Seropositivity and risk factors of Brucella infection in small ruminants that had history of recent abortion in the afar region of north-eastern Ethiopia

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Abstract

Background: Brucellosis is one of the most important reproductive disease causes abortion and breeding failure in small ruminants and also causes severe systemic diseases in exposed humans. In Ethiopia, several studies of seroprevalence shows the magnitude and distribution of brucellosis both in animals and humans vary in different geographical localities. However, except few studies in Ethiopia all these serological studies was limited to RBPT and CFT, so far not supplemented with a varities of serological tests like ELISA to detect brucella infection, which is increase the likehood of detecting infected individulas and also improve the reliability of epidemiological data for appropriate control strategies. Hence, the present study was conducted in Amibara district of Afar Region, Ethiopia to detect the seropositivity and risk factor of *Brucella* infection in small ruminants that had history of recent abortion using mRBPT, cELISA and CFT.

Materials and methods: Sera were collected from 226 animals (195 goats and 31sheep) and assessed for seropositivity of *Brucella* infection using modified Rose Bengal Plate Test (mRBPT), Complement Fixation Test (CFT) and competitive Enzyme Linked Immuno Sorbent Assay (cELISA).

Results : In this study the over all seroprevalence was 12.0% (27 out of 226), 7.5% (17 out of 226) and 26.6% (60 out of 226) by mRBPT, CFT and cELISA, respectively. Out of 27 sera which were reactive by mRBPT, 17 (63.0%) were also reactive by (CFT). Out of the 17 sera which were reactive by CFT and mRBPT, 14 (82.4%) were reactive by cELISA. Out of the 29 sera which were non-reactive both by mRBPT and CFT, 10 (34.5%) were found to be reactive by cELISA. Out of the 226 sera which were tested both by 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). A high seropositivity for *Brucella* infection was significantly associated with the presence of retained placenta in the study animals (adjusted OR= 2.2, 95%CI, 1.1-4.4, P=0.030) as detected by cELISA.

Conclusion: The findings of this study could suggest that brucellosis is main cause of abortion and breeding failure in small ruminants that had histry of recent abortion in the pastoral communities' andwarrants the need for proactive measures to reduce its economic impact and risk of zoonotic transmission. This study indicates that cELISA based seroepidemiological survey increase the likehood of detecting infected individulas of brucellosis and also would be useful to provide reliable evidence for *Brucella* infection in small ruminants compared to mRBPT.

Introduction

Brucellosis is a common disease in the tropical and subtropical countries, and causes an 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 considred as the commonest 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 giograpical localities [7]. Hence, 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 culture method, which is a reliable method for the diagnosis of *Brucella* infection in animals and human requires advanced laboratory, trained manpower and high biosafety suitations. In addition, culture method is not feasible to carry out an epidemiological study of brucellosis [8]. Hence, serological tests such as Rose Bengal Plate Test (RBPT), Complement Fixation Test (CFT) and Enzyme Linked Immuno sorbent Assay (ELISA) in a single or combination are commonly used for the secreening of *Brucella* infection [8]. Among others, RBPT with or without CFT is the most commnoly used for the screening of *Brucella* infection in many resource limitted 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 sero-epidemiological survey of brucellosis as compared to RBPT and good diagnostic results have been obtained in sheep and goats with indirect (I-) or competitive (C-) enzymelinked 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 [15-19 and 41].

In Ethiopia, several seroprevalence studies of brucellosis have shown the occurrence of brucellosis among livestock using mRBPT and CFT [20-23]. Nevertheless, few studies have used ELISA to assess the seroprevalence of brucellosis in animals [22, 24]. Previous studies showed that cELISA is highly specific compared to indirect ELISA and mRBPT for the diagnosis of brucellosis in animals [18, 25, and 26]. Therefore, the present study was conducted in the afar region of north-eastern Ethiopia to detect the seropositivity and risk factor of *Brucella* infection in small ruminants that had history of recent abortion using mRBPT, cELISA and CFT.

Methods

Study area and animal population

This study was conducted in Amibara district of the Afar Region, north-east Ethiopia. Detailed description of the study area has been given elsewhere [27]. In Ethiopia to date, no brucellosis vaccination has been undertaken in animals [24, 28, and 29].

Sample size estimation

With the assumption of 16% seroprevalence of *Brucella* infection in small ruminants in the study area [24] 5% precision and 95% confidence level, about 207 animals were intended to be included in the study.

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 history of recent abortion (abortion occured in last 30days at the time of data collection) were included in the study. After the aim of the survey had been explained, the owners were interviewed using structured questionnaire regarding history of abortion, the duration of abortion, age of the animal, frequency of abortion and retained placenta. Then, about 3 ml blood samples was collected from each animal, serum was separated and stored at -20^{O}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. Rapsgatan 7, 751 74 Uppsala, Sweden). All sera found positive by mRBPT were further tested by CFT. In addition, 29 randomly selected sera which 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 were 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.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (IRB) of the College of Veterinary Medicine and Agriculture (CVMA), Addis Ababa University. The purpose of the study was explained to all small ruminant owners who participated in the study and verbal consent was obtained from the animal owners before collecting samples from small ruminants. The IRB approved the verbal consent from owners would be enough for blood sample collection from aborted goats since the procedure was taken as a routine clinical procedure to confirm *Brucella* diagnosis.

Results

Baseline characteristics of the study animals and seroprevalence of brucellosis

According to the report by the owners, 98 (43.4%) animals had abortion history for two or more times and about half (49.6%). More than half (52.7%) of the study animals had retained placenta during the data collection.Table 1 shows the baseline characteristics of the 226 study animals and seroprevalence of brucellosis as detected by RBPT and CFT. 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 which were negative by mRBPT found positive by CFT.

Table 1. Baseline characteristics of the study animals and seropositivity for *Brucella* infection by mRBPT and CFT

Characterstics	RBPT		CFT	
	Number tested (%)	Number Positive (%)	Number tested (%)	Number Positive (%)
Animal species				
Goat	195 (86.3)	26 (13.3)	50 (25.6)	16 (32.0)
Sheep	31 (13.7)	1 (3.2)	6 (19.4)	1(16.7)
Age in years				
2.0-3.9	82 (36.3)	6 (7.3)	15 (18.3)	5 (33.3)
4.0-5.9	89 (39.4)	9 (10.1)	26 (29.2)	4 (15.4)
≥ 6.0	55 (24.3)	12 (21.8)*	15 (27.3)	8 (53.3)*
Parity				
None	46 (20.4)	6 (13.0)	6 (13.0)	6 (100)*
1-2	87 (38.7)	13 (14.9)	24 (27.6)	6 (25.0)
≥3	92 (40.9)	8 (8.7)	25 (27.2)	5 (20.0)
Stage of abortion				
First month	34 (15.0)	1 (2.9)	1(2.9)	1 (100)
Second month	80 (35.4)	1 (1.3)	11(13.8)	0(0)
Third month	112 (49.6)	25 (22.3)*	44 (39.3)	16 (36.4)
Frequency of abortion				
Once	128 (56.6)	11 (8.6)	32 (25.0)	8 (25.0)
≥ 2 times	98 (43.4)	16 (16.3)	24 (24.5)	9 (37.5)
Retained placenta				
Absent	107(47.3)	1(0.9)	12 (11.2)	0 (0)
Present	119 (52.7)	26 (21.9)*	44 (37.0)	17 (38.6)*

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

On the other hand, 60 (26.6%) animals were found positive for *Brucella* infection by cELISA. Out of the 27 animals which were positive for *Brucella* infection by mRBPT, 7(25.9%) were negative by cELISA. Out

of the 17 animals which were positive for *Brucella* infection by CFT and mRBPT, 14 (82.4%) were positive by cELISA.

In general, cELISA revealed a high seropositivity for *Brucella* infection in the study animals with retained plancenta compared to animals without retained placenta (34.5% versus 17.8%, x^2 = 8.05, p= 0.005). In bivariate logestic 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 assocated with a 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 a high seropositivity for *Brucella* infection (Table 2).

Table 2. Association between baseline characteristics of the study animals and seropositivity for *Brucella* infection as detected by cELISA

iable	Number (%)	Number Positive (%)	Adjusted OR (95% CI)	P value
e				
2-3.9 years	82 (36.3)	16 (19.5)	Reference	
4-5.9 years	89 (39.4)	24 (27.0)	1.38 (0.54-3.52)	0.505
≥ 6 years	55 (24.3)	20 (36.4)	1.92 (0.66-5.57)	0.230
rity				
None	46 (20.4)	10 (21.7)	Reference	
1-2	87 (38.7)	23 (26.4)	1.00 (0.39-2.56)	0.998
≥3	92 (40.9)	27 (29.4)	0.99 (0.34-2.87)	0.895
ge of abortion				
st month	34 (15.0)	7 (20.6)	Refrence	
cond month	80 (35.4)	16 (20.0)	0.94 (0.33-2.69)	0.906
rd month and above	112 (49.6)	37 (33.0)	1.44 (0.55-3.77)	0.463
equency of abortion				
nce	128 (56.6)	29 (22.7)	Reference	
2 times	98 (43.4)	31 (31.6)	1.46 (0.71-2.98)	0.301
tained placenta				
Absent	107 (47.3)	19 (17.8)	Reference	
Present	119 (52.7)	41 (34.5)*	2.17 (1.08-4.37)	0.030

Agreement of the tests for the screening of *Brucella* infection in small ruminants

Table 3 shows test agreement between mRBPT and cELISA for the screening of *Brucella* infection in the study animals. Out of the 226 sera which were tested both by 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 which

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 3. Tests agreement for the sero-diagnosis of *Brucella* infection in the study animals.

	CFT result		
mRBPT (n= 56)	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 history of recent abortion is between 12.% 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 [21,31], but relatively lower than previously reported overall seroprevalence of brucellosis in small ruminants using CFT in the Afar Region [28,29].

In this study, we assessed a seroprevalence of brucellosis in small ruminants with history of recent abortion by mRBPT and cELISA. The seroprevalence of brucellosis was 12.0% in the study animals as detected by mRBPT which is similar to a recently reported seroprevalene of brucellosis in small ruminants in other districts of the Afar Region [28,29]. Another study in Afar Region showed a low seroprevalence (3.1%) of brucellosis in small ruminants using mRBPT [24]. Study in other pastoral areas of Ethiopia also revealed relatively a low seroprevalence (8.5%) of the diseases using mRBPT [21]. On the other hand, Tadeg et al. [30] reported a high seroprevalence of brucellosis (17.4%) using mRBPT in small ruminants in other area of the Afar Region.

In this study, cELISA revealed a high seroprevalence for *Brucella* infection in the study animals as compared to that of mRBPT (26.6% VS 12.0%, x^2 =35.5, p< 0.001). Previous studies in Ethiopia also showed a significantly high seroprevalence of brucellosis in small ruminants using iELISA as compared to mRBPT [24]. Similar to the findings of the present study, previous studies in small ruminants suggested that ELISA based test is more effective for the 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, previous study in Ethiopia revealed a fair agreement between mRBPT and indirect ELISA for the sero-diagnosis of *Brucella* infection in small ruminants [24]. On the other hand, study in Rwanda has shown an excellent agreement between mRBPT and cELISA (K= 0.92) for the serodiagnosis of *Brucella* infection in cattle [32]. The low agreement between mRBPT and cELISA in the present study was attributed to the high seroprevalence of brucellosis detected by the cELISA which might be due to the ability of this ELISA based technique to detect low level of antibody even at early stage of infection [17,33].

In this study, some sera which were positive either by mRBPT or both by mRBPT and CFT were found negative by cELISA. In recent studies conducted in small ruminants elsewhere a considerable number of sera which were positive by mRBPT were found negative by ELISA [34-36]. In this study, a high seroprevalence for *Brucella* infection was detected in those animals with retained placenta which is similar to the results of previous studies elsewher [37-40]. 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].

In order to evaluate the reliability of the mRBPT and/or cELISA for the serodiagnosis of brucellosis, gold standard (bacteria culture) need 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

In this study demonstrated high seropositivity of brucella infection in small ruminants with the history of recent abortion could suggest that brucellosis is main responsible cause of abortion and breeding failure in small ruminants of pastoral communities. Beside this a 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 suggest that cELISA based test is more effective for the serological based survey of brucellosis in small ruminants as compared to mRBPT in the present study area though additional confirmational studies are important. And also this finding warrants appropriate control strategies to reduce its economic impact and risk of zoonotic transmission of the disease in the study area.

Declarations

Competing Interests:

The authors declared that they have no competing interests.

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