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Sero-prevalence of small ruminants' brucellosis in four districts of Afar National Regional State, Northeast Ethiopia

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A cross-sectional study was conducted in four districts of Afar region to determine the prevalence of brucellosis in small ruminants. One thousand fifty sera were tested using modified Rose Bengal plate test (mRBPT) and complement fixation test (CFT) as screening and confirmatory tests, respectively. The results showed that 7.1 and 13.6% of sheep and goats were sero-positive, respectively. District level sero-prevalence ranged from 3.6 to 13.6% and 3.3 to 6.7% in sheep and goats, respectively. The logistic regression model for small ruminants identified goats (odds ratio (OR) = 2.36; 95% CI: 1.46 to 3.82) are at higher risk of brucellosis as compared to sheep. In addition, small ruminants greater than two years (OR = 3.132; 95% CI: 1.6 to 6.15), and larger flock size (OR = 2.04; 95% CI: 1.35 to 3.1) are at higher risk of brucellosis than their counter categories. The results of this study demonstrated that livestock brucellosis is widely prevalent in the study areas. Hence, the study suggests the need for implementing control measures and raising public awareness on prevention methods of brucellosis.

Key words: Afar region, brucellosis, complement fixation test, modified Rose Bengal precipitation test, small ruminants.

INTRODUCTION

Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World animal Health Organization as the second most important zoonotic disease in the world accounting for the annual occurrence of more than 500,000 cases (Schelling et al., 2003; Pappas et al., 2006). The disease can affect almost all domestic and wild mammal species and cross transmission can occur between cattle, sheep, goats, camels and other species (Ghanem et al., 2009). It is an important zoonotic disease and causes significant reproductive losses in sexually mature animals (Radostits et al., 1994). The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis females, epididymitis and orchitis in males, with excretion of the organisms in uterine discharges and milk in female animals. It also causes considerable loss of productivity through high morbidity (Pappas et al., 2006). *Brucella melitensis*, *Brucella abortus*, and *Brucella suis* are zoonotic pathogens which can infect humans. *Brucella canis* may cause infections in immune-suppressed individuals (Young, 2000).

Globally, this disease is under-reported because of its vague clinical symptoms, difficult laboratory diagnosis and lack of familiarity by the medical professionals (Corbel, 2006). It has been stated that in Sub-Saharan

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Africa (SSA), the epidemiology of brucellosis in humans and livestock are not well understood and available data are limited (McDermott et al., 1999; Mangen et al., 2002; McDermott and Arimi, 2002; Schelling et al., 2003).

Ministry of Agriculture and Rural Development (MoARD) estimated that pastoralists own 7.05 million (73%) goats, 4.25 million (25%) sheep, 7.70 million (20%) cattle and 100% of the camels (MoARD, 2008). The rest of livestock is reared by highland mixed crop-livestock production system farmers. Under Ethiopian context, livestock of different species usually share pastures and dwellings. This may play a role in maintenance and transmission of endemic diseases such as tuberculosis and brucellosis (Amenu et al., 2010). Moreover, brucellosis is common in rural areas because farmers live in close contact with their animals and often consume raw dairy products. However, the vending of dairy products may also bring the disease to urban areas (Mantur and Amarnath, 2008).

Very few reports have been published about brucellosis in small ruminants of Ethiopia as compared to its economic and public health importance. Ashenafi et al. (2007) revealed 9.4 and 4.8% prevalence using Rose Bengal plate test (RBPT) and complement fixation test (CFT), respectively. Teshale et al. (2006) documented 1.9% (n = 38) positive using RBPT and 9.7% (n = 193) positive by enzyme linked immunosorbent assay (i-ELISA) in pastoral areas.

In Afar region activities such as habit of consuming raw milk, unsafe handling of aborted materials and other infected excretions of animals, rearing of diversified animal species together, herding of large number of animals collectively is widely practiced. Moreover because of repeatedly occurring natural phenomenal such as concurrent drought, earth quake and flooding in the region the introduction of new animals from outside the districts as a replacement stock by different non-governmental organizations (NGOs) and food security programs for vulnerable communities may also be causes of introduction of infection to the area. There is paucity of current situation about the disease in livestock and humans in the region. Therefore the objectives of this study were to determine the sero-prevalence of brucellosis in shoats and assess the possible risk factors for the disease.

MATERIALS AND METHODS

Description of study area

The Afar National Regional State (ARS) is located in the northeast part of Ethiopia. Administratively, the region is divided into five zones which are further subdivided into 32 districts and 358 peasant associations (PAs). Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. The population is estimated to be 1.2 million of which 90% are pastoralists and 10% agro-pastoralists. The total surface area of the region is estimated to be 97,970 Km^2 (ARFEB, 2007). The study was conducted in four districts namely, Afambo, Assaiyta, Teru and Awura.

Assayita and Afambo are parts of the administrative Zone 1 located at about east of Semera town. The district has 11 and 8 rural PAs and 2 and 1 urban PAs, respectively. Assayita and Afambo have altitudes of 350 and 280 to 850 m above sea level (masl), respectively. Assayita is a destination for pastoralists from zone four and neighboring districts of zone one in search of pasture for their livestock as it is endowed with several large scale irrigation farms using Awash River which attracts the pastoralists to feed agricultural leftovers. Afambo district is known by Lake Abbi which is the final destination for Awash River (ARFEB, 2007).

Awura and Teru districs are found in Zone 4 and located 250 and 350 km from Semera town and established with eleven and twelve PAs, respectively. Some PAs practice agricultural activity and they are on the transit to agro-pastoral arena. The climate of Teru is arid with minimum and maximum temperatures of 28 and 50°C, respectively. The climate of Awra is generally arid to semiarid, with high temperature. The districts have two main rainy seasons, karma (July to September) and sugum (March to April) and also short rainy season called Dadaa within January. There are dry seasons called Gillal and Hagay. The annual rainfall is from 200 to 800 mm. There are no perennial rivers in these districts. The main water sources are seasonal rivers, Ela and pond. The districts are covered by sparse *Acacia* species and extensive grazing land (ARFEB, 2007).

Study population

The approximate number of sheep and goats in Awesi-Resu (zone one) were 687,551 and 1,083,567, respectively (Community-supported agriculture (CSA), 2010, 2011); while in Fanteyna Resu (zone four) approximately 418,206 sheep and 398,127 goats populations exist in the area and considered as study population (ARFEB, 2007). Sheep and goats which were above 6 months of age, with no history of vaccination against brucellosis were included in the study. Then individual animal age, species, sex category and flock size were recorded. Afterwards, herd size per household was classified as ≤ 30 and > 30. Moreover, based on their sexual maturity animals were classified into ≤ 2 years and > 2 years, respectively.

Study design

A cross-sectional study design was conducted from November, 2011 to April, 2012, to determine the sero-prevalence of brucellosis in shoats of selected pastoral and agro-pastoral residences of the Afambo, Assayita, Awura and Teru districts and to identify potential risk factors associated with sero-positivity. Four districts and about 30% PAs per district were selected purposively based on easier accessibility as well as sheep and goat population. At the present, there are 7, 11, 9 and 12 PAs in Afambo, Assayita, Awura, and Teru districts, respectively. Peasant associaion is the lowest administrative unit within a district considered. A total of 1,050 sera samples were collected from 132 flocks of small ruminants with no history of previous vaccination against brucellosis.

Experimentals

Multistage sampling technique was used in the survey of sheep and goat brucellosis. The PA was considered as primary unit, the herds as secondary units and individual animals as tertiary units. An average of 30% of small ruminants aged 6 month and above were picked randomly from each selected herd until the calculated sample size was achieved.

Sheep and goats herds in 13 PAs from four districts were sampled during the study based on the livestock population of each

Table 1. Herd level sero-prevalence of small ruminants brucellosis.

Zone	District	Species	Number tested	RBPT positive (%)	CFT positive (%)	Herd Level	
						Number Tested	Positive (%)
1	Afambo	Ovine	100	12 (12)	9 (9)	43	22 (51.16)
		Caprine	237	34 (14.35)	30 (12.66)		
	Assayita	Ovine	69	5 (7.24)	4 (5.8)	33	17 (51.5)
		Caprine	193	32 (16.58)	30 (15.54)		
4	Awura	Ovine	110	4 (3.64)	4 (3.64)	29	12 (41.38)
		Caprine	128	18 (14.06)	15 (11.71)		
	Teru	Ovine	45	6 (13.33)	6 (13.33)	27	17 (62.96)
		Caprine	168	25 (14.88)	24 (14.28)		
Total		Ovine	324	27 (8.33)	23 (7.1)	132	68 (51.51)
		Caprine	726	109 (15.0)	99 (13.64)		

district. In order to determine the desired sample size there was no previous reports of prevalence in the districts except the 0% (n = 32) report by Ashenafi et al. (2007) in Assayita. Hence, the average expected prevalence rate was assumed to be 50% for the area within 95% confidence intervals (CI) at 5% desired accuracy as stated by Thrusfield (2005) as shown in the formula below:

$$n = \frac{1.96^2 \times P_{ex} \times (1 - P_{ex})}{d^2}$$

Where n = sample size, d = desired absolute precision (0.05), P_{ex} = expected prevalence (50%), thus the desired sample size for P_{ex} = 0.5 is n = 384. But, we inflate the sample size to 1,050 to increase the representativeness of the samples to the wider population. Hence, n = 1050 goats and sheep were sampled. Sampling was proportionally distributed based on the total small ruminant population in the study districts and PAs. Blood samples were collected from jugular vein of each animal of selected herds using plain vacutainer tubes and allowed to clot at room temperature. Serum was separated from clotted blood by decanting to other tubes and stored at -20°C until laboratory test was performed.

Rose Bengal plate test (RBPT)

The modified Rose Bengal plate test (mRBPT) was done in Semera Regional Veterinary Laboratory in order to screen positive samples by RBPT using RBPT antigen (Institue Pourquier 325, rue de la galèra 34097 Montpellier cedex 5, France). Positive sera were then retested using complement fixation test (CFT) of same origin at the National Veterinary Institute (NVI), Debre Zeit. Samples were considered positive for brucellosis if they were positive for RBPT and CFT. For the mRBPT, the procedure described by Alton et al. (1975) was followed with little modification by Blasco et al. (1994). Reactions were categorized as 0, +, ++, +++, according to Nielson and Dunkan (1990), where: 0 = means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing, and +++ = clumping, definite clearing. Those samples identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were regarded as positive.

Complement fixation test (CFT)

Positive sera with RBPT were further tested with CFT for confirmation using standard *Brucella abortus* antigen (New Haw, Addleston, Surrey KT15 3NB, UK). The CFT test proper and reagent preparation procedures were following the procedures outlined by OIE (2004). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (OIE, 2004).

Data processing and statistical analysis

The data were entered into Microsoft Excel 2007 and coded data were stored and finally transferred to SPSS® version 16 for statistical analysis. Descriptive statistics were used to analyze the sero-positivity at individual animal and flock levels. Logistic regression and Chi-square test (χ^2) were employed to see the association of risk factors with that of sero-positivity to brucella antibody and the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI). A test value was considered as statistically significant when $P < 0.05$. Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals. Variable reduction was performed by fitting univairiate logistic regression for each covariate and variables with p-value > 0.25 were dropped.

RESULTS

The sero-positivity of sheep and goats in study districts was 7.1 and 13.6%, respectively (Table 1).

Effect of risk factors on sero-prevalence of brucellosis in small ruminant

Higher prevalence was seen in goat than sheep with a statistically significant difference ($X^2 = 24.91$, $P < 0.05$).

Table 2. Association of risk factors with brucellosis reactivity in small ruminants.

Variable	Category	No. sampled	Complement fixation test		
			Positive (%)	Chi-square	P-value
	4	451	49 (10.9)	0.44	0.508
	1	599	73 (12.2)		
Zone	Afambo	337	39 (11.6)	4.8	0.187
	Assayita	262	34 (13)		
	Awura	238	19 (8)		
	Teru	213	30 (14.1)		
Species	Goat	726	99 (13.6)	9.32	0.002
	Sheep	324	23 (7.1)		
Sex	Male	204	16 (7.8)	3.52	0.061
	Female	846	106 (12.5)		
Age	≤2 years	200	10 (5)	10.54	0.001
	>2 years	850	112 (13.2)		
Flock size	≤30 animals	462	37 (8)	10.47	0.001
	>30 animals	588	85 (14.5)		

Table 3. Association of risk factors with brucellosis reactivity in goats in the study areas.

Variable	Category	No. sampled	Complement fixation test		
			Positive (%)	Chi-square	P-value
Zone	1	430	60(14)	0.90	0.764
	4	296	39(13.2)		
District	Afambo	237	30(12.7)	23.43	0.054
	Assayita	193	30(15.5)		
	Awura	128	15(11.7)		
	Teru	168	24(14.3)		
Sex	Male	169	13(7.7)	6.61	0.01
	Female	557	86(15.4)		
Age	≤2 years	158	9(5.7)	10.81	0.001
	>2 years	568	90(15.8)		
Flock size	≤30 animals	313	31(9.9)	6.50	0.011
	>30 animals	413	68(16.5)		

The association of large flock size and sero-positivity was statistically significant (X^2 = 10.47, P < 0.05) (Table 2). Moreover, the difference in sero-prevalence between the sex groups of goats was statistically significant (X^2= 6.61, P < 0.05) (Table 3). In sheep only flock size has significant difference (P < 0.05).

Univaraite logistic regression analysis of risk factors

The univariable logistic regression analysis of the putative risk factors showed statistically significant (P < 0.05) difference on brucella reactivity between small ruminants with small and large flock size (Table 4).

Table 4. Effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis using CFT.

Risk factors		Complement fixation test		
Variable	**Category**	**OR**	**95% CI**	**P-value**
Zone	4*	-	-	0.508
	1	1.14	0.78-1.67	
District	Teru*	-	-	0.194
	Afambo	1.25	0.75-2.09	0.387
	Assayita	1.09	0.65-1.87	0.725
	Awura	1.89	1.03-3.47	0.04
Species	Sheep*	-	-	
	Goat	2.07	1.29-3.32	0.003
Sex	Male*	-	--	-
	Female	1.68	0.97-2.92	0.063
Age	≤2 years*	-	-	-
	>2 years	2.88	1.48-5.61	0.002
Flock size	≤30*	-	-	-
	>30	1.94	1.29-2.92	0.001

*Reference category.

Multivariable stepwise logistic regression analysis of risk factors for brucellosis reactivity

The difference for age-group, sex and herd size based infection rates observed in goat in the initial logistic regression model were also found evident after the control of confounding factors in the stepwise multi-variable logistic regression analysis. On the other hand, in sheep only flock size was used while sex and age of animals were excluded from the model indicating that both were not significantly affecting reaction to bruce-llosis. Table 5 shows the result of stepwise multivariate logistic regression analysis of risk factors and brucellosis reactivity of sheep and goat in the study area.

DISCUSSION

The overall sero-prevalence in this finding is slightly lower than reports previously by Teshale et al. (2006) and Kaoud et al. (2010) in Ethiopia and Egypt, respectively. The variation might be due to geographical differences, number of animals included and methods implemented. Brucellosis was detected in all the four districts of the two zones. The difference in prevalence between zones and among the districts was not statistically significant. It could be due to the similarity in the agro-ecological conditions and livestock management system in the area. The herd level prevalence is higher than individual animal level and this characterizes the nature and importance of the disease in the large flock size. This signifies that brucellosis has significant economic implication in its ability to bring about morbidity at flock level.

Epidemiology of the disease at individual and herd level show wider spread of the disease in different species of animals. In Afambo and Assayita districts of zone one, animals are kept in confinement around cultivation fields than the other two districts, as the districts are largely dominated by agricultural irrigation using Awash River. This may be responsible for the high prevalence in zone one as infection is easily transmitted within the entire herd under this management system. Teru and Awura districts are mostly pastoralist settings and are dominated by free range management system.

In sheep, the study is fairly in agreement with different findings. Shehu et al. (1999) reported a prevalence of 6.6% in sheep in Nigeria. However, the findings disagree with that of Yesuf et al. (2010) who reported a sero-prevalence of 1.5% in south Wollo, Teshale et al. (2006) and Ashenafi et al. (2007) who reported seo-prevalence of 14.6 and 3.2% in Mille and Dalifage districts of Afar region and in Afar region, respectively. Such differences might be attributed to methodologies followed by number of animals and geographical and management differences. In other countries, Bale et al. (1982) reported 15.9% prevalence in a study conducted in Northern Nigeria.

Higher prevalence in goats compared to this finding was reported by Teshale et al. (2006) (16.45%), Bale et al. (1982) (34.8%) and Ojo et al. (2007) (45.75%) in Afar region of Ethiopia, northern Nigeria and Abeokuta, respectively. However, a lower prevalence of 5.8% was reported by Ashenafi et al. (2007). The high prevalence and wide distribution are not surprising since small ruminants are not being vaccinated against brucellosis, coupled with the traditional practice of communal grazing in most part of the region.

Statistically significant difference in sero-prevalence was observed between sheep and goats where goats were found to be at higher risk than sheep. This finding is in agreement with results of Omer et al. (2000) and Radostitis et al. (1994). The higher prevalence in goats than in sheep may be in part due to the greater susceptibility of goats to *Brucella* infection than sheep and partly it may be due to the fact that sheep unlike goats do not excrete the *Brucella* organisms for longer periods of time. This can reduce the potential of the spread of the disease among sheep flock (Radostitis et al., 1994).

There is no statistically significant difference between male and female animals. Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection, due to the absence of erythritol. However, in support of the present findings, Teshale et

Table 5. Multivariable stepwise logistic regression analysis of risk factors for brucellosis in small ruminants.

Species	Variable*	Complement fixation test		
		OR	95% CI	P-value
Goat	Female	2.075	1.12-3.85	0.021
	>2 years	3.061	1.498-6.258	0.002
	>30 flock size	1.91	1.23-3.02	0.006
Goat and sheep	Goat	2.361	1.458-3.825	0.000
	Female	1.785	1.018-3.13	0.043
	>2 years	3.132	1.595-6.149	0.001
	>30 flock size	2.036	1.348-3.075	0.001

Reference categories were omitted to avoid repetitions.

al. (2006) and Ashenafi et al. (2007) have also reported no observable difference in the prevalence of brucellosis between male and female sheep and goats. Statistically significant difference was observed between age and sero-positivity in goats but not in sheep. In the latter, the reason may be few number of the young sheep included in the sampling. This result was consistent with Ashenafi et al. (2007). Brucellosis infection may occur in animals of all age groups but persists commonly in sexually mature animals (Radostitis et al., 1994). Younger animals tend to be more resistant to infection and frequently clear infections although few latent infections may occur (Radostitis et al., 1994; Walker, 1999).

The prevalence has increased in Assayita district when it is compared with a previous study done by Ashenafi et al. (2007) who found 0% in small ruminants. This increase may be due to the seasonal migration of livestock from Chifera, Awura, Teru and Golina to Assayita and Dubiti districts of the region in search of cotton, maize and sorgume leftover as an animal feed. Mixing of the different species during migration, at watering or night enclosures (resting) among different species is a common practice in Afar area. The other contributing factor to the spread of brucellosis may be the movement of animals for grazing and watering as aggregating the animals around watering point will increase the contact between infected and healthy animals thereby facilitating the spread of the disease. The disease is a herd wide problem rather than individual animals and this should call our attention to its economic impact on the region and the nation at large.

Conclusion

The sero-prevalence described in this study shows that brucellosis is a widespread and well-established infection among goats and sheep across the two zones and all the study districts of Afar region. The most important risk factors identified for individual animal sero-prevalence were age, species and flock size. Sero-prevalence of brucellosis is common in very old aged goats and animals within large flock size. Thus, there is a need to design and implement control measures aiming at preventing further spread of the disease in the region. Critical assessment of the economic impact of the disease, which emanates from its effect on reproductive and production performance of animals, is worthy. Studies to investigate the link between livestock and human brucellosis and cross infection among species in the region should be conducted to devise appropriate preventive mechanisms. Isolation and identification of the biotypes of brucella responsible for infection in the region should be carried out to look for effective vaccine and treatment. Herd vaccination program should be implemented to prevent the impact of the disease on economy as well as human health hazards.

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