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Sero-Prevalence of Bovine and Human Brucellosis in Adami Tulu, Central Ethiopia

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Abstract: A cross-sectional study was carried out to investigate the prevalence and risk factors of bovine and human brucellosis from November 2010 to March 2011 in and around Adami Tulu, Ethiopia. A total of 690 bovine and 93 human sera samples were randomly collected from selected 11 kebeles of the study area. The sera samples were screened by Rose Bengal Plate Test (RBPT) and all positive samples by the RBPT were subjected to Complement Fixation Test (CFT) for confirmation. Accordingly, 31(4.5%) bovine and 2(2.15%) human sera were found to be positive using RBPT. When retested with CFT, 30(4.3%) bovine and 2(2.15%) human sera were confirmed to be positive. There was statistically significant ($P<0.05$) variation in prevalence of the disease between sex, age and management system of animals. However, no statistically significant ($P>0.05$) variation was observed between sex and age of humans. Even though, the observed overall individual sero-prevalence of bovine brucellosis in the study area was not high, it deserves due attention due to its public health significance and thus physicians should also consider brucellosis as differential diagnosis of non-specific febrile diseases.

Key words: Bovine · Brucellosis · Humans · Seroprevalence · Adami Tulu · Ethiopia

INTRODUCTION

Brucellosis is an infectious contagious bacterial disease usually caused by *Brucella abortus* in cattle. *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs. The disease is an important zoonosis that exists worldwide and is more or less endemic in most African countries and still exists in some southern European countries [1, 2]. It causes significant reproductive losses in animals. Abortions, placentitis, stillbirth and birth of weak offspring in female and epididymitis and orchitis in male are the most common consequences [3]. Brucellosis in humans also known as Undulant fever' or Malta fever' is primarily a disease of those whose occupations bring them into direct or indirect contact with domestic animals. It causes orchitis/epididymitis in men and spontaneous abortion in pregnant woman [20].

In Africa, Brucellosis is considered to be one of the most serious health problems facing the veterinary professionals [4]. It creates a serious economic problem in both intensive and extensive livestock production system in the topics and a threat to public health. Brucellosis is regarded as a true zoonosis in that almost all human cases are acquired from animals through either direct contact with infected animals or consumption of contaminated milk and dairy products [5].

Brucellosis is endemic in Ethiopia since 1970. Since then, few fragmented studies have demonstrated the presence of antibodies against Brucella in animals and humans in different parts of the country. The prevalence of brucellosis has been found to range from 0.2% to 38% in cattle [6, 7]. Few serological studies on human brucellosis reported prevalence as high as 34.9% [8, 9]. Brucellosis is of particular public health importance in societies that live closely together with their livestock. as it can easily be transmitted via raw milk, the predominant consumption pattern for this commodity in Ethiopia. Any strategy for the control of brucellosis should begin by assessing its importance through establishing a different epidemiological contexts like determination of the frequency of the disease and assessment of the

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predisposing factors within a given area The objectives of this study were therefore, to determine the sero-prevalence of bovine and human brucellosis and to identify risk factors that are likely to influence the occurrence of the diseasein and around Adami Tulu.

MATERIALS AND METHODS

Description of Study Area: The study was conducted in and around Adami Tulu. Adami Tulu is located at 7.58°N latitude and 38.43°E longitudes in the central part of Oromia situated in mid rift valley, 167kms south of Addis Ababa. Its altitude ranges 1500-2000 meter above sea level. It has 1403.3km^2 of land inhabited by about 14, 1745 people of which more than 85% are living in the rural areas. Agro-ecologically, the area is categorized under the semi arid with mean minimum and means maximum temperature of 12.7°C and 27.2°C with relative humidity of 60%.

Study Population: The study subjects were cattle and humans. The bovine species were under extensive management system and were kept with other species such as sheep and goats. Human patients with non-specific febrile condition that were presented to Adami Tulu health center of the study area as well as those apparently healthy individuals that were thought to have close contact with the animal (at higher risk group) were sampled. The study was conducted on 93 humans and 690 bovine species of exotic and local breeds of different age, gender and body condition score. All individuals greater than 10 years old and both sex were considered in case of human being.

Study Design: A cross-sectional study was the design used in the study. Risk factors like age, sex and management system of animal were assessed from November 2010 to March 2011. Convenient sampling was employed to select kebeles (smallest administrative unit). Eleven kebeles were selected based on their accessibility and population of the study animals. This is followed by systematic random sampling of households keeping cattle in and around Adami Tulu. On average, three animals were selected using systematic random sampling from the selected households.

Sample Size Determination: The sample size for the bovine sero-prevalence study was determined by assuming 10% prevalence based on previous study in and around Adami Tulu district [10]. The sample size was calculated using the formula given by [11] with 95% confidence interval and desired precision level of 0.05. Hence, p_{exp} = 10%, d=5%, substituting these figures we had n=138. However, to increase the precision, the sample size was increased by 5 fold and a total of 690 cattle were sampled in this study.

The sample size for the humans was determined using the previous prevalence reported, 5.3% [13]. Accordingly, the minimum number of humans should be sampled was 78, however to increase the precision an additional 15 individuals were included in the study.

Ethical Clearance: The study was approved by the Research Ethics Committee and the letter of clearance was obtained from Jimma University and East Shewa Zone administration office. The sample was taken after written informed consent is made with all study participants. All the rights of privacy and confidentiality of participants are protected.

Serum Collection: Approximately, 10 ml of blood sample was drawn from jugular vein of each selected bovine and 5 ml from radial vein in case of humans. During the sampling, animals were restrained, the area was first disinfected by using 70% alcohol before puncturing and blood was collected in vacutainer tube. Each tube was identified using numbers and was taken to Adami Tulu agricultural research center. The blood was allowed to clot by placing it overnight at room temperature and the next day sera was separated. The sera were stored at -20°C until analyses. All sera samples collected were screened by Rose Bengal Plate Test (RBPT). In case of humans, after the blood sample was taken from radial vein, it was allowed to clot only for one hour and then the serum was separated. After the serum was separated, it was directly subjected to RBPT [3].

Serological Tests

Rose Bengal Plate Test (RBPT): For the RBPT, the procedure described [3] was followed. The sera were removed from the refrigerator and left at room temperature for 30 minutes before the test was performed. Briefly, serum of 30 µl was mixed with an equal volume of antigen on enamel plate by using applicator stick. The mixture was shaked gently for four minutes at ambient temperature and then observed for agglutination. After four minutes of rocking, any visible agglutination was considered a positive result. For interpretation of the results, both positive and negative control sera were employed.

Complement Fixation Test (CFT): All sera which tested positive to the RBPT were further tested using CFT for confirmation. The CFT test was conducted at the National Veterinary Institute, Debre Zeit. A standard *Brucella antigen* for CFT was employed to detect the presence of antibodies against *Brucella* in the sera. 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 Management and Analysis: Data collected from field and laboratory results were stored in a Microsoft excel spreadsheet and analyzed using SPSS version 16 software program. The prevalence was calculated as the number of animals/humans tested positive by the RBPT/CFT divided by the total number of animals/humans tested. The association between the risk factors and the outcome variables was assessed using chi-square (x^2) test. For all analysis, a P-value less than 0.05 were considered significant.

RESULT

Prevalence and Potential Risk Factors: From total of 690 bovine sera samples tested, 31(4.5%) were positive for brucellosis by RBPT. The 31 RBPT positive sera were retested using CFT and 30(4.3%) were positive. From a total of 93 human sera sample tested, 2(2.15%) were positive for brucellosis both by RBPT and CFT. Both positive samples were taken from human female (Table 1).

The prevalence of the disease in male and female animals was 2.6% and 5.8% respectively. There was statistically significant (P=0.041) difference in sero-positivity between the two sexes. There was also significant difference in sero-positivity between the age groups (P=0.007). The prevalence of the disease in semi-intensive management system was 1.8%, while that of extensive system was 6.1%. Statistical analysis revealed that there was significant difference in sero-positivity between the two systems (P<0.05) (Table 1).

In human cases, the prevalence of the disease in male and female were found to be 0% and 2.15% respectively. Statistical analysis revealed that there is no significant difference in seropositivity between sexes and age group (P>0.05) (Table 2).

DISCUSSION

The overall sero-prevalence of bovine brucellosis in Adami Tulu district was 4.5% by the RBPT and 4.3% by the CFT. This result is lower than the reports of [12, 10] (10%) and [13] (14.96%) in Addis Ababa and northwestern parts of Ethiopia respectively. Higher prevalence (24.5%) was also reported in the northern Sudan [14] and 6.9% in Tanzania [15]). The prevalence recorded in this study was lower than the finding of [10] who reported a prevalence of 10%. This difference might be due to absence of confirmatory test (CFT) in their serological test and the prevalence was reported only with the result of RBPT which lacks specificity.

In this study, statistically significant difference was observed in the prevalence of bovine brucellosis between the two sexes (P=0.041). Similarly, a higher sero-prevalence of bovine brucellosis in females was also reported in different studies [13, 16-19] in Ethiopia and Eretria. The reason could be due to the fact that males are kept for relatively shorter time in breeding herd than females and thus the chance of exposure was lower for males. Other authors [18] have reported that male animals are less susceptible to *Brucella* infection due to low level of erythritol.

The overall sero-prevalence of brucellosis in humans was 2.2% which is lower than the reports of [8] (34.9%) in Boran and (29.4%) in Hamar, Ethiopia. These authors reported that living in close proximity of livestock, keeping and attending to livestock and consumption of raw milk and fresh cheese as risk factors for having brucellosis. The lower prevalence in our study could be due the fact that the number of livestock, level of contact with animals and frequency of consumption of low dairy products is low as compared to the mentioned study subjects which are purely pastoralists. There was no statistically significant difference in the prevalence of human brucellosis between both sexes (P=0.35). This result showed that the prevalence of human brucellosis is a bit higher in females than male, this insignificant difference might be due to a relatively larger number of females included in the study as compared to males.

In conclusion, the observed overall individual seroprevalence of bovine brucellosis in and around Adami Tulu was not high. However, it deserves due attention because of the public health significance of the disease. Thus, epidemiological studies like isolation and characterization of the Brucella species should be carried

Table 1: Prevalence of bovine *Brucella* antibodies with respect to different risk factors (n=690)

Variables	No tested	RBPT positive (%)	CFT positive (%)	X^2	P-value
Sex				4.16	0.041
Male	309	8(2.6)	8(2.6)		
Female	381	23(6)	22(5.8)		
Age				9.788	0.007
< 3 years	149	0(0)	0 (0)		
3-6 years	244	11(4.5)	11(4.5)		
>6	297	24(6.7)	19(6.4)		
Management system				7.356	0.007
Semi-intensive	279	5(1.8)	5(1.8)		
Extensive	411	26(6.3)	25 (6.1)		

Table 2: Prevalence of Human Brucellosis in the area

Factors	Group	No tested	Prevalence(no)	X^2	P-value
Age	10-25 years	35	0	1.2	0.54
	26-50	58	3.4% (2)		
Sex	Male	28	0	0.9	0.35
	Female	65	3.1% (2)		

out in l to investigate the link between bovine and human brucellosis in the present study area to formulate strategic control measures to reduce associated reproductive wastage and the public health risks.

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