

Seropositivity, Comparison Between the Efficiency of Serological Tests and Risk Factors of *Brucella* Infection in Small Ruminants with History of Abortion in the Afar Region of North-Eastern Ethiopia

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Purpose: Brucellosis is one of the most important reproductive diseases that cause abortion and breeding failure in small ruminants in Ethiopia. Therefore, our objective was to detect the seropositivity and risk factors of *Brucella* infection in small ruminants with history of abortion using modified RBPT, cELISA, and CFT in the Amibara district of the Afar Region, Ethiopia.

Methods: Sera were collected from 226 animals (195 goats and 31 ewes) and assessed for seropositivity of *Brucella* infection using modified RBPT, CFT, and competitive ELISA.

Results: The overall seroprevalence was 12.0% (27 out of 226), 7.5% (17 out of 226), and 26.5% (60 out of 226) by mRBPT, CFT, and cELISA, respectively. Out of 27 sera that were reactive by mRBPT, 17 (63.0%) were also reactive by (CFT). Out of the 17 sera that were reactive by CFT and mRBPT, 14 (82.4%) were reactive by cELISA. Out of the 29 sera that were non-reactive by both mRBPT and CFT, 10 (34.5%) were found reactive by cELISA. Out of the 226 sera that were tested by both mRBPT and cELISA, 20 (8.9%) were reactive by both tests, while 159 (70.4%) were non-reactive by both tests. The percentage of test agreement (79.2%) between mRBPT and cELISA was poor ($k=0.353$). High seropositivity for *Brucella* infection was significantly associated with the presence of retained placenta in the studied animals (adjusted OR=2.2, 95% CI, 1.1–4.4, $P=0.030$) as detected by cELISA.

Conclusion: The current study revealed that a cELISA-based seroepidemiological survey increases the likelihood of detecting individuals with brucellosis and provides reliable evidence for mRBPT. Furthermore, there was a significant association between seropositivity for *Brucella* infection and retained placenta. These findings emphasize the necessity for proactive measures to reduce the economic impact of brucellosis and mitigate the risk of zoonotic transmission.

Keywords: abortion, brucellosis, seropositivity, small ruminants

Introduction

Brucellosis is a common disease in tropical and subtropical countries and causes enormous economic losses due to it causes abortion and breeding failure in small ruminants and also it affects the health of livestock and diminishes their products.^{1,2} It has also been considered the most common re-emerging zoonotic disease in many areas of the world.^{3–6} However, the magnitude and distribution of brucellosis both in animals and humans vary in different geographical localities.⁷ Studies on small ruminant brucellosis in pastoral areas of Ethiopia determined seroprevalences ranging from 1.5% to 16% in Afar, Oromia, and Somali Regional States using the rose Bengal plate (RBPT) and complement fixation (CFT) tests.^{8,9} A higher risk of *Brucella* infection in goats has been associated with agro-pastoral and pastoral production systems. In pastoral areas, the contribution of brucellosis to abortion in goats has been highlighted and an upsurge of infection has been associated with an increased abortion rate.^{10,11} Hence, an extensive epidemiological survey of brucellosis in humans and animals in different settings using reliable diagnostic methods would be useful to provide reliable epidemiological data.

Bacteriological isolation using the culture method, which is a reliable method for the diagnosis of *Brucella* infection in animals and humans. However, as the *Brucella* organism grows very slowly in vitro, it requires advanced laboratory, trained manpower, and high biosafety situations. In addition, the culture method is not feasible to carry out an epidemiological study of brucellosis.¹² Hence, serological tests such as modified Rose Bengal Plate Test (mRBPT), Complement Fixation Test (CFT), and Enzyme Linked Immuno Sorbent Assay (ELISA) in a single or combination are commonly used for the screening of *Brucella* infection.¹² Among others, RBPT with or without CFT is the most commonly used for preliminary diagnosis of brucellosis in many resource-limited countries including Ethiopia.^{9–12} However, RBPT has been criticized for its drawbacks such as false-positive results due to cross-reactivity with other bacteria.^{13,14}

Several studies have also suggested that ELISA is more effective for a seroepidemiological survey of brucellosis as compared to RBPT and good diagnostic results have been obtained in sheep and goats with indirect (I-) or competitive (C-) enzyme linked immunosorbent assays (ELISAs) using various antigens, but generally the ELISAs that use antigens with a high content of smooth lipopolysaccharide (sLPS) are the most useful.^{15–20}

In Ethiopia, several seroprevalence studies of brucellosis have shown the occurrence of brucellosis among livestock using mRBPT and CFT.^{21–24} Nevertheless, few studies have used ELISA to assess the seroprevalence of brucellosis in animals.^{23,25} Previous studies have shown that cELISA is highly specific compared to mRBPT for the diagnosis of brucellosis in animals.^{18,26,27}

Therefore, the present study was conducted in the Amibara district of the Afar Region, Ethiopia, to detect the seropositivity and risk factors of *Brucella* infection in small ruminants with a recent history of abortion using modified RBPT, cELISA, and CFT.

Methods

Study Area and Animal Population

The study was conducted in a pastoralist area of Amibara district of Afar National Regional State, Ethiopia. Amibara district is located in the Middle Awash Valley about 260 km to the North East of Addis Ababa. The livestock populations of the Amibara district are 103,959 cattle, 122,526 goats, 48,043 sheep, 3888 donkeys, and 39,995 camels. The production system of the Afar region is dominated by pastoralism (90%) from which agro-pastoralism (10%) is now emerging, following some permanent and temporary rivers on which small-scale irrigation is developed.^{28,29} In Ethiopia to date, no brucellosis vaccination has been undertaken in animals.^{25,30,31}

Sample Size Estimation

With the assumption of 18% seroprevalence of *Brucella* infection in small ruminants in the study area²⁵ 5% precision and 95% confidence level, about 226 animals were intended to be included in the study.

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2}$$

Where,

n =required sample size,

P_{exp} =expected prevalence, and

d =desired absolute precision

Hence, based on the above formula, taking the expected prevalence of brucellosis in clinically aborted small ruminants as 18%²⁵ a desired absolute precision of 5% and 95% confidence level, 226 animals are required.

$$n = \frac{3.84 \times 0.18(1 - 0.18)}{(0.05)^2} = 226$$

Therefore, a total of 226 clinically aborted small ruminants were considered for this study from Pastoral *kebeles*.

Study Animals and Procedure of Data Collection

A community-based cross-sectional study was conducted from October 2015 to April 2016. A house-to-house survey was conducted to include goats and/or sheep that had a history of recent abortion (abortion occurred in the last 30 days at the time of data collection) and were included in the study. After the aim of the survey had been explained, the owners were interviewed using a structured questionnaire regarding the history of abortion, the duration of abortion, age of the animal, frequency of abortion, and retained placenta. Then, about 3 mL of blood samples were collected from each animal, serum was separated and stored at -20°C until processed for serological analysis.

Serological Tests

All sera were screened using mRBPT and cELISA as per the manufacturers' instruction (Svanova, Brucella-ab c-ELISA Uppsala Business Park, Rapskatan 7,75174 Uppsala, Sweden). All sera found positive by mRBPT are further tested by CFT. In addition, 29 randomly selected sera that were negative by mRBPT were also tested by CFT.

Data Analysis

Data were entered into EpiData Software v.3.1 and analyzed using Stata version 11. Frequencies and percentages were used to summarise baseline characteristics of the study animals and the seroprevalence of brucellosis as diagnosed using mRBPT, CFT, and cELISA. Univariable and multivariable logistic regression analyses were used to assess the effect of each of the independent variables (such as age, history of abortion, and retained placenta). A p-value less than 0.05 was considered statistically significant. Agreement between the tests was assessed using Cohen's Kappa (k) coefficient. K values greater than 0.75 between 0.4 and 0.75 and less than 0.4 were considered excellent, fair, and poor agreement, respectively.

Results

Baseline Characteristics of the Study Animals and Seroprevalence of Brucellosis

According to a report by the owners, 98/226 (43.4%) animals had abortion history for two or more times. More than half 119/226 (52.7%) of the studied animals had retained their placenta during the data collection. Out of the total 226 animals, 27 (12.0%) were positive for *Brucella* infection by the mRBPT. Out of those 27, 17 (63.0%) were positive for *Brucella* infection by the CFT. None of the 29 animals that were negative by mRBPT were found to be positive by CFT.

In addition, out of 226 animals, 60 (26.6%) animals were found to be positive for *Brucella* infection by cELISA. Out of the 27 animals that were positive for *Brucella* infection by mRBPT, 7(25.9%) were negative by cELISA. Out of the 17 animals that were positive for *Brucella* infection by CFT and mRBPT, 14 (82.4%) were positive by cELISA.

In general, cELISA revealed high seropositivity for *Brucella* infection in the studied animals with retained placenta compared to animals without retained placenta (34.5% versus 17.8%, $\chi^2=8.05$, $P=0.005$). In bivariate logistic regression analysis, age over 6 years (Crude OR=2.3, 95% CI, 1.1–5.1, $P=0.030$) and retained placenta (crude OR=2.4, 95% CI, 1.3–4.5, $P=0.005$) were significantly associated with high seropositivity for *Brucella* infection as detected by cELISA. In multivariable logistic regression analysis, only retained placenta (adjusted OR=2.2, 95% CI, 1.1–4.4, $P=0.030$) was significantly associated with high seropositivity for *Brucella* infection (Table 1).

Agreement of the Tests for the Screening of *Brucella* Infection in Small Ruminants

Table 2 shows the test agreement between mRBPT and cELISA for the screening of *Brucella* infection in the studied animals. Out of the 226 sera that were tested by both mRBPT and cELISA, 20 (8.9%) were positive by both tests, while 159 (70.4%) were negative by both tests. Hence, the percentage of agreement (79.2%) between mRBPT and cELISA was poor ($k=0.353$). Out of the total 56 sera that were tested both by CFT and cELISA, 14 (25%) were positive by both tests, while 29 (51.8%) were negative by both tests and the percentage of agreement (76.8%) between cELISA and CFT was also poor ($k=0.193$).

Table 1 Association Between Baseline Characteristics of the Studied Animals and Seropositivity for Brucella Infection by mRBPT, CFT, and cELISA

Variables	mRBPT		CFT		cELISA			P value
	Number Tested (%)	Number Positive (%)	Number Tested (%)	Number Positive (%)	Number Tested (%)	Number Positive (%)	Adjusted OR (95% CI)	
Age								
2–3.9 years	82 (36.3)	6 (7.3)	15 (18.3)	5 (33.3)	82 (36.3)	16 (19.5)	Reference	
4–5.9 years	89 (39.4)	9 (10.1)	26 (29.2)	4 (15.4)	89 (39.4)	24 (27.0)	1.38 (0.54–3.52)	0.505
≥ 6 years	55 (24.3)	12 (21.8)*	15 (27.3)	8 (53.3)*	55 (24.3)	20 (36.4)	1.92 (0.66–5.57)	0.230
Parity								
None	46 (20.4)	6 (13.0)	6 (13.0)	6 (100)*	46 (20.4)	10 (21.7)	Reference	
1–2	87 (38.7)	13 (14.9)	24 (27.6)	6 (25.0)	87 (38.7)	23 (26.4)	1.00 (0.39–2.56)	0.998
≥3	92 (40.9)	8 (8.7)	25 (27.2)	5 (20.0)	92 (40.9)	27 (29.4)	0.99 (0.34–2.87)	0.895
Stage of abortion								
First month	34 (15.0)	1 (2.9)	1 (2.9)	1 (100)	34 (15.0)	7 (20.6)	Reference	
Second month	80 (35.4)	1 (1.3)	11 (13.8)	0 (0)	80 (35.4)	16 (20.0)	0.94 (0.33–2.69)	0.906
Third month and above	112 (49.6)	25 (22.3)*	44 (39.3)	16 (36.4)	112 (49.6)	37 (33.0)	1.44 (0.55–3.77)	0.463
Frequency of abortion								
Once	128 (56.6)	11 (8.6)	32 (25.0)	8 (25.0)	128 (56.6)	29 (22.7)	Reference	
≥ 2 times	98 (43.4)	16 (16.3)	24 (24.5)	9 (37.5)	98 (43.4)	31 (31.6)	1.46 (0.71–2.98)	0.301
Retained placenta								
Absent	107 (47.3)	1 (0.9)	12 (11.2)	0 (0)	107 (47.3)	19 (17.8)	Reference	
Present	119 (52.7)	26 (21.9)*	44 (37.0)	17 (38.6)*	119 (52.7)	41 (34.5)*	2.17 (1.08–4.37)	0.030

Notes: *Pearson's chi-square test was statistically significant at $p < 0.05$.

Table 2 Tests Agreement for the Serodiagnosis of Brucella Infection in the Studied Animals

mRBPT (n= 56)	CFT result		
	Number Positive	Number Negative	Total number Tested
Number Positive	17	10	17
Number Negative	0	29	29
	cELISA result		
mRBPT (n=226)	Number Positive	Number Negative	Total number tested
Number Positive	20	7	27
Number Negative	40	159	199
	CFT result		
cELISA (n=56)	Number Positive	Number Negative	Total number tested
Number Positive	14	10	24
Number Negative	3	29	32

Discussion

The present study determined the overall seroprevalence of brucellosis in sheep and goats with a history of recent abortion is between 12.0% and 7.5% with mRBPT alone and using combined mRBPT CFT tests, respectively. The observed seroprevalence is higher than seroprevalence reported in small ruminants in other areas of Ethiopia using CFT^{22,32} but relatively lower than previously reported overall seroprevalence of brucellosis in small ruminants using CFT in the Afar Region.^{30,31}

In this study, we assessed a seroprevalence of brucellosis in small ruminants with a history of recent abortion by mRBPT and cELISA. The seroprevalence of brucellosis was 12.0% in the studied animals as detected by mRBPT, which is similar to a recently reported seroprevalence of brucellosis in small ruminants in other districts of the Afar Region.^{25,26} The similarities could be due to resemblance to animal husbandry, communal grazing of rangelands and watering areas, and possibly similar climatic conditions.²⁵ Another study in Afar Region showed a low seroprevalence (3.1%) of brucellosis in small ruminants using mRBPT.²¹ Studies in other pastoral areas of Ethiopia also revealed relatively a low seroprevalence (8.5%) of the diseases using mRBPT.²² In addition, Tadeg et al³³ reported a high seroprevalence of brucellosis (17.4%) using mRBPT in small ruminants in another area of the Afar Region. This difference might have originated mainly from variation in the sample type and method of detection.²³

In this study, cELISA revealed a high seroprevalence for *Brucella* infection in the studied animals as compared to that of mRBPT (26.6% VS 12.0%, $\chi^2=35.5$, $p<0.001$). Previous studies in Ethiopia also showed a significantly higher seroprevalence of brucellosis in small ruminants using iELISA as compared to mRBPT.²⁵ Similar to the findings of the present study, previous studies on small ruminants suggested that ELISA-based test is more effective for serological-based survey of brucellosis as compared to mRBPT.^{16–19}

In this study, the agreement between mRBPT and cELISA for the serodiagnosis of *Brucella* infection in small ruminants was poor. However, a previous study in Ethiopia revealed a fair agreement between mRBPT and indirect ELISA for the serodiagnosis of *Brucella* infection in small ruminants.²⁵ On the other hand, a study in Rwanda has shown an excellent agreement between mRBPT and cELISA ($K=0.92$) for the serodiagnosis of *Brucella* infection in cattle.³⁴ The low agreement between mRBPT and cELISA in the present study was attributed to the high seroprevalence of brucellosis detected by cELISA, which might be due to the ability of this ELISA-based technique to detect low level of antibody even at the early stage of infection.^{17,35}

In this study, a high seroprevalence for *Brucella* infection was detected in those animals with the retained placenta, which is similar to the results of previous studies elsewhere.^{36–39} This indicates that abortion and retained placenta are typical outcomes of brucellosis.⁴⁰ A review on clinical features of brucellosis also showed that placenta retention is one of the main clinical signs of *B. melitensis* infection in aborted small ruminants.¹

To evaluate the reliability of the mRBPT and/or cELISA for the serodiagnosis of brucellosis, the gold standard (bacteria culture) needs to be used. However, in this study, the seroprevalence of brucellosis in animals with a history of recent abortion was assessed and compared using mRBPT and cELISA without using a gold standard like several previous studies and this could be one of the limitations of this study.

Conclusion

This study demonstrated high seropositivity of *Brucella* infection in small ruminants with a history of recent abortion could suggest that brucellosis is the main responsible cause of abortion and breeding failure in small ruminants of pastoral communities. The results associated with the presence of abortion are consistent with serological investigations and indicate a high level of exposure to *Brucella* infection. Besides this, high seropositivity of *Brucella* infection in small ruminants was detected by cELISA as compared to mRBPT. Moreover, seropositivity for *Brucella* infection was significantly associated with retained placenta.

Hence, this finding suggests that cELISA-based test is more effective for serological-based surveys of brucellosis in small ruminants as compared to mRBPT in the present study area though additional conformational studies are important. This finding also warrants appropriate control strategies to reduce its economic impact and risk of zoonotic transmission of the disease in the study area.

Abbreviations

AAU, Addis Ababa University; ALIPB, Aklilu Lemma Institute of Pathobiology; CVMA, College of Veterinary Medicine Agriculture; ITM, Institute of Tropical Medicine.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request and with permission from IRB and/or other responsible bodies in Ethiopia.

Ethics Approval and Consent to Participate

We confirmed that the study protocol was approved by the Institutional Review Board (IRB) of the College of Veterinary Medicine and Agriculture, Addis Ababa University. The purpose of the study was clearly explained to all goat and sheep owners who participated in the study, and their verbal consent was obtained before collecting samples from goats. The IRB specifically approved the use of verbal consent for obtaining blood samples from aborted goats, as this procedure was considered a routine clinical practice for confirming *Brucella* diagnosis.

Acknowledgments

We acknowledge Dr. Mekonin Bayissa (Amibara District Pastoral Office, Afar Region) for his positive cooperation during data collection; Administration of Afar Pastoral Regional State of Amibara district, animal owners, and Melka Werer Agriculture Research Center are highly acknowledged. We acknowledge the Animal Health Institute (AHI) in Sebeta, Ethiopia, for their support in serology laboratory analysis.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This Study was financially supported by a collaborative research project entitled “Integrated Community and Health facility-based study of brucellosis in Pastoralists and their livestock in Afar Regional State of Ethiopia” between ALIPB and CVMA of Addis Ababa University funded by the Institute of Tropical Medicine (ITM-Belgium) No: 1123/2016. The funding organization had no any role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Disclosure

The authors declare that they have no competing interests

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