Seroepidemiological Investigation of Bovine Brucellosis in the Extensive Cattle Production System of Tigray Region of Ethiopia

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ABSTRACT

A cross-sectional epidemiological study was carried out from September 2004 to March 2005 to determine the seroprevalence and identify risk factors for seropositivity of bovine brucellosis in the extensive cattle production systems of Tigray Region. The study populations comprised indigenous breed cattle in the region, and samples were selected by 2-stage cluster sampling. Serum samples collected from 816 extensively managed cattle herds above 6 months of age were screened for *Brucella* antibodies by the Rose Bengal Plate Test and reactor sera were further tested by the Complement Fixation Test (CFT). Moreover, information was gathered on individual animal and farm-level risk factors and other farm characteristics using a questionnaire. In this study, the overall seroprevalence of *Brucella* antibodies in the extensively managed cattle was 3.19% based on CFT. The overall herd-level prevalence was 42.31% and the within-herd prevalence varies from 0% to 15.15% based on CFT. The results of univariate logistic regression analysis revealed that seropositivity to brucellosis was significantly higher in animals kept under the transhumance management system than animals in the sedentary system ($P < 0.001$). The results also indicated that there was a statistically significant increase in seroprevalence to brucellosis with increasing age ($P < 0.01$) but not parity ($P > 0.05$). Significant increment of seropositivity was also observed as herd size increases from small to medium ($P < 0.05$) and then to large sizes ($P < 0.001$). In addition, a significantly higher seroprevalence was found in animals in the lowland than those in the highland agro-climatic zones. Nevertheless, in the multivariate logistic regression analysis, systemic factor (odds ratio [OR] = 10.6%, 95% confidence interval [CI] = 2.3-49.3, $P < 0.01$) and age (OR = 4.2, 95% CI = 2.3-49.3, $P < 0.01$) were identified as the major risk factors for individual animal seroprevalence. Furthermore, Fisher's Exact Test revealed that seropositivity to brucellosis had statistically significant association with history of previous abortions and stillbirths. The results of this study showed that brucellosis is an endemic and widely distributed disease in Tigray Region.

INTRODUCTION

Brucellosis is an infectious bacterial disease caused by members of the genus *Brucella*.[1-3] Brucellosis has a considerable impact on animal and human health, as well as wide socio-economic impacts, especially in countries in which rural income relies largely on livestock breeding and dairy products.[4]

Brucellosis has a worldwide distribution and it is an important disease among livestock and people in sub-Saharan Africa. In Ethiopia, there is no documented information on how and when brucellosis was introduced and established. However, in the last 2 decades, several serological surveys have showed that bovine brucellosis is an endemic and widespread disease in the country.[5-10]

The objective of this preliminary study was to determine the prevalence of bovine brucellosis and to identify the associated risk factors under extensive husbandry system in the Tigray Region of Ethiopia.

MATERIALS AND METHODS

Study Area and Animals

The study evaluated 1 herd from each of 26 administrative areas (Tabias) found in 12 Districts. The Districts represent 4 major agro-ecological zones that possess more than 95% of the livestock population in the Region. Cattle production in the Region is mainly characterized by extensive type of management system, which includes sedentary and transhumance cattle husbandry systems. Sedentary farming is a feature of the highlands while transhumance prevails in the northwestern and southeastern lowlands. Cattle population more than 6 months old represents the study animals.

Study Design

A cross-sectional epidemiological study was carried out on indigenous cattle using serological tests (Rose Bengal Plate Test [RBPT] and Complement Fixation Test [CFT]) and a questionnaire survey from September 2004 to March 2005 in Tigray Region, Northern Ethiopia. Sample size was determined using a method recommended for 2-stage cluster

sampling.[11] Accordingly, the number of animals required by the method was 718; however, 816 animals (8 males) belonging to 26 herds were sampled to increase the precision.

Sampling Procedures

In this study, 2-stage cluster sampling technique was used where smallest administrative units (Tabias) and herds were the established clusters. First, the study areas were stratified by cattle husbandry system into sedentary (sub-system of the extensive management system where livestock owners and their livestock remain permanently settled in one area without practicing seasonal migration to other areas in search of feed and/or water) and transhumance systems (sub-system of the extensive management system where there is seasonal movement of herds following precise routes and repeated each year), subsequently; the total sample size was proportionally allocated to the size of cattle population in the 2 systems (Table 1). The following procedure was followed during sampling.

- From each cattle husbandry system, random sample of Tabias were selected (10 from the transhumance and 16 from the sedentary system) using random number method.
- In each Tabia, one herd was randomly selected by a lottery method.
- From each herd, at least 30 animals above 6 months of age were sampled. A herd, in this study, was defined as group of animals sharing the same grazing area and/or watering point.

Blood Sample Collection

About 10 mL of blood was collected form the jugular or coccigeal vein of each selected animal using plain vacutainer tubes and allowed to clot overnight at room temperature. The serum samples were separated and transported in iceboxes to Mekelle Veterinary Research and Diagnostic Laboratory, Tigray, and stored at -20°C until testing.

Serological Tests

The RBPT was performed according to the standard procedure.[12] The antigen was

Table 1. Proportion and Number of Animals Sampled From Each Cattle Husbandry System.

Husbandry Systems	Cattle Population	Percentage of Sample	Calculated Sample Size	Actually Sampled
Transhumance	1,004,997	38	273	312
Sedentary	1,663,081	62	445	504
Total	2,668,078	100	718	816

obtained from Institut Pourquer, 3409 Montpellier Cedex 5, France. Sera found positive to RBPT were retested by CFT. The CFT was done at National Veterinary Institute, Debre Zeit, Ethiopia according to the protocols recommended.[3] Antigen, control sera, and complement were obtained from the BgVV, Berlin, Germany.

Questionnaire Survey

A structured questionnaire was prepared and administered in person to 26 farm owners in a group interview. The questionnaire was pre-tested in the field and adjusted as required. Purposive sampling was used to select the key informants. Data on breed, sex, age, herd size, abortion, presence of swollen joints, animal management, and agro climate of the area were collected. Moreover, the full history herd level risk factors like farm attributes, grazing, watering points, and disease conditions were recorded.

Data Analysis

Data was stored in Microsoft (MS) Excel Spread Sheet program and analysis was done using standard software programs.[13,14] The total prevalence was calculated by dividing the number of RBPT- and CFT-positive animals by the total number of animals tested. Herd prevalence was calculated by dividing the number of herds with at least one reactor in RBPT and CFT by the number of all herds tested. The within-herd prevalence was calculated by dividing the number of RBPT and CFT reactors within a herd by the number of serum samples tested in the herd.[11] Odds ratio (OR) was utilized to measure the degree of association between risk factors such as age, herd size, parity number, management factor, agro-ecology, and farm or herd-level risk factors with brucellosis

seroprevalence. All risk factors that had P value <0.20 in the univariate logistic regression analysis were subjected to multivariate logistic regression analysis.

RESULTS

Individual Animal Seroprevalence

Of the 816 sera examined, 27 (3.3%) were seropositive to RBPT out of which 26 (3.19%) reacted positively to CFT with a titer >1:20. The entire seropositive animals were female animals. Among the 12 Districts included in the study, *Brucella* antibodies were detected in 6 districts.

A univariate logistic regression showed statistically significant effect of systemic factor ($P < 0.001$), age ($P < 0.001$), herd size ($P < 0.001$) and agro-climate ($P < 0.001$) on the individual animal seroprevalence. However, significant difference in seropositivity was not observed among the 3 parity groups ($P > 0.05$) (Table 2). In the extensive cattle production system, cattle in the transhumance management sub-system (7.37%) had a significantly higher seroprevalence as compared to cattle in the sedentary system (0.60%). The OR indicated that animals in the transhumance management sub-system were 13 times more likely to develop brucellosis than animals in the sedentary system. Regarding the effect of age, animals above 5 years of age (n = 425) had significantly higher prevalence (5.18%) than those 0.6-5 years of age (n = 391) (1.02%) ($P < 0.001$). The OR indicated that older animals were about 5 times more likely to develop brucellosis than younger animals. There was also a trend of increment in individual animal seroprevalence with herd size. The risk of seropositivity was 8.5 and 4.3 times higher in the large and medium size herds, respec-

Table 2. The Effect of Risk Factors on Individual Animal Seropositivity to Brucellosis in the Extensive Management System.

Variables	N	Number (%) Positives	Univariate Analysis			Multivariate Analysis		
			OR	P Value	95% CI	OR	P Value	95% CI
Age (year)								
0.6-5	391	4 (1.02)						
>5	425	22 (5.18)	5.3	0.002	1.8-15.5	4.2	0.009	2.3-49.3
Herd size								
1-100	438	4 (0.91)						
101-200	157	6 (3.97)	4.3	0.025	1.2-15.5	1.5	0.607	0.3-6.3
>200	221	16 (7.80)	8.5	0.000	2.8-25.6	1.2	0.835	0.3-4.8
Systemic factor								
Sedentary	504	3 (0.60)						
Transhumance	312	23 (7.37)	13.3	0.000	3.9-44.8	10.6	0.003	2.3-49.3
Agro-climate								
Highland (>1500 masl)	438	3 (0.68)						
Lowland (<1500 masl)	378	23 (6.08)	9.4	0.000	2.8-31.5			
Parity number								
No parturition	144	1 (0.69)						
Single parturition	172	7 (4.07)	6.1	0.094	0.7-49.9			
Multiple parturition	500	18 (3.6)	5.3	0.104	0.7-40.3			
>1 parturition	672	25 (3.72)	5.5	0.095	0.7-41.1			

N = number of observations; OR = odds ratio; masl = meters above sea level.

tively, in comparison to the small herd size. Furthermore, higher seroprevalence was recorded in the lowlands (6.08%) than in the highland agro-climatic zones (0.68%). Odds ratio indicated that animals in the lowlands were 9.4 times more likely to be seropositive to brucellosis than those in the highland agro-climatic zone (Table 2).

Risk factors that showed significant effect in the univariate logistic regression were fitted in a model for multivariate logistic regression except agro-climatic factor, which was regarded to be confounded with systemic effects (Table 2). The result revealed that systemic effect was the major risk factor that was found to be significantly associated with individual animal seroprevalence to brucellosis ($P < 0.001$). The OR value showed that animals in the transhumance system were about 11 times more at risk to brucellosis than those in the sedentary

system. In the multivariate analysis, age also exerted a significant effect on individual animal seroprevalence to brucellosis ($P < 0.01$). Older animals were approximately 4 times more likely to be affected by brucellosis than younger ones. However, the effect of herd size on brucellosis seropositivity was not important in the multivariate logistic regression analysis.

Results of Fisher's Exact Test showed that history of previous abortions ($P < 0.001$) and stillbirths ($P < 0.05$) in the individual animal were significantly associated with brucellosis seropositivity.

Herd-Level Seroprevalence

Out of 26 herds studied, 11 (42.31%) were positive using CFT. The within-herd prevalence varied between none to 15.15% (5/33) based on CFT. Herd-level seroprevalence in the transhumance management

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sub-system (80%) was significantly higher than prevalence in the sedentary system ($P < 0.01$). The values of OR indicated that herds in the transhumance sub-system were about 17 times more likely to be seropositive than herds in the sedentary system. However, herd size was not associated with herd-level seropositivity to brucellosis ($P > 0.05$) (Table 3).

Table 3. Herd-Level Risk Factors to Brucellosis Seropositivity in the Extensive Management System.

Variables	N	Number of Herds (%) Positives	Univariate Analysis		
			OR	*P* Value	95% CI
Systemic factor					
Sedentary	16	3 (18.75)			
Transhumance	10	8 (80)	17.3	0.005	2.3-127.3
Herd size factor					
1-100	14	4 (28.57)			
101-200	6	3 (50.00)	2.5	0.368	0.3-18.0
>200	6	4 (66.67)	5	0.125	0.6-39.0

N = number of observations; OR = odds ratio.

DISCUSSION

The prevalence of brucellosis in cattle in the extensive management system in this study agrees with reports from other areas of the country and countries with similar cattle husbandry systems.[8,9,15-18] In general, in the extensive cattle management system in Ethiopia, prevalence figures reported varies with ranges from 0.77% to 8.2%.[9,19]

In the extensive cattle production system, significantly higher seroprevalence of *Brucella* antibodies was detected among cattle in the transhumance management system. This could be attributed mainly to the large herd size of cattle in this system and the mobility of herds. The higher prevalence observed in large herd size is similar to observations made by several researchers.[7-10,20-22] According to one finding, large herd size enhances the exposure potential, especially following abortions through increased contact and common feeding and watering points promoting transmission of *Brucella* organisms.[21] Moreover, it was explained that mobile herds have greater opportunity to come into contact with other potentially infected herds during their movement into the different areas.[18] Furthermore, migration increases the chance of coming into contact with geographically limited or seasonally abundant diseases and also increases the opportunity for interactions of domestic and wild animals.[23]

On the other hand, the finding of low brucellosis prevalence in the sedentary husbandry system is consistent with several previous reports.[21,22,24,25] It was observed that cattle herds in this system are small in size and sedentary with little possibility of contact with other infected herds, thus, there was less risk of acquiring the disease. In general, it was described that the incidence of brucellosis is relatively high in pastoral production systems, and decreased as herd size and size of land holding decreased.[25] Similarly, in other study, it was stated that herds of bigger size were found to be more frequently infected than smaller herds.[21]

In the extensive management system, the significantly higher seroprevalence of *Brucella* antibodies in older than in younger animals is in accordance with several reports. According to some workers, cattle become increasingly susceptible as they approach breeding age.[1,2] Our finding is also consistent with the findings of several researchers who reported significantly higher proportion of positive reactors in older animals.[7,8,21,26]

The absence of male reactor animals in this study could probably be due to the smaller number of male (n = 8) animals studied as compared to females (n = 808). It was also reported that serological response of male animals to *Brucella* infection is

limited. It was indicated that the testes of infected male animals were usually observed to be non-reactors or showed low antibody titers.[27] Similarly, one research finding showed that male cattle are more resistant than females.[28] However, the apparently high seroprevalence figure in female animals compared to males in this study agrees with other works.[7,8,9,26]

The significantly higher seropositivity result in the large herd size categories is consistent with several authors. Large herd size was reported as one of the major risk factor for occurrence and higher prevalence of bovine brucellosis.[7-9,21,22,25] In contrast, a significantly higher seropositivity to brucellosis in animals in the hotter lowland agroclimate, which is unsuitable for survival of *Brucella* organisms,[1] is unexpected. However, this could probably show that the effect of agro-climate may have been confounded with management system.

A history of previous abortions or stillbirths was significantly associated with brucellosis seropositivity. This could be explained by the fact that abortions or stillbirths and retained placenta are typical outcomes of brucellosis.[1,29] Similar results were also obtained by other investigators.[25,26,30]

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