *Brucella* organism between the two breeds was statistically significant ($p < 0.05$). The local breed of cattle being almost four times (OR=4.04) more likely to harbor the anti-Brucella antibody compared to crossbred animals. Similarly, the variation in the serostatus of *Brucella* organism between pregnant and non-pregnant cows was statistically significant, where pregnant cows were three times (OR=3) more likely to harbor anti-Brucella antibody compared to non-pregnant ones. However, body condition, abortion period, retained fetal membrane and parity were not able to explain the seroprevalence distribution of the anti-Brucella antibody (Table 1).

Statistically significant variation ($p < 0.05$) was observed between Brucella serostatus and herd sizes. Cattle from large herd size category were almost eight times (OR= 8.29) more likely to be seropositive for anti-Brucella antibody than the cattle from the small herd size category. Similarly, a statistically significant difference in the serostatus of *Brucella* organism ($p < 0.05$) was observed in cattle herded with sheep and/ goats; those having close contact with small ruminants had about four times (OR=4.42) more chance to be infected with *Brucella* organism than those with no contact. However, the study districts, agro-ecology, management system and introduction of the new animal were not significantly associated with anti-Brucella antibody distribution among cattle (Table 2).

No significant interactions ($p > 0.05$) between variables were detected. A Hosmer-Lemeshow goodness-of-fit value ($p = 0.94$), indicated that the model was fit the data. The model had the good predictive ability (ROC=0.82). The final multivariable logistic regression model showed that age, breed, herd size and species composition of domestic ruminants were independently associated ($p < 0.05$) with the seroprevalence of cattle (Table 3).

Discussion

A case-control study is paramount for the *Brucella* organism to investigate associated risk factors of brucellosis [24]. An overall of 4.02% seroprevalence of anti-Brucella antibody was recorded in the present study. A similar level of prevalence was reported by previous studies [27, 38], that reported a seroprevalence of 3.2% from the central and 4.8% from the southern Ethiopia. Likewise, other studies [40, 41]

Table 1
Univariable logistic regression analysis of host-related risk factors of cattle brucellosis in the study areas

Variables	Category	Cases	Controls	OR (95% CI)	*p*-value
Age					0.035
	<3 years (Ref)	11	18		
	3-6 years	67	137	3.92 (1.10-13.96)	0.035
	>6 years	63	127	5.92 (1.49-23.52)	0.012
Breed					
	Cross (Ref)	49	67		
	Local	92	215	4.04 (1.5-10.89)	0.003
BCS					0.269
	Poor (Ref)	21	33		
	Medium	91	173	1.84 (0.56-6.01)	0.313
	Good	29	76	4.12 (0.76-23.26)	0.109
Parity					0.216
	Nulliparous (Ref)	72	112		
	Monoparous	40	72	2.31 (0.63-8.47)	0.206
	Pluriparous	29	98	2.63 (0.72-9.62)	0.144
Pregnancy status					
	Non-Pregnant (Ref)	54	111		
	Pregnant	87	171	3.0 (1.09-8.28)	0.034
Abortion period					0.070
	No history (Ref)	0	282		
	After 5th month	44	0	1.26 (0.15-5.29)	0.832
	Before 5th month	97	0	0.33 (0.12-0.89)	0.029
Retained placenta					
	No (Ref)	75	219		
	Yes	66	63	0.478 (0.18-1.27)	0.138

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference; BCS: Body condition score

reported 4.6% seroprevalence in selected regions of Ethiopia; [34] reported 3.1% in Jimma zone; [42] reported 4.3% in Adami Tulu and [43] reported 4.9% in northwest Ethiopia. Comparable prevalence was also reported by [44] 4.2% and 3.3% [45] in Eritrea and the Central African Republic, respectively. However, the seroprevalence report in this study is lower than some previous studies carried out in the country: 11.2% in East Shewa [46]; 6.1% in western Tigray [13]; 14.1% in Assela [47]; and 10.6% in Borana [21]. Similarly, higher seroprevalence was also reported in other African countries, For instance, 6.6% in Ghana [48], 41% in Togo [49], 6.6% in Chad [50] and 46.8% in Uganda [51]. On the other hand, the seroprevalence reported in the current study was higher than the 2.9% reported in central Ethiopia [52]; 1.7% in Sidama zone [17]; 2.6% in Arsi zone [23] and 1.4% in central Ethiopia [22, 53]. The variation in seroprevalence of brucellosis may be related to the prevalence of brucellosis that may vary based on the breed involved, management and environmental factors that influence the transmission rate of *Brucella* organism. This result is in agreement with a study [10] that reported that *Brucella* infection varies from country to country and

Table 2
Univariable logistic regression analysis of managemental and environmental-related risk factors of cattle brucellosis in the study areas

Variables	Category	Cases	Controls	OR (95% CI)	*p*-value
District					
	Limu Seka (Ref)	83	166		
	Chora Boter	58	116	1.71 (0.59-4.95)	0.321
Agro-ecology					
	Mid-altitude (Ref)	117	234		
	Lowland	24	48	3.39 (0.44-25.99)	0.240
Management system					
	Semi-intensive (Ref)	91	182		
	Extensive	50	100	1.61 (0.60-4.31)	0.345
Introduction of new animal					
	No (Ref)	68			
	Yes	73	146	2.71 (0.98-7.46)	0.054
Herd size					0.036
	Small (Ref)	60	120		
	Medium	34	68	2.18 (0.69-6.92)	0.187
	Large	47	94	8.29 (1.06-64.60)	0.044
Species composition					
	Only cattle (Ref)	8	16		
	Mixed with sheep and/ goat	133	266	4.42 (1.47-13.26)	0.008

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference

also between regions even within a country.

In this study, an increase in age is associated with the increased risk of being *Brucella* seropositive; older animals (>6 years) were fourteen times (OR=14.16) more likely to be infected by brucellosis compared to their younger age groups. Similarly, several studies indicated age as one of the important risk factors influencing *Brucella* serostatus in cattle [12, 15, 16, 21, 32, 37, 54, 55, 56] in Ethiopia and elsewhere. This report is in line with the standard veterinary literature which supports younger animals tend to be more resistant to infection and being frequently infection-clear. Older animals are more susceptible to brucellosis than younger animals, which are due to sex hormones and erythritol that stimulate the growth and multiplication of bacteria [9].

There is an argument among different researchers

on the issue of breed susceptibility to brucellosis. This study revealed that breed caused statistically significant variations in *Brucella* serostatus with the odd of the disease being five times (OR=5.36) higher in local than the crossbred breeds. The better management in the crossbred herds, intensive feeding that minimizes contacts between animals may be responsible for this difference. This finding is consistent with some previous studies in Ethiopia and elsewhere [12, 22, 43, 55, 57, 58] showing that the seropositivity for the anti-Brucella antibody was significantly associated with the breed in cattle. However, several studies [37, 39, 59, 60,] report that breed was not significantly associated with *Brucella* seropositivity in cattle in different parts of the country. Similarly, a few other studies [11, 61, 62] also reported no significant association between *Brucella* seropositivity and cattle breed in Zambia, Ni-

Table 3
Final multivariable logistic regression model of risk factors associated with cattle brucellosis in the study areas

Variables	Cases	Controls	Adjusted OR (95% CI)	*p*-value
Age				0.004
<3 years (Ref)	11	18		
3-6 years	67	137	6.43 (1.46-12.34)	0.014
>6 years	63	127	14.16 (2.91-28.84)	0.001
Breed				
Cross (Ref)	49	67		
Local	92	215	5.36 (1.76-11.33)	0.003
Herd size				0.037
Small (Ref)	60	120		
Medium	34	68	2.77 (0.62-2.93)	0.109
Large	47	94	11.82 (1.31-16.17)	0.024
Species composition				
Only cattle (Ref)	8	16		
Mixed with sheep and/ goat	133	266	5.10 (1.49-13.43)	0.009

OR: Odds Ratio; CI: Confidence Interval, Ref: Reference

geria, and Malaysia, respectively. This variation could be due to the difference in environmental factors and management systems.

In the present study, statistically significant variation has been observed in the seroprevalence of anti-Brucella antibody between different herd sizes; larger herd sizes were almost twelve times (OR=11.82) more likely to be seropositive. Herd size has previously been reported in Eritrea as an important determinant for transmission of *Brucella* organism between susceptible and infected animals [44] and thus; larger herds were more likely to have at least one positive animal than smaller herds [63]. Several authors in Ethiopia and Zimbabwe also reported that large herd size enhances the exposure to and maintenance of *Brucella* organisms following abortions through increased contact at common feeding and watering points [12, 16, 17, 18, 64]. However, contrary to this another study [37] reported that the risk of seropositivity was independent of herd size in the central Ethiopia. The observed variation could be attributed to various factors including agro-ecology and management system.

In this study, cows from households herding cattle together with goats and/ sheep had five times (OR=5.10) more odds of brucellosis than those kept without other species. Herding of these animals together increases the chance of cross-species trans-

mission of *Brucella* organisms. *Brucella* organism is not strictly host-specific; *Brucella melitensis* has been isolated from cattle [65] and thus, herding together might have increased the spillover of the pathogen from small ruminants to cattle. Moreover, herding more cattle at one farm may increase animal density and chance of contact among animals, as a result, facilitating exposure to *Brucella* species and increasing the chance of acquiring the disease [66]. This finding is in line with the previous study [21], that reported the mixing of sheep and/ goats with cattle increased risk of *Brucella* seropositivity in cattle in Borana zone, Ethiopia. Moreover, other reports from Eritrea [44], Malaysia [62] and Jordan [63] also confirmed that mixed farming especially raising sheep and/ goats along with cattle was a risk for *Brucella* spread among different animal species. This is different from the findings of another study [67], that reported that keeping sheep and/ goats with cattle is not significantly associated with *Brucella* seropositivity in Sudan. This variation could be due to the difference in environmental factors, breed of animals, and management system.

In conclusion, the present study shows that cases have higher *Brucella* seropositive status than control cattle groups. Higher *Brucella* seropositivity was recorded in this study. This indicates that brucellosis causes huge economic losses and serious public health

problem. The present study identified that age, breed, herd size, and species compositions as risk factors for *Brucella* seropositivity in cattle. Hence, different livestock species need to be kept and maintained separately to reduce the risk of transmission of *Brucella* among them. It is also important to conduct applicable control methods and increasing public awareness of the zoonotic transmission of brucellosis. Moreover, further study should be carried out to identify the specific causes of abortion in cattle for the preparation of the appropriate vaccine.

Material and methods

Study areas

The study was conducted from October 2016 to October 2017 in selected districts of Jimma zone. These districts are one of the potential areas for cattle production in the zone. Limu Seka district is situated 109 km from Jimma town. The district is located at an altitude of 1400-2200 meter above sea level, 09°29' North latitude and 37°26' East longitudes. The agroecology is characterized by 13% highland and 55% mid-highland and 32% lowland. The average temperature varies from a minimum of 15.1°C to a maximum 31°C. There are two distinct seasons in Limu Seka: the rainy season (from late March to October), and the dry season (November to early March). Limu Seka district has 295,627 cattle, 104,892 sheep, 89,079 goats and 134,370 human populations. Chora Boter district is located 112 km from Jimma town. The district is located at 9°-10°24' North latitude and 37°56'-40°35' East longitude with an altitude range of 1100-2200 meter above sea level. The agroecology is characterized by 25% highland, 73.5% mid-highland and 2.3% lowland. The annual average temperature ranges from 18.3°C to 26.7°C. Similar to the Limu Seka district, the district

has two seasons. The rainfall is often more than 1,800-2,200 mm per annum. Chora Boter district has 228,846 cattle, 47,854 sheep, 68,037 goats and 215,348 human populations. There are two management systems in the area: these are extensive (crop-livestock production) systems and semi-intensive (urban production). Local cattle are the dominant breed in the area and crossbred Holstein- Friesian also present (Figure 1).

Study population, design and methods

Target populations were female cattle in the selected districts of Jimma zone. The study population was breeding cattle in selected peasant associations of the study districts. Animals in this study were female cattle from herd having three and above cows and/or heifers with a history of abortion. Case-Control study design was used, where cows or heifers that had experienced abortion were defined as cases. Controls were cows or heifers from the same herd but had no record of abortion with the age of two years and above. Abortion was the loss of pregnancy from 42 to 260 days of gestation [1] for this study. Jimma zone was selected purposively based on the dominant of cattle population, while the peasant associations, village, and herd were selected randomly. A simple sampling method was used to select a sample of animals. Cattle involved in this study had no vaccination against brucellosis.

Sampling procedure and sample size determination

Limu Seka and Chora Boter districts were selected purposively based on the history of abortion. A total of ten peasant associations were included from these districts using a random sampling technique, where six peasant associations were from Limu Seka and four of them from Chora Boter. Cows/heifers with a history of abortion in the herd were selected purposely based on districts' veterinary clinic case book and the owners' information. Before the selection of herds with the history of abortion, the availability of cases were checked. To incorporate more herds (clusters),
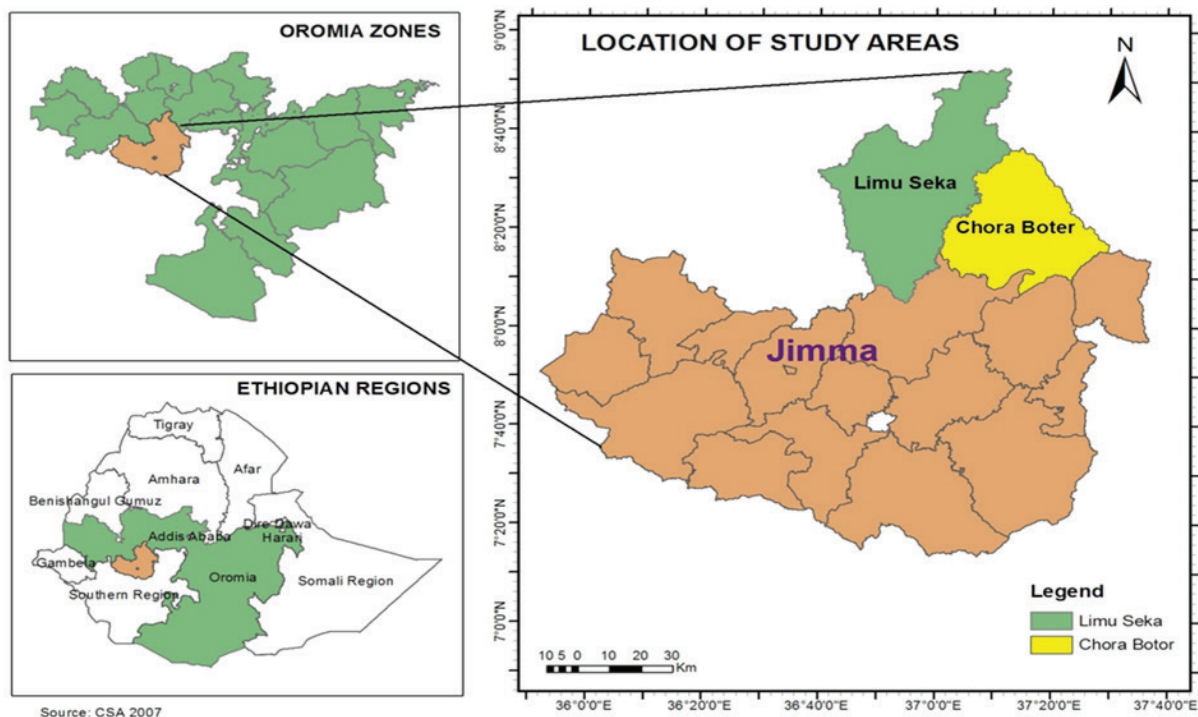

Figure 1
Map of the study areas (Limu Seka and Chora Boter districts).

the number of cases per herd was limited to a maximum of two and four control per herd. The necessary minimum sample size was calculated using [25] base on case-control study design with a predetermined odds ratio (OR) of 3, an expected prevalence of exposure in control groups of 10%, a desired level of 95% confidence, 5% precision, and a power of 80% [26, 27], thus leading to a sample size of 97 cases. With two controls selected per case, the number of controls should have been 194. To adjust the difference among the clusters, the sample size was multiplied by the design effect (D) by using the formula $D = \rho (n-1) + 1$, where n is average number of dairy cattle in cluster (6), and intra-cluster correlation coefficient of $\rho = 0.09$ has been reported for *B. abortus* in cattle [28]. The design effect (D) was 1.45 and increasing the power by using two controls per case. Thus, a minimum of 141 cases and 282 controls were selected to be enrolled in this study. Eventually, a total of 423 cows were involved in the study. Selecting sample animals, a sampling frame of the herd with abortion was prepared in collaboration with district veterinary departments and a total of 118 herds were chosen at random. In each herd where one or two cases were found, these were selected and controls were chosen at random using a lottery method. On the other hand, a random sampling method was used where large cases and controls were available in the herd.

Data collection

Information related to district, agro-ecology, age, body condition, breed, parity, pregnancy status, history of retained fetal membrane, abortion period, herd size, management system, introduction of new animal and species composition (mixed of cattle with sheep and/ goats) were gathered using a separate format prepared for this purpose. Classification of management systems (extensive and semi-intensive) was done based on the criteria adopted by [29]. Body condition score was based on the criteria adopted by [30] and for all cows, under the study, their body condition grouped into three groups (poor, medium and good). Age of animals was categorized into <3, 3-6 and >6 years and groups were chosen because optimal age at first calving cattle reared under tropical conditions was estimated to be 2-3 years [31]. Herd size was categorized into small (3-5 heads of cattle), medium (6-10 heads of cattle) and large (>10 heads of cattle). Those cattle that kept in the same barns grouped and considered as one herd [16, 32]. Parity number was categorized as nulliparous (zero parity), monoparous (parity one) and pluriparous (≥ two parities) [33, 34].

Blood sample collection

Approximately 10 milliliters of blood samples were collected from the jugular vein of each animal, using sterile needles and plain vacutainer tubes. The identification of each animal was labeled on the corresponding vacutainer tubes and blood samples were allowed to stand overnight (12 hours) at room temperature to obtain the serum. The animals' identification codes were transferred to the cryovials to which the serum was decanted and serum samples were kept at -20°C [35] in Jimma University microbiology laboratory until they transported to National Veterinary Institute, Debrezeite using icebox for serological analysis.

Rose Bengal Plate Test

The serum samples were screened by using Rose Bengal Plate Test (RBPT) (KT153NB, UK) for the presence of *Brucella* agglutinins according to the previously published procedure [35]. Serum samples and antigens when taken out from the refrigerator, will be kept at room temperature for half an hour and processed following the recommended procedure. A total of 30 microliters of serum sample was dispensed onto the plate and 30 microliters of RBPT antigen were dropped on the slide with sera. The inter-pretation of both positive and negative control results was done according to the degree of agglutination and the reaction was read in a good light source or by a magnifying glass when micro agglutination was suspected. The RBPT results were interpreted 0, +, ++ and +++ as has been described by [26], where 0 indicates no agglutination, + indicates barely visible agglutination (using magnifying glasses), ++ indicates fine agglutination and +++ indicates coarse clumping. Those serums identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were considered as positive.

Complement fixation test

All RBPT positive sera were further tested using a complement fixation test (CFT) using standard *B. abortus* antigen S99 and control sera (positive and negative) (KT15 3NB, United Kingdom). The antigen dilution was standardized at 1:10. Two-fold dilutions (1:5, 1:10, 1:20 and 1:40) of test sera were ready in standard 96-well U-bottom microtiter plates before adding *Brucella* antigen, guinea pigs complement and 3% sensitized sheep red blood cells. The preparation of the reagent was evaluated by titration and performed according to the recommended protocols by [7]. The plates were incubated at 37°C for thirty-minute with agitations and results were read after the plates have been centrifuged at 2500 rpm for five minutes at 4°C. Sera with a strong reaction, more than 75% fixation of complement (3+) at 1:5 dilution or at least with 50% complement fixation (2+) at 1:10 dilution and above were considered as positive and lack of fixation/complete hemolysis was considered as negative [35]. An animal was considered positive if tested seropositive on both RBPT and CFT in serial interpretation. Both the Rose Bengal plate test and the Complement fixation test were done in the National Veterinary Institute (NVI). The combination of RBPT and CFT in serial most widely used is commonly recommended to maximize the specificity of the test result by ruling out false-positive serological cross-reactions [26].

Data management and analysis

The collected data were stored in Microsoft Excel for Windows 2010 and then transferred to SPSS version 20.0 (IBM SPSS, 2011) for analysis. The seroprevalence of brucellosis was calculated by dividing the number of seropositive samples to the total of cattle samples. The association between brucellosis and associated risk factors were analyzed using logistic regression model. Risk factors associated with brucellosis were identified by using a multivariable logistic regression model and the strength of their association was assessed using adjusted odds ratios (OR). Variable with a *p*-value less than or equal to 0.25 in the univariable analysis were involved in the multivariable logistic model. The backward elimination procedure was used for a further selection of variables. The variables were tested for interaction effect using cross-product terms and for multiple-collinearity using the collinear matrix index before building the final model [36]. Hosmer-Lemeshow test was used to evaluate the validity of the model. Similarly, the predictive ability of the model was assessed using the ROC curve. Confidence level (CL) was at 95% and $p \leq 0.05$ were set for significance for all analyses.

Author Contributions

D.T. contributed to sample collection, laboratory tests, data analysis and drafting the manuscript. B.D. and F.B. contributed to the main design of the study, and reviewed and edited the manuscript. All authors approved the final version of the manuscript for publication.

Conflict of Interest

The authors declare that there is no conflict of interest.

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