

Seroprevalence and risk factors for brucellosis in cattle in selected districts of Jimma zone, Ethiopia

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Abstract A cross-sectional study was carried out in Jimma town and Chora Botor district of Jimma zone from February 2014 to May 2014 to determine seroprevalence and risk factors of brucellosis in cattle. A total of 348 blood samples (174 each from zebu and crossbreed) were collected. The sera were separated and screened by Rose Bengal plate test (RBPT), and positive sera were retested by complement fixation test (CFT) for confirmation. The overall seroprevalence of bovine brucellosis was 1.4 and 0.3 % as tested by RBPT and CFT, respectively. The seroprevalence of bovine brucellosis in indigenous and crossbreed cattle was 1.1 and 0.6 % and 1.7 and 0 % using RBPT and CFT, respectively. Retained fetal membrane was the only risk factor found to be significantly associated with seropositivity of brucellosis in this study ($p=0.019$). The overall seroprevalence of brucellosis was very low. However, due to the zoonotic and economic importance of the disease, prevention and control measures are required to stop further spread of the disease. To effectively implement this, the One Health (OH) is the most constructive approach we recommend.

Keywords Brucellosis · Cattle · Chora Botor · Ethiopia · Jimma · Prevalence

Introduction

Brucellosis is a major zoonosis worldwide and remains a major source of disease in humans and domesticated animals (FAO 2009). Brucellosis is an infectious bacterial disease caused by members of the genus *Brucella*. The disease is usually caused by *Brucella abortus*—in cattle, *Brucella melitensis* or *Brucella ovis*—in small ruminants, *Brucella suis*—in pigs, and *Brucella canis*—in dogs (OIE 2008).

Brucellosis continues to be an important source of morbidity primarily in the Mediterranean region, Arabian Peninsula, India, Mexico, Central America, and South America (Hurtado 2001). The prevalence of bovine brucellosis is variable in cattle. In livestock, it causes abortion, late first calving age, long inter-calving interval, low herd fertility, and comparatively low milk production. Sources of infection include aborted fetuses, fetal membranes, vaginal discharges, and milk from infected cows (Radostits et al. 2000).

In Ethiopia, the varying level of brucellosis seroprevalence has been reported from different parts of the country. For instance, in Borena zone 50 % (Alem and Solomon 2002), in central Ethiopia 11 % (Kebede et al. 2008), in southern and eastern Ethiopia 3.5 % (Megersa et al. 2011), and in northern Ethiopia 7.7 % (Haileselassie et al. 2010). However, in Jimma zone, there is no recent information on the status of brucellosis in cattle and where there is poor hygienic practice. Therefore, this study was aimed to investigate the seroprevalence of brucellosis and factors associated with the occurrence of the disease in cattle. We hope that the output of this study will help in initiating further study and setting priority to the zonal livestock health agency.

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Materials and methods

Study area and population

The study was carried out between February 2014 and May 2014 in Jimma town and Chora Botor district, located in Jimma zone, southwestern Ethiopia. Jimma zone is found about 355 km southwest of Addis Ababa at 7° 41' N and 36° 10' E. The altitude of the zone ranges from 1000 to 3360 m above sea level. This zone is categorized as a humid tropical climate with a heavy annual rainfall that ranges from 1200 to 2000 mm that comes from the long and short rainy seasons. The mean annual minimum and maximum temperature ranges from 7 to 12 °C and from 25 to 30 °C. Jimma zone has an estimated livestock population of 2,016,823 cattle, 942,908 sheep, and 288,411 goats (CSA 2009).

Study design and sampling

To avoid a false-positive reaction resulting from previous *Brucella* vaccination, Jimma Zone Livestock Production and Health Agency Office was asked to check documented vaccination reports and therefore, none of *Brucella* vaccines were provided to animals in this zone.

A cross-sectional study was conducted to determine seroprevalence and risk factors for bovine brucellosis by using RBPT and CFT. A total of 348 cattle both indigenous and crossbreeds, at least 6 months of age and above were selected. From Jimma town, 174 samples and 50 % from Chora Botor district were sampled. The sample size was calculated using the formula recommended for cross-sectional study described by (Martin et al. 1987) which was $n = \lceil Z\alpha \sqrt{2p} (1-p) + Z\beta \sqrt{p_1(1-p_1) + p_2(1-p_2)} \rceil^2 / (p_2 - p_1)^2$, where n = sample size for each group, $Z\alpha$ = Z value for type I error (1.96 at 5 % levels), $Z\beta$ = Z value for type II error (0.84 at 20 % levels), p_1 = estimate of outcome for one group, p_2 = estimate of outcome for second group, and total sample size used was $(2n)$. Study areas and individual animals were selected by purposive and systematic random sampling methods, respectively. Approximately 10 ml of blood samples were collected from the jugular vein of selected cattle by using plain Vacutainer tubes and left at room temperature overnight to clot for serum separation. Sera samples were collected into cry-vials with plastic pipettes and transported in cold ice box to the National Veterinary institute (NVI), Ethiopia, and stored at -20 °C until tested.

Serological tests

All serum samples were screened by Rose Bengal plate test. The sera that tested positive to the RBPT were further subjected to the CFT for confirmation. A standard *B. abortus* antigen

(OIE 2008) for CFT was used to detect the presence of antibody against *B. abortus* in the sera. Antigen used: Veterinary Laboratories Agency, UK. Standardized *B. abortus* Ag Batch 16 and Product code PA0066. Sera with a strong reaction more than 75 % fixation of the complement (3+) at a dilution of 1:5 and with at least 50 % fixation of the complement (2+) at dilutions of 1:10 and 1:20 were classified as positive (+), according to the guidelines of (OIE 2008).

Data analysis

Statistical analyses were performed using SPSS version 20 software. The overall seroprevalence of brucellosis and demographic characteristics of the herd owners was performed using descriptive statistics. A herd level and individual animal seroprevalence were calculated by dividing the number of positive test results by the total number of animals and herds sampled, respectively. Chi-square test was used to determine association between explanatory variables and outcome variables. A 95 % level of confidence and a 0.05 p value were used to consider the variable for significance test.

Ethical consideration

The study was approved by the Research and Ethics Committee and the letters of clearance were obtained from Makerere University. The data were collected after written informed consent was made with all owners' of study animals.

Results

Demographic characteristics

A total of 105 cattle herd's owners interviewed, the majority (92.5 %) were male. The age of respondents ranged from 19 to 86 years with mean age of 43 years, SD ± 12 and SE of 0.6. Their educational qualification ranged from nonformal education (24.1 %) to university graduate (17.8 %). The highest (40.2 %) numbers of participants were in primary level of education. A total of 348 cattle samples (174 from Jimma town and 174 from Chora Botor district) were sampled.

Prevalence of bovine brucellosis

The overall seroprevalence of bovine brucellosis in Jimma zone was found to be 1.4 and 0.3 % based on RBPT as a screening test and CFT as a confirmatory test, respectively. The prevalence of bovine brucellosis in Chora Botor district and Jimma town was 1.1 and 0.6 % and 1.7 and 0 % as tested by RBPT and CFT, respectively. Herd level seroprevalence of brucellosis based on RBPT and CFT was found to be 4.76 and 0.95 %, respectively (Table 1).

Table 1 Individual animal- and herd-level seroprevalence of bovine brucellosis in the study areas

Description	Tests	N	Positive reactors	Prevalence (%)
Individual cattle seroprevalence				
Overall	RBPT	348	5	1.4
	CFT	5	1	0.3
Jimma town	RBPT	174	3	1.7
	CFT	3	0	0
Chora Botor	RBPT	174	2	1.1
	CFT	2	1	0.6
Herd-level seroprevalence				
Jimma town	RBPT	20	3	15
	CFT	3	0	0.0
Chora Botor	RBPT	85	2	2.35
	CFT	2	1	1.18
Overall	RBPT	105	5	4.76
	CFT	5	1	0.95

Risk factors associated with occurrence of brucellosis in cattle

From 348 cattle sampled, 46.6 % were below 2 years of age and the rest (53.4 %) were above 2 years. Seropositivity to *Brucella* was (0.62 and 2.15 %) for cattle below and above 2 years, respectively. However, there was no statistically significant variation ($p>0.05$) in seroprevalence of brucellosis between the age groups.

The seropositivity of brucellosis in female animals was 1.8 % ($n=5$) while no seroreactive male cattle obtained. No statistically significant difference was detected in seroprevalence of the disease between the two sexes ($p>0.05$). The majority (3.1 %) of seropositive cattle to *Brucella* was recorded in medium (11–50 cattle) herd size, and 0.625 and 0.0 %

were found in small (2–10 cattle) and large (>50 cattle) herd sizes, respectively. However, no statistically significant difference was found among the three categories of herd sizes ($p>0.05$).

In the extensive management, 83 herds were freely grazing on the communal pasture land and sharing water together. All herdsmen reported to mix different species of animals together in the barn at the night. In intensive management type, respondents reported to clean the barn at least 2–3 times per day in small herds and 4–5 times in medium and large herds. Mixing of different species of animal was not practiced in this management system. However, there were no statistically significant differences in seroprevalence of brucellosis perceived between the management system ($p>0.05$).

From 14.7 % abortions reported, 0.6, 5.7, and 8.3 % occurred at first trimester, second trimester, and third trimester of pregnancy, respectively. However, seroprevalence of brucellosis was not associated with abortion ($p>0.05$). The presence of retained fetal membrane was significantly associated with seropositivity of brucellosis ($p=0.019$) (Table 2).

Discussion

The overall seroprevalence of brucellosis in cattle was found 1.4 and 0.3 % using RBPT and CFT, respectively. The result of this study is comparable with other findings reported in different parts of Ethiopia using CFT as a confirmatory test: in North Gonder 0.14 % by Tadese (2003), in Tigray region 0.69 % by Tesfaye (2003), in Jimma 0.61 % by Tolosa (2004), in central highlands 0.45 % by Lidia (2008), in Arsi zone 0.05 % by Degefa et al. (2011), and in Dibate and Wembera districts 1 % by Adugna et al. (2013).

The current finding is also in line with the works done in other countries: Rahman et al. (2006) in Bangladesh 2.4 %,

Table 2 Association of the potential risk factors with seropositivity of brucellosis in cattle

Factors	Category	N	Frequency (positive)	Seropositive (%)	Chi-square (p value)
Age	<2 years	162	1	0.62	0.377
	>2 years	186	4	2.15	
Sex	Female	279	5	1.8	0.587
	Male	69	0	0.0	
Herd size	2–10 cattle	160	1	0.625	0.138
	11–50 cattle	129	4	3.1	
	Above 50 cattle	59	0	0.0	
Abortion	Yes	51	1	1.96	1.000
	No	297	4	1.35	
Retained fetal membrane	Yes	159	5	3.14	0.019
	No	189	0	0.0	
Management	Extensive	174	2	1.1	1.000
	Intensive	174	3	1.7	

Unger et al. (2003) in Senegal 0.6 % and 0 % in Guinea, and Nizeyimana et al. (2013) in Uganda 1.2 % using tube agglutination test, CFT, and I-ELISA, respectively. However, the current prevalence report is relatively smaller than previous reports in some parts of Ethiopia: 4.1, 7.7, 3.5, and 1.5 % by Hunduma and Regassa (2009), Haileselassie et al. (2010), Megersa et al. (2011), and Tesfaye et al. (2011), respectively. The fact that our prevalence is lower than the previous studies could be due to the variation in management practices, use of different diagnostic tests, population dynamics, and biological factors.

In this study, the herd level overall seroprevalence of brucellosis was 4.76 and 0.95 % based on RBPT and CFT, respectively. Different studies reported herd level seroprevalence of brucellosis as 2.9 % by Tolosa (2004), 4.9 % by Adugna et al. (2013). However, higher prevalences were also reported by various authors: 42.3 % by Berhe and Belihu and Asfaw (2007), 45.9 % by Kebede et al. (2008), 13.6 % by Jergefa et al. (2009), 13.7 % by Asmare et al. (2010), 12 % by Amenu et al. (2010), 6.5 % by Makita et al. (2011), and 26.1 % by Megersa et al. (2011) using CFT. The result of present study was very low compared to the previous findings reported. The difference could be due to the difference in hygienic practices, herd size, presence of infected animals in the herds, animal movement, and breeding systems practicing.

Age is one of the possible factors associated with the occurrence of brucellosis. In the present study, the seropositivity of brucellosis occurred more in cattle older than 2 years. However, none of animals less than 2 years tested positive with CFT, and the difference was not statistically significant ($p > 0.05$). Brucellosis appears to be more associated with sexual maturity (Roberts 1971). The current finding is in agreement with the result reported by Zubairu et al. (2014). Findings of other studies revealed that older cattle (>2 years) are more likely to be seropositive than the younger ones (Asmare et al. 2010; Hailu et al. 2011; Moti et al. 2012).

The seroprevalence of brucellosis in female cattle was 1.8 and 0.4 % in RBPT and CFT, respectively, but none of the male cattle were positive to *brucella* antibody. The difference in prevalence between two sexes was insignificant ($p > 0.05$). This finding is in agreement with the work reported by (Bayemi et al. 2009; Matope et al. 2011; Zubairu et al. 2014). In fact, that serological data may underestimate *Brucella* infection in males as infected bulls tested might be generally nonreactors (Nicoletti 1980; FAO/WHO 1986).

In the current study, the seroprevalence of brucellosis was 0.625 % in small herd size, 3.1 % in medium herd size, and no seropositive animals in large herd sizes. Seroprevalence of brucellosis was not significantly different among herd sizes ($p > 0.05$). This result is in agreement with the findings of Kebede (2000). However, Tolosa (2004) reported significant variation ($p = 0.001$) between herds having 1 to 5 cattle and with >5 cattle.

In the extensive management, 97.6 % of herds were freely grazing on the communal pasture land, sharing water together

and are kept together (cattle, sheep, and/or goats) in the barn at the night, whereas in intensive management type, keeping different species of animals together was not practiced in. However, there were no statistically significant differences in seroprevalence of brucellosis perceived between the management system ($p > 0.05$). In fact, mixed farming, particularly raising sheep and/or goats along with cattle, was reported to be a risk factor for *Brucella* transmission between different animal species (Omer et al. 2002; Al-Majali et al. 2009; Ghanem et al. 2009).

Retained fetal membrane was associated with seropositivity of brucellosis ($p < 0.05$). The majority (45.7 %) of the cattle owners interviewed reported to have retained fetal membrane problems in their cattle herds. Among the cattle reported to have previous history of retained fetal membrane problem, 3.15 % ($n = 5$) were positive to *brucella* antibodies. This finding is in agreement with the findings elsewhere (Kubuafor et al. 2000; Aulakh et al. 2008).

Conclusion

Retained fetal membrane was the only risk factor associated with the occurrence of brucellosis identified by this study. The overall seroprevalence of brucellosis in the study area was low. However, due to the zoonotic and economic importance of the disease, prevention and control measures are required to stop further spreads of the disease. In addition, further epidemiological studies of brucellosis in cattle, shoats, and humans and identification of the *Brucella* species and biotypes involved is recommended. To effectively implement this, the One Health (OH) is the most constructive approach we recommend.

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Conflict of interest The authors declare that they have no competing interests.

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