Libyan Agriculture Research Center Journal International 3 (2): 47-52, 2012
ISSN 2219-4304
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DOI: 10.5829/idosi.larcji.2012.3.2.56358

Assessment of Risk Factors and Seroprevalence of Small Ruminant Brucellosis in Adamitulu-Jido-Kombolcha District, Oromia Regional State, Ethiopia

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Abstract: A cross-sectional seroprevalence study was conducted at Adamitulu-Jido-Kombolcha district, Oromia Regional State, Ethiopia from October 2008 to March 2009 to determine the seroprevalence of brucellosis as well as to investigate the potential risk factors associated with seroprevalence of brucellosis in small ruminants. A total of 2,340 sera were collected from small ruminants (775 from sheep and 1,565 from goats). All the serum samples were screened using modified Rose Bengal plate test (mRBPT) and all the positive sera were further tested using CFT. Out of 2,340 blood samples, 107 (4.6%) sera were found positive by mRBPT, of which only 90 (3.8%) sera were found positive using CFT. The overall individual and herd level seroprevalence were 3.8% and 28.1%, respectively. Individual seroprevalence was 4.8% in goats and 1.9% in sheep, whereas, 4.2% in females and 2.2% in males. Statistically significant difference ($p < 0.05$) was observed among different age groups, in which the seroprevalence was higher (6.5%) in young age groups and lower in adults (3.1%). Both univariable and multivariable logistic regression analyses showed the significant association between species with the seropositivity of small ruminant brucellosis.

Key words: mRBPT · CFT · Brucellosis · Small Ruminants · Seroprevalence

INTRODUCTION

Livestock plays a crucial role in the livelihoods of the majority of Africans. It accounts for 16% of the national and 27-30% of the agricultural GDPs and 13% of the country's export earning. The greatest share of this income is from small ruminants [1].

Small ruminants play a big role in supporting the livelihood system of the poorest men and women livestock keepers, especially in the marginalized areas. This sub-sector receives only very small attention in the poor countries and diseases of small ruminants affect the incomes of smallholder farmers in sub-Saharan Africa by reducing productivity or through loss of the animals [2]. Particularly in Ethiopia, they play major economic roles in the low land pastoral areas of the country, where they serve as sources of milk and meat. Milk from sheep and goats is consumed raw.

Good quality data on the impacts of different diseases and their control on animals and humans populations in Sub-Saharan Africa are usually lacking [3-4]. Only few studies on small ruminant brucellosis have revealed the occurrence of small ruminant brucellosis in Ethiopia [5-9]. Therefore, the objectives of this study were to determine the seroprevalence of brucellosis and to investigate the potential risk factors associations with the brucellosis in small ruminants.

MATERIALS AND METHODS

Study Design and Sampling Procedures: The study was conducted in selected peasant associations (PA) in Adamitulu-Jido- Kombolcha district from October 2008 to March 2009. Cross-sectional study design was conducted in the selected PAs of Adamitulu-Jido-Kombolcha district to determine the seroprevalence of brucellosis in small ruminants. Sheep and goats above 6 months of age were included in the study population. The information on potential risk factors of small ruminant brucellosis was gathered using a pre-tested questionnaire. Risk factors such as species, sex, age, herd size, pregnancy status, source of replacement stock, grazing and breeding

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systems together with the reproductive disorders such as history of abortion, stillbirths and other potential risk factors were recorded. Age of animals was categorized into 0.6-1year (young), 1- < 4 years (adult) and ≥ 4 years (old). Herd size was categorized into < 10, 10-20 and > 20 heads of small ruminants.

Proportional sampling procedure was employed to include 160 households/flocks from the selected 10 PAs of the district to obtain a total samples of 2,340 small ruminants (775 sheep and 1,565 goats) as described by Thrusfield [10]. This sampling approach was based on accessibility of flocks and other logistics.

Sampling and Serological Test: Whole blood was collected from the jugular vein of each animal randomly selected from the herd and the blood was stored overnight at +4°C (when conditions allowed) or at room temperature until the serum was separated (3-4 hours on average). In the laboratory, the sera were stored at -20°C until tested for the presence of *Brucella* antibodies.

Modified Rose Bengal Plate Test (mRBPT): All serum samples (N = 2,340) were screened using mRBPT according to the procedures described by OIE [11]. The antigen used was Rose Bengal antigen, which constitutes a suspension of *Brucella abortus* (obtained from the Institute Pourquier, Montpellier, France), inactivated by heat and 0.5% phenol, adjusted to pH 3.65 and colored with Rose Bengal. Briefly, 75µl of serum was mixed with 25µl of antigen on a glass plate and shacked. After 4 minutes of gentle shacking, any visible agglutination was considered as positive [11].

Complement Fixation Test (CFT): All sera tested positive by mRBPT were further tested using CFT for confirmation. Since there was no *Brucella melitensis* antigen, Standard *Brucella abortus* antigen S99 for CFT (from the Veterinary Laboratory Agency, Addlestone, United Kingdom) and 2% sheep red blood cells (SRBCs) (National Veterinary Institute, Ethiopia), were obtained to detect the presence of anti-brucella antibodies in the sera. The control sera and complement were obtained from the Federal Institute for Health Protection for Consumers and Veterinary Medicine, Berlin, Germany. All the reagents used in CFT were titrated. The preparation of reagents and CFT procedures were performed according to the protocols of the Federal Institute for Health Protection for Consumers and Veterinary Medicine Service Laboratory, Berlin, Germany [12].

Interpretation of results were as followed: sera were classified as positive sera with a strong reaction of approximately 100% fixation of the complement (4+) at a dilution of 1:50; about 75% fixation of the complement (3+) at a dilution of 1:10; about 50% fixation of the complement (2+) at a dilution of 1:20 and about 25% fixation of the complement (1+) at a dilution of 1:40 [10, 13].

Questionnaire Survey: In all the study households, 160 individuals were asked about awareness of brucellosis, sources of replacement stock, presence of regular veterinary services, grazing and breeding systems and safe disposal of fetal membrane to find out the presence of association between risk factors and prevalence of small ruminant brucellosis.

Data Management and Analysis: Data obtained from both serological tests and questionnaire survey were stored in Microsoft excel spreadsheet program. Descriptive statistical analysis for seroprevalence was carried out using SPSS version 15.0 for windows. Chi-square test was used to determine presence of association of different risk factors with seropositivity and, 95% confidence interval (CI) at 5% cut-off value were set for significance. Univariable and multivariable logistic regression were also used to analyze associations of various risk factors with the seroprevalence of the disease. Sheep and goats tested positive to both mRBT and CFT tests serially were said to be positive. Clusters (flocks) having at least one seropositive animal were considered as positive. The degree of association between potential risk factors and seroprevalence were computed using Odds ratio.

RESULTS

The overall herd level seroprevalence of *Brucella* antibodies in the study area was 28.1%. Statistically significant ($p < 0.05$) variations were observed among herds with the highest seroprevalence recorded in K/Garbi (75%) and no positive cases were recorded in Bochesa as shown in Figure 1.

A total of 2,340 sheep and goats sera were tested with mRBPT for the presence of antibodies against *Brucella* infection from which 107 (4.6%) sera were positive. These 107 positive sera were tested further using CFT for confirmation, of these 90 (3.8%) were found positive. There were statistically significant ($p < 0.05$) associations among PAs with the highest seroprevalence of 12.1% in A/Shisho and no positive cases were recorded in Bochessa.

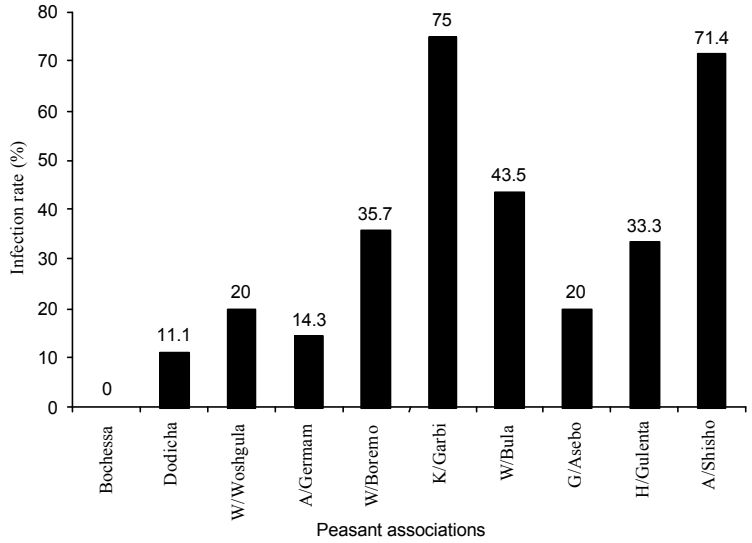

Fig. 1: Herd level seroprevaence of *Brucella* antibody by the serial tests in the different peasant associations of the study area

Table 1: Influence of some risk factors on sero-prevalence of small ruminant brucellosis at individual animal level

				Univariate			Multivariate		
Risk factor	No of animals	No. of positive	Prevalence %	OR	CI (95%)	P-value	OR	CI (95%)	P-value
Species									
Goats	775	15	1.9	2.55	1.45-4.47	0.001	3.51	1.67-7.38	0.001
*Sheep	1565	75	4.8						
Sex									
Female	1891	80	4.2	1.94	0.99-3.77	0.051			
*Male	449	10	2.2						
Age									
0.6-1yr	277	18	6.5	1.73	0.24-0.83	0.057	1.50	0.68-3.34	0.308
1-<4yrs	946	29	3.1	0.79	0.32-1.02	0.330	0.70	0.37-1.31	0.262
*≥4yrs	1117	43	3.8						
Herd size									
<10	36	1	2.8	0.77	0.11-11.22	0.860			
10-20	96	3	3.1	0.87	0.08-21.68	0.910			
*>20	28	1	3.6						

* Reference category

Table 2: Association between reproductive status and/or disorders and seroprevalence of brucellosis

				Univariate			Multivariate		
Factors	No of animals	No. of positive	Prevalence (%)	OR	CI (95%)	P-value	OR	CI (95%)	P-value
Pregnancy status									
Non-prignant	1502	70	4.7	1.850	0.95-3.63	0.072	2.05	0.90-4.68	0.088
*Pregnant	389	10	2.6						
Abortion									
Absent	1837	47	2.6	0.017	0.01-0.03	0.001	0.02	0.01-0.35	0.088
*Present	54	33	61.1						
Still birth									
Absent	1853	58	3.1	0.023	0.01-0.05	0.001	0.02	0.01-0.05	0.001
*Present	38	22	57.9						

* Reference category

Table 3: Association of management risk factors on sero-positivity of small ruminant brucellosis

Management variables		No. of Respondents and (%)	No. of positive cases and (%)	χ^2 -value	P-value
Awareness about brucellosis					
	Absent	150 (93.75)	40 (26.7)	2.525	0.112
	Present	10 (6.25)	5 (50.0)		
Source of stock replacement					
	Village	11 (6.9)	6 (54.5)	4.079	0.130
	Own stock	130 (81.3)	34 (26.2)		
	Market	19 (11.88)	5 (26.3)		
Presence of regular veterinary service					
	Absent	150 (93.75)	40 (26.7)	2.525	0.112
	Present	10 (6.25)	5 (50.0)		
Grazing system					
	Communal	160 (100.0)	45 (28.1)		
	Individual	0	0		
Breeding system					
	Natural	160 (100.0)	45 (28.1)		
	Artificial	0	0		
Disposal of fetal membrane					
	Unsafe	144 (90.0)	40 (27.8)	0.086	0.769
	Safe	16 (10.0)	5 (31.3)		

There was statistically significant ($p < 0.05$) difference between species of small ruminants where higher seroprevalence of the disease was observed in goats (4.8%) than in sheep (1.9%). The univariable logistic regrassion analysis showed that the odds of the disease in goats was 2.55 times the odds of the disease for the reference category (sheep) as shown in Table 1.

There was significant difference in susceptibility to brucellosis between sex groups. The seroprevalence was (4.2%) and (2.2%) in female and male, respectively. Statistically significant ($p < 0.05$) difference was observed between sexes, where the odds of the disease in females was 1.94 times the odds of the disease for the reference category (male) (Table 1).

A high variation in seroprevalence of *Brucella* antibody among different age groups was observed. The seroprevalence in age groups were (6.5%), (3.1%) and (3.8%), in young, adult and old age groups, respectively. Statistically significant ($p < 0.05$) difference was found among different age groups, in which the odds of the disease in younger group was 1.73 times the odds of the disease in old age group (Table 1).

The seroprevalence at herd level was 2.8, 3.1 and 3.6% with the herd size categories of < 10, 10-20 and >20, respectively. There was no statistically significant ($p > 0.05$) variation among different herd groups. The univariable logistic regression analyais model was not statistically significant ($p > 0.98$).

There was no statistically significant association with pregnancy status. The seroprevalence was (57.9%) in female small ruminants with still births and 47 (3.1%) in those without still births. There was statistically significant ($p <0.05$) variation among small ruminants with abortion and still births using multivariable logistic regression analysis as shown in Table 2.

All households used natural breeding and communal pasture. There were no significant ($p > 0.05$) variations among households in relation to awareness about brucellosis, sources of replacement stock, presence of veterinary service and safe disposal of fetal membrane as shown in Table 3.

DISCUSSION

The herd level seroprevalence of the serial tests was 28.1%. This finding is higher than flock seroprevalence of 22.3% reported in eastern Amhara region, Ethiopia [14] and 12.1% in Moroco [15]. Higher herd prevalences of 43% in Uganda [16], 37% in West Asia and, 49% in North Africa [15] were reported. This difference in herd seroprevalence might be attributable to poor animal production and management systems, movement of animals in search of pasture and water, trade within and among countries, mixing of animals at market places and watering points.

This study demonstrated that the overall individual animal level seroprevalence of brucellosis in small ruminant was 3.8% (4.8% in goats and 1.9% in sheep). This is comparable to the seroprevalence of 2.8% (3.2% in goats and 1.6% in sheep) reported in Southern Ethiopia [8], 3.4% in Afar (Ethiopia) [7], 3.9% in Borena [17], 1.6% in sheep and 4.1% in goats in Morocco [15] and 3.8% in goats and 1.4% in sheep in Eritrea [18].

The present investigation recorded a higher seroprevalence of brucellosis in goats (4.8%) than in sheep (1.9%). There was significant difference between these species. Similar results were recorded; 3.2% in goats and 1.6% in sheep in Southern Ethiopia [8], 3.8% in goats and 1.4% in sheep in Eritrea [18] and 4.1% in goats and 1.6% in sheep in East Morocco [15]. Higher seroprevalences of 16.7% in goats and 14.2% in sheep in Afar [7] and 5.8% in goats and 3.2% in sheep in Afar, Ethiopia [9].

Most beerds of goats are fully susceptible but the susceptibility of sheep breeds differes widely [19]. This difference might be due to the differences in flock sizes and proportions of goats and sheep in the herd (1,565 goats and 775 sheep in the present study). In addition, sheep are more resistant than goats and they do not shade the bacteria for long time. Flocks with high numbers of sheep would have low prevalence [20]. Excretion from the vagina in goats is more copious and prolonged than sheep and lasts for at least 2-3 months. In addition, goats are considered as the principal host of *Brucella melitensis*, whereas, sheep are not significantly infected even when kept in close contact with goats [13]. In goats, infection can vary from a short time occurrence to persistent occurrence for years. In sheep, the course of infection depends upon the dose of infection and after recovery they are resistant to re-infection [19].

Statistical significant difference ($p < 0.05$) was observed between sex groups to seropositivity. Higher prevalence was recorded in females than in males. Females were two times more affected than males in this study. This finding is in agreement to Mengistu [8] who reported brucellosis in 3.2 and 1.2% of females and males, respectively, in Southern Ethiopia. The high prevalence of brucellosis in females might be due to high concentration of erythritol, which is scarcely produced in males reproductive organs [19, 21].

In this study, *Brucella* seropositivity was stronglly associated with the presence of abortion and still births. Statistically significant difference ($p < 0.05$) in seroprevalence was observed between the status of shoats in which the seropositivity was (61.1%) in aborted and (2.6%) in non-aborted ones. The odds of the disease indicated that the aborted shoats were almost 0.02 times more likely to be seropositive than non-aborted. The present study demonstrated that the sera of sheep and goats obtained from these PAs showed a higher seroprevalence of *Brucella* antibodies in with still births. Because in both conditions *Brucella* organisms can be dissiminated in the environment by dogs, carnivorous birds and other contaminated materials, by which the feeding and watering places could be contaminated indirectly for the transmission of the disease.

Therefore, in conclusion, the existing traditional husbandry practices of handling multiple species support the spread of brucellosis in the area. This study indicated that brucellosis is becoming one of the impediment to the exploitation of the huge small ruminant population in the country and impairs the export of live sheep and goats, as the importing coutries strictly require *Brucella* free animals. Therefore, public awareness about the economic and public health importance as well as well structured and financed veterinary services for the control of small ruminant brucellosis in the areas are reqired.

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