academicJournals

Vol. 9(19), pp. 1338-1344, 13 May, 2015
DOI: 10.5897/AJMR2015.7400
Article Number: 469C2C953254
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Seroprevalence and risk factors of small ruminant brucellosis in selected districts of Arsi and East Shoa zones, Oromia region, Ethiopia

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Received 28 January, 2015; Accepted 7 May, 2015

A cross-sectional study was conducted in two districts in Arsi zone and one district in East Shoa zone, Ethiopia, to determine seroprevalence and assess the possible risk factors associated with small ruminant brucellosis. A total of 840 blood samples (409 sheep and 431goats) were collected. All sera samples were screened by modified Rose Bengal Test (mRBT) and all positive reactors were further tested by indirect enzyme linked immunosorbent assay (iELISA) test for confirmation. All 39 (4.6%) mRBT positive samples tested positive in iELISA. The individual animal and herd level seroprevalences of small ruminant brucellosis in the study area were 4.6 and 26%, respectively. Individual animal and herd level seroprevalences were highest in Adami Tulu-Jido Kombolcha district in East Shoa and lowest in Dodota Sire district in Arsi zone but differences were not statistically significant. In univariate logistic regression, statistically significant difference in seropositivity were found between different age groups, pregnancy status and parity number but not between flock size, species and sex. Upon multivariate logistic, regression analysis parity and pregnancy status remained significant. A survey among 80 owners revealed general lack of awareness of the disease and showed that they practiced improper handling, disposal of aborted materials and consumption of raw milk as potential risk behaviours. Hence, the study suggests the need for implementing control measures and raising public awareness on prevention methods of the disease.

Key words: Brucellosis, Ethiopia, risk factors, small ruminant, zoonosis.

INTRODUCTION

The small ruminant population of Ethiopia is estimated to be nearly 23.33 million goats and 23.62 million sheep playing an important role in the livelihood of resource poor farmers. They provide their owners with a vast range of products such as meat, milk, skin, hair, horns and manure for cash. Sheep and goats are highly adaptable to broad range of environmental conditions. Moreover, low cost of production, requirement of little land and higher

prolificacy made them attractive asset for development. Investment in sheep and goats avoid losses due to high inflation rates that are found in unstable economies of many developing countries like Ethiopia. There is also a growing export market for sheep and goat meat in the Middle East Gulf states and some African countries. Despite all these, the country fails to optimally utilize this huge resource because of different constraints among which disease stands in the front line. Brucellosis is one of such diseases that hamper the productivity of small ruminants (Yami and Merkel, 2008; Central Statistical Agency, 2012).

Brucellosis is an infectious bacterial disease caused by members of genus *Brucella*. It is a disease of worldwide importance and affects a number of animal species. Brucellosis in small ruminants is mainly caused by *Brucella melitensis,* and rarely by *Brucella abortus* or *Brucella suis* (Hirsh and Zee, 1999; Benkirane, 2006, Verma, 2013). The disease in naturally infected sheep and goats is characterized by abortion, stillbirth and birth of weak offspring in females and acute orchitis and epididymitis in males. Brucellosis is an important zoonosis causing chronic debilitating disease in man. Groups at higher risk for brucellosis are animal health workers, butchers, farmers and those who habitually consume raw milk and come in contact with animals (Radostits et al., 2006; Gupta et al., 2006).

In Ethiopia, few studies have been published so far on small ruminant brucellosis (Tekelye and Kasali, 1990; Teshale et al. 2006; Ashenafi et al., 2007; Ferede et al., 2011; Bekele et al., 2011; Yohannes et al., 2013). Particularly, there is no published data on small ruminant brucellosis in the study area. On the other hand, there is high population of sheep and goat in the study area (CSA, 2012). Therefore, the objectives of this study were to determine the seroprevalence and assess possible risk factors of small ruminant brucellosis in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Tiyo and Dodota Sire districts of Arsi zone, and Adami Tulu-Jido Kombolcha district of East Shoa zone of Oromia region, Ethiopia. Arsi zone is found at 6°45′N to 8°58′N and 38°32′ E to 40°50′ E. Asella, the capital of the zone is found at 175 km Southeast of Addis Ababa. The animal population of Arsi zone is 2,295,138 cattle, 1,207,182 sheep, 653,327 goats, 202,467 horses, 369,218 donkeys, 21,587 mule and 1,449,583 poultry. The mean annual temperature of the zone is 20-25°c in the low land and 10-15°C in the central high land. On average, the zone gets annual mean rainfall of 1020 mm. The altitude of the zone ranges from 805 m above sea level to 4195 meters at mountain peak of mount Kaka (CSA, 2012; OFEDB, 2007).

Adami Tulu-Jido Kombolcha district is found at 7°9′ N latitude and 38°7′ E longitude. The district is situated in the mid-rift valley, East Showa zone of Oromia region, 167 km South of Addis Ababa. According to CSA (2012) the animal population of East Shoa zone is 973,563 cattle, 299,284 sheep, 488,512 goats, 13,000 horses, 247,399 donkeys, 7,087 mules and 926,465 poultry. The zone is found at an altitude of 1650 m above sea level with a bimodal unevenly distributed rainfall pattern. The average annual rainfall for the last 10 years was 760.9 mm. AdamiTulu-Jido Kombolcha district has a minimum mean temperature of 12.7°C (ALDHA, 2012).

Study animals and study design

A cross-sectional study was conducted from January to June, 2012 to study seroprevalence and associated risk factors of small ruminant brucellosis. The predominant sheep and goat breeds in the study area are Arsi-Bale breeds which are managed under extensive management system. Traditional housing, feeding and milking practices are generally practiced. Vaccination against brucellosis is not practiced in Ethiopia (Figure 1). Blood sample was collected from goats and sheep of above six month age and laboratory tests were done. Questionnaire survey was conducted on randomly selected small ruminant owners.

Sample size and sampling methodology

The sample size was calculated using the formula recommended by Thrusfield (1995) for simple random sampling considering 95% confidence interval level and 5% desired absolute precision. 50% expected prevalence was used as there was no previous study in the area.

$$N = \frac{(CI)^2 \, Pexp \, (1\text{-}Pexp)}{d^2}$$

Where, N-the required sample size, Pexp-expected prevalence rate, CI-confidence interval and d-desired absolute precision
The sample size required as per the above formula is 384 heads for each species. However, the sample size was increased to 840 (409 sheep and 431goats) to increase precision. The zones and districts were selected purposively based on their small ruminant population and accessibility. Simple random sampling technique was used to select peasant associations (PAs) and herds. Nine PAs (three from each district) were selected by lottery method. 131 herds were included in the study. The number of animals and herds tested in each district is indicated in Table 1.

Questionnaire survey

Eighty (80) small ruminant owners were selected randomly by lottery method (out of 131 owners) and interviewed using structured

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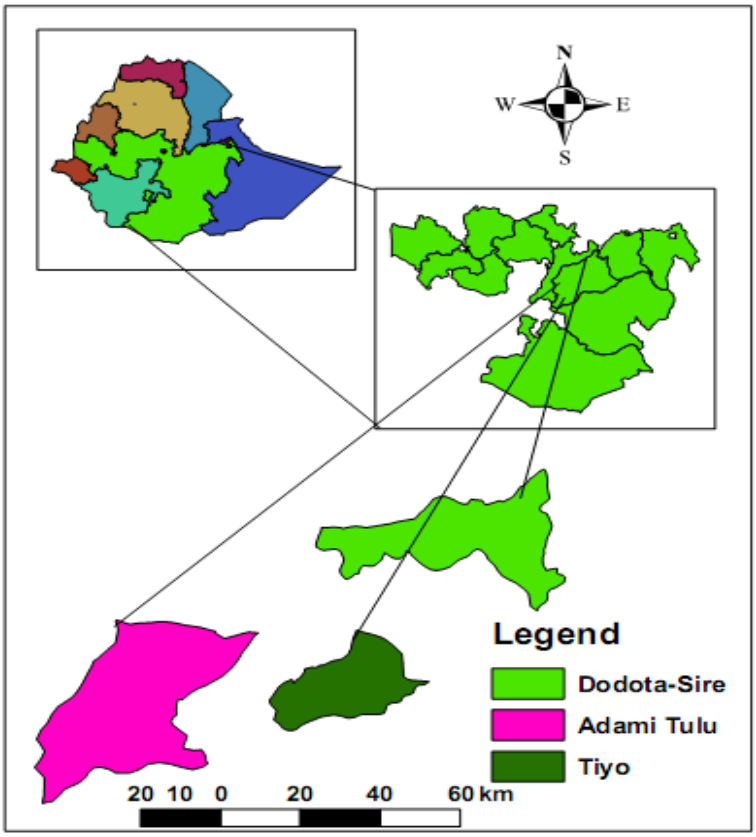

Figure 1. Map of study districts.

Table 1. Individual animal and herd level seroprevalence of small ruminant brucellosis in the three study districts.

Zone	Individual animal seroprevalence				Herd level seroprevalence		
	District	No. animals tested	No. Positive (%)	P-value	No. of tested herds	No. Positive (%)	P-value
Arsi	Tiyo	326	17(5.2)	0.17	44	13(29.5)	0.14
	Dodota Sire	287	8(2.8)	0.14	49	8(16.3)	0.13
E/Shoa	Adami Tulu-Jido kombolcha	227	14(6.2)	0.63	38	13(34.2)	0.65
	Total	840	39(4.6)		131	34(26)	

pre-tasted questionnaire. By doing so, management practices that may predispose the public to infection by brucellosis were accessed.

Blood sample collection and handling

About 7-10 ml of blood samples were collected from jugular vein of each animal using properly labeled plain vacutainer tubes. The individual animal details such as species of animal, sex, age, herd size, source of animal and history of abortion were recorded along with blood sample collection. Samples were allowed to stand tilted overnight at room temperature. Then, serum was separated from clotted blood and transferred to cryogenic vials. Separated sera were stored at -20°C until being tested.

Laboratory tests

The sera samples were removed from the refrigerator and left at room temperature for at least 30 minutes before performing the test. All sera samples were screened by mRBT antigen (Lillidale

diagnostics, UK) according to the modified procedure of Blasco et al. (1994), mixing 75µl of serum and 25 µl of antigen. The interpretation of the results was done according to the degree of agglutination. Samples with no agglutination were recorded as negative while those with agglutination were recorded as positive (Nielsen and Dunkan, 1990). Confirmation of mRBT positive sera was done by iELISA (SERELISA® *Brucella* OCB Ab Mono Indirect kit, SYNBIOTICS EUROPE SAS, France). Indirect ELISA test was performed according to manufacturer's manual. Both mRBT and iELISA were performed at Sebeta National Animal Health Diagnostic and Investigation Center.

Data analysis

Data obtained was stored into Microsoft Excel® spreadsheet. The individual animal level seroprevalence was calculated by dividing RBPT and iELISA positive results by total number of animals tested. Logistic regression analysis using SPSS 16 for windows was used to determine association of risk factors with the disease. All risk factors with P-value <0.2 in the univariable logistic regression analysis were subjected to multivariate logistic regression analysis.

RESULTS

Overall seroprevalence of small ruminant brucellosis

Out of total 840 sera samples collected from the three districts, 39(4.6 %) were positive for mRBT. Upon further testing, all were found to be iELISA positive. The individual animal and herd level seroprevalence of the disease in the study area were 4.6% and 26% respectively. The individual animal level seroprevalence of the disease was found to be higher in Adami Tulu-Jido Kombolcha district (6.2%) than Tiyo (5.2%) and Dodota Sire (2.8%) districts. Herd level seroprevalence was also higher in Adami Tulu-Jido Kombolcha district (34.2%) followed by Tiyo (29.5%) and Dodota Sire was lowest (16.3%). However, there was no statistically significant difference observed between districts both at individual animal and herd level (Table 1).

Association of risk factors with seroprevalence of small ruminant brucellosis

The prevalence of *Brucella* antibodies in goats and sheep was 4.9 and 4.4%, respectively and it was higher in male (6.7%) than females (4.4%). Seroprevalences of 4.1, 4.8 and 6.6% were found for herd sizes of [0-10], [11-20] and >20, respectively. There was higher rate of infection in adult (5.4%) than young age group(1.3%) and in pregnant (6.9%) than non-pregnant(2.3%). The prevalence of the disease was 3.7% and 4.58% in shoats with and without previous abortion respectively. The prevalence of the disease was 1.46, 5.59 and 8.4% for [0-1], [2-4] and>4 parities, respectively. There was a statistically significant difference in seropositivity between different parity groups, age groups and pregnancy status in univariable logistic regression analysis. However, there was no statistically significant difference in seropositivity between different sex, species, herd sizes and abortion status (Table 2). Pregnancy and parity status remained significant in multivariable logistic regression analysis. The odds ratio indicated that pregnant sheep and goats were 3.28 times more likely to be infected with brucellosis than the non pregnant ones. The risk of seropositivity was 6.19 and 3.89 times higher in >4 and [2-4] parity groups respectively in comparison to [0-1] parity group. Multivariable logistic regression analysis of potential risk factors for small ruminant brucellosis is indicated in Table 3.

Questionnaire survey result

Pre-tasted questionnaire was presented to 80 randomly selected farmers to assess association of management risk factors with the disease. Univariable logistic regression analysis of management risk factors obtained through questionnaire survey showed that only seasonal migration of herds was significantly associated with the disease (Table 4). All of the respondents have no knowledge about the disease. Neither did they use any protective material while handling aborted fetus or fetal membranes. Moreover, none of them practiced safe disposal of aborted material. Raw milk consumption was practiced by some of the herd owners interviewed.

DISCUSSION

The seroprevalence obtained in the present study was higher than the report of Tekelye and Kasali (1990) who reported prevalence rates of 1.5% in sheep and 1.3% in goats in Central Ethiopia; and Ferede et al. (2011) who reported prevalence proportions of 0.87% in goats and 0% in sheep in and around Bahir Dar. The report of Ashenafi et al. (2007) with prevalence rate of 5.8% in goats and 3.2% in sheep in pastoral regions of Afar and that of Ashagre et al. (2011) with prevalence of 4.2% in goats in South Omo zone showed fair agreement with this finding. Bekele et al. (2011) reported lower prevalence rate of 1.2% in sheep and 1.9% in goats in Jijiga area. The difference in prevalence might be attributed to the differences in animal husbandry and serological tests employed. Most of the above findings used standard rose bengal plate test (RBPT) and Compliment Fixation Test (CFT) for screening and confirmation of sera samples respectively; but, in this study mRBT and iELISA were used for screening and confirmation respectively.

Table 2. Univariable logistic regression analysis of potential risk factors.

Risk factor	Category	Animals tested	No.of positives	Prevalence (%)	P-value	OR (95% CI)
Species	Goat	431	21	4.9	0.746	1.11 (0.584-2.12)
	Sheep	409	18	4.4		
Sex	Male	88	5	5.68	0.625	1.27 (0.48-3.34)
	Female	752	34	4.5		
Age	(0.6-1year)	160	2	1.3		
	(>1year)	680	37	5.4	0.038	4.55 (1.08-19.06)
Flock size	[1-10]	438	18	4.1	0.59	
	[11-20]	311	15	4.8	0.64	1.18 (0.59-2.38)
	>20	91	6	6.6	0.31	1.65 (0.64-4.27)
Pregnancy	Pregnant	362	2	6.9	0.004	3.14 (1.45-6.82)
	Non-pregnant	390	9	2.3		
Parity	0-1parity	274	4	1.46	0.01	
	2-4parity	358	20	5.59	0.013	3.98 (1.35-11.79)
	>4 parity	119	10	8.4	0.002	6.19 (1.9-20.20
Abortion	No	698	32	4.58	0.77	1.25 (0.3-5.4)
	Yes	54	2	3.7		

*Male goats and sheep aged less than or equal to one year and female animals that had not yet given birth were included in the younger age group.

Table 3. Multivariable logistic regression analysis of potential risk factors.

Risk factor	Level	OR (95%CI for OR)	P-value
Pregnancy	Non-pregnant		
	Pregnant	3.28(1.5-7.2)	0.003
Parity	[0-1]		0.008
	[2-4]	3.89(1.31-11.55)	0.015
	>4	6.58(2.01-21.57)	0.002

The higher prevalence rates recorded by Verma et al. (2012), Bertu et al. (2010), Falade and Hussein (1997) and Waghela (1976) in India, Nigeria, Somalia and Kenya, respectively could be due to differences in agro-ecology and animal husbandry system. Teshale et al. (2006) reported higher prevalence rate of 5.6 and 13.2% in sheep and goats respectively in Afar and Somali areas. Arsi and East Shoa zones are characterized by mixed farming, in which fewer animals are raised in separate herds; however, pastoralists in Afar and Somali regions keep large number of different species of animals.

There was no statistically significant difference in seropositivity between the two species in the study area which agrees with the findings of Bekele et al. (2011) in Jijiga district, Tekleye and Kasali, 1999 in central Ethiopia and Bertu et al. (2010) in plateau state in Nigeria. However, Omer et al. (2002), Teshale et al. (2006) and Ashenafi et al. (2007) reported significantly higher prevalence in goats than in sheep.

No statistically significant difference observed between males and females. However, in support of the present finding, Teshale et al. (2006), Ashenafi et al. (2007),

Table 4. Univariable logistic regression analysis of management risk factors.

Risk factor	Category	Herd owner response (%)	No.of positive herds	Prevalence in herd	P-value	OR (95% CI)
Herd migration	Yes	20 (25)	7	35	0.038	3.5 (1.1-11.4)
	No	60 (75)	8	13.3		
Decision on aborting animal	Sell	62 (77.5)	12	19.5	0.8	1.2 (0.3-4.8)
	Keep	18 (22.5)	3	16.7		
Abortion encountered	Yes	58 (72.5)	14	24.13	0.075	6.7 (0.8-54.3)
	No	22 (27.5)	1	4.5		
Delivery assisted	Yes	33 (41.25)	5	15.2		
	No	47 (58.75)	10	21.3	0.49	0.66 (0.2-2.2)
Knowledge of disease	Yes	0 (0)	0	0		
	No	80 (100)	15	18.75		
Aborted material disposal	Throw into field	58 (100)	14	24.13		
	Burning/burying	0 (0)	0	0		
Handling of aborted fetus/membrane	Bare hand	58 (100)	14	24.13		
	Protective material	0	0	0		
Raw milk consumption	Yes	14 (17.5)	5			
	No	66 (82.5)	10		0.075	6.68 (0.8-54)

Ashagrie et al. (2011) and Bekele et al. (2011) also reported the absence of statistically significant difference between the two sexes. This could be due to the small sample size of males. Males are also kept in the herd for shorter period which decrease their exposure to the disease.

Pregnant sheep and goats showed significantly higher rate of infection than non-pregnant ones. Higher parity was also significantly associated with the disease which agrees with the report of Ashagrie et al. (2011). Age was found to be significantly associated with the disease in univariable logistic regression which agrees with the findings of Bekele et al. (2011) and Ashenafi et al. (2007). Sexually mature and pregnant animals are more susceptible to infection with the organism than sexually immature animals of either of sex, which is due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organism, tend to increase in concentration with age and sexual maturity (Radostits et al. 2006; Quinn et al., 2004). In this finding, seasonal migration of herds showed significant association with the disease.

Conclusion and recommendations

This study reveals that small ruminant brucellosis was distributed at a moderately higher rate in all studied peasant associations and districts. The result of questionnaire survey also showed that the owners of small ruminants lack knowledge about the disease; nor did they practice proper disposal of aborted materials. They assisted delivery with bare hand. Moreover, some of the owners practiced the habit of drinking raw milk. Generally, this finding revealed high risk of transmission of the disease in the small ruminants and people of the studied area. Hence, we recommend the implementation of control measures and raising public awareness on prevention methods of the disease in the area.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank Sebeta National Animal Health Diagnostic and Investigation Center and Asella Regional Animal Health Diagnostic and Research Laboratory for their technical support and for allowing us to use their laboratory facilities.

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