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REGULAR ARTICLES

Sero-prevalence and risk factors study of brucellosis in small ruminants in Southern Zone of Tigray Region, Northern Ethiopia

Teshale Teklue · Tadele Tolosa · Getachew Tuli · Belay Beyene · Birhanu Hailu

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Abstract This study reports a prevalence and risk factor survey of brucellosis in small ruminants in Southern Zone of Tigray Region, Northern Ethiopia between October 2011 and April 2012 to determine the sero-prevalence of small-ruminant brucellosis and to identify associated risk factors for the occurrence of disease in small ruminants under extensive production system. Multistage random sampling was followed to select locations, flocks, and individual animals. Laboratory analysis of serum samples provided sero-prevalence estimates for flocks and geographic location. Information on risk factors at the individual and flock level was obtained by examination of individual animal and a questionnaire interview to flock owners. The overall individual animal-level sero-prevalence of brucellosis in small ruminants was 3.5 % and flock level sero-prevalence was 28.3 %, and the within-flock sero-prevalence was ranged from 0 % to 22.2 % based on the Complement Fixation Test. Multivariable logistic regression showed that the major risk factors for flock level sero-positivity were flock size and abortion history. This study showed that small-ruminant brucellosis is prevalent in the study area. Larger flock size and history of previous abortion in the flock were major risk factors identified for sero-positivity of small-ruminant brucellosis.

Keywords Brucellosis · Risk factor · Sero-prevalence · Small ruminants · Southern Zone Tigray · Northern Ethiopia

T. Teklue · T. Tolosa (✉) · B. Beyene · B. Hailu
Department of Microbiology and Veterinary Public Health, College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box 307, Jimma, Ethiopia
e-mail: tadeletolosa@yahoo.com

T. Teklue
Alamata Agricultural Research Center, P.O. Box 56, Alamata, Ethiopia

G. Tuli
National Animal Health Diagnostic and Investigation Center, P.O. Box 04, Sebeta, Ethiopia

B. Beyene
Wollega University, College of Agriculture and Natural Resources, Shambu Campus, Shambu, Ethiopia

B. Hailu
Semera University, Faculty of Veterinary Medicine, P.O. Box 132, Semera, Ethiopia

T. Tolosa
M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics, and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Introduction

Agriculture is the mainstay of Ethiopian economy which comprise crop and livestock sub-sectors. Small-ruminant production is one of the important agricultural activities in the livestock sub-sector. The small-ruminant population of Ethiopia is estimated to be 48.3 million (CSA 2011) and they are a local breed kept under extensive production system. This type of production system is characterized by low productivity with limited commercial offtake. Among many factors, production diseases are the one which hinder the economic return of small ruminants in Ethiopia.

Of all the production diseases, brucellosis is the one that causes low reproductive efficiency characterized by repeated breeding. Brucellosis is an infectious bacterial disease caused by genus *Brucella*, has got worldwide significance, and affects many animal species (Seleem et al. 2009; Sonawane et al. 2011). Brucellosis in small ruminants is a serious disease primarily in goats which is caused by *Brucella melitensis* and in sheep by *Brucella ovis*. It is the disease of sexually matured animals with predilection for placentas and genital organs in male animals (OIE 2009; Mariana et al. 2010).

In Ethiopia, studies conducted on small-ruminant brucellosis have indicated that there is variation in sero-prevalence of the disease from place to place (Amenu et al. 2010; Ashagirie et al. 2011; Bekele et al. 2011). Tigray region is one of the regional states located in the northern part of Ethiopia. In this region, so far a document on small-ruminant brucellosis is scarce. Besides, in the Southern Zone of Tigray there is no report on the disease except farmers that raise abortion as priority problems. Thus, the objectives of this study were to determine the sero-prevalence of small-ruminant brucellosis and to identify associated risk factors for the occurrence of disease in small ruminants under extensive production system in the Southern Zone of Tigray Region, Northern Ethiopia.

Materials and methods

Description of study area and study animals

This study was conducted in Southern zone of Tigray Regional State, Northern Ethiopia between October 2011 and April 2012. The zone is geographically located at 12°15′ and 13°41′ north latitude and 38°59′ and 39°54′ east longitude, constituting an area of 9,446 km^2. It consists of five administrative districts namely Alamata, Alaje, Endamohoni, Ofla, and Raya Azebo under different agro-ecological zones. It shares a common border with Eastern Tigray zone in the north, Amhara regional state from the south and west, and Afar Regional state from the east. The study area has a total human population of 1,004,558 (124,813 from urban and 879,745 from rural) (CSA 2007). The livestock population approximately consists of 699,559 cattle, 269,098 sheep, 398,503 goats, 950 horses, 160,761 donkeys, 2,154 mules, 130,303 camels, 845,548 poultry, and 52,699 beehives (CSA 2011). Mixed crop–livestock farming system is an agricultural production system practiced in the area, and livestock production is a major component of the livelihood system and provides draught power, food, and income. Small-ruminant production is one the components of the livestock production system. Small ruminants that are managed under extensive production system which comprise the local breeds of sheep and goat with no history of vaccination against brucellosis were the study animals. From both sexes, animals more than 6 months of age were included in the study.

Study design and sampling

A cross-sectional study was conducted to determine the overall individual animal- and flock-level sero-prevalence of small-ruminant brucellosis and associated risk factors. At the start of the study, community identification and assessment of complete list of flock distribution was conducted and then both blood sample collection and questionnaire were conducted at the same time between October 2011 and April 2012.

Multistage random sampling was followed to select locations, flocks, and individual animals (Dohoo et al. 2009). From each location (district), 10 % of peasant associations, from each peasant associations 10 % of the flocks (four to seven flocks from the peasant association), were included and all animals more than 6 months of age were sampled. Accordingly, four districts, 10 peasant associations, 53 flocks, and 985 individual animals were included in the study.

A pre-tested semi-structured questionnaire was developed to gather information on factors influencing the spread of *Brucella* infection within or between flocks using face-to-face interview by the first author. Data on breed, sex, age, herd size, previous abortion history, and presence of swollen joints, animal management, grazing type, watering points, and agro-ecology of the area were recorded.

Individual animal blood sample was collected from the jugular vein in a 10-ml sterile vacutainer tube by following aseptic procedure, and samples were properly labeled and left for 24 h at room temperature to allow clotting. After 24 h, the serum was separated by gently decanting and transferring it to sterile cryovials. The serum was kept at −20 °C until serological testing (OIE 2009).

Serological tests

All the samples were initially screened using Modified Rose Bengal Plate Test (mRBPT) for the presence of positive agglutinin sera as described by OIE (2009) and *Brucella abortus* antigen Strain 99 (Lilliale Diagnostics, Badbury View, Bothenwood, Wimborne, Dorset, BH21 4HU, UK) was used for screening. Results of RBPT were interpreted as 0, +, ++, and +++ as described by OIE (2009) with 0 = no agglutination, + = barely visible agglutination (seen using magnifying glasses), ++ = fine agglutination, and +++ = coarse agglutination. Those samples with no agglutination (0) were recorded as negative while those with +, ++, and +++ were recorded as positive.

All sera tested positive to mRBPT were then subjected to Complement Fixation Test (CFT) for confirmation. A known antigen was incubated with a test and control sera to see formation of immune complexes. A defined amount of complement was added to the reaction mixture. In positive reactions of antigen and antibody create immune complexes, and the complement would be fixed or consumed. In negative sera, there was no immune complex formation and there was no consumption of the complement. In the second reaction step, red blood cells and their specific antibodies were added and form complexes. In the positive sera, no complement was left to hemolyze red blood cells. In negative sera, the complement will cause visible hemolysis of red blood cell.

Statistical analysis

Data obtained from both serological tests and questionnaire surveys were entered and stored in Microsoft (MS) Excel spreadsheet program. Analysis was done using SPSS software programs version 16 (2007). Samples positive for both mRBPT and CFT were considered as true sero-positive. The total sero-prevalence was calculated by dividing the number of CFT-positive animals by the total number of animals tested. Flock sero-prevalence was also calculated by dividing the number of flock with at least one positive reactor in CFT by the number of all flocks tested. The within-flock seroprevalence was calculated by dividing the number of positive CFT reactors within a flock by the number of serum samples tested in the flock.

Chi-square was used to associate risk factors with sero-prevalence, and for sample parameters resulting in small numbers in some of the cells of a 2×2 contingency table, i.e., when the expected values are less than 5, Fisher's exact test was used (Thrusfield 2008). Univariable logistic regression was used to analyze the association of exposure variables with *Brucella* sero-positivity. Univariable associations were analyzed between the binary outcome variable (sero-prevalence) and all independent variables. Statistical significance in this step was assessed at $P<0.2$. All variables $P<0.2$ were subjected to multivariable logistic regression analysis to construct the likely model ($P<0.05$) by forward selection method. The overall fitness of the model was assessed using maximum likelihood (probability) estimation (Dohoo et al. 2009).

Results

Farm characteristics

Twenty-five (47 %) of the interviewed farmers were caprine owners with minimum flock size 60 and maximum of 350. Twenty-eight (53 %) of them were ovine owners with minimum and maximum flock sizes of 60 and 400, respectively. All the respondents reported that communal type of grazing system and the same watering points were used in the area. Keeping animals separately is not yet fully practiced (Table 1).

Observation of testicular swelling on their flocks were mentioned in few respondents (4 %) (Table 1). More abortions were observed in goats than in sheep in which 80 % of the goat owners indicated the existence of abortion in their flock and only 32 % of sheep owners respond similarly (Table 1). Most of the farmers (81 %) claimed that the aborted materials are simply thrown on fields which could facilitate further contamination of other flocks or herd. Almost all the farmers mentioned that mobilization to far distant areas for search of grazing and water in small ruminants is not practiced (Table 1). Some of the farmers (10 %) also used to eat raw meat. Nevertheless, about 18 % of the respondents had less habit of raw milk consumption. Assisting animals during birth delivery without glove is also practiced in 17 % of the community. The questionnaire survey showed that most respondents (81 %) had no basic knowledge about the transmission of brucellosis. It was also found that some of the animal husbandry systems would potentially facilitate easily the transmission of disease among flocks, and generally community awareness regarding small-ruminant brucellosis is less in which only 10 % of the farmers have the concept that the disease could cause abortion.

Table 1 The result of questionnaire survey on small ruminant brucellosis in Southern Zone of Tigray Region, Northern Ethiopia between October 2011 and April 2012

Parameters	Number of respondents		
	Total	Yes (%)	No (%)
Sharing of ram/buck			
Goats	25	24 (96)	1 (4)
Sheep	28	27 (96.4)	1 (3.6)
Abortion in flock			
Goats	25	20 (80)	5 (20)
Sheep	28	9 (32)	19 (68)
Abortion management	53	6 (11)	47 (89)
Mobility	53	1 (1.9)	52 (98.1)
Testicular swelling	53	2 (4)	51 (96)
Raw meat consumption	53	10 (18.9)	43 (81.1)
Milking	53	18 (33.9)	35 (66.1)
Goats	25	20 (80)	5 (20)
Sheep	28	9 (32)	19 (68)
Milk consumption			
Raw milk	16	1 (6.3)	15 (93.7)
Heat treated	37	17 (46.0)	20 (54.0)
Disease transmitted by eating raw meat and milk	53	10 (18.9)	43 (81.1)
Observed dog eating aborted fetus and membranes	53	31 (58.4)	22 (41.5)
Common watering	53	51 (96.2)	2 (3.80)
Perception on causes of abortions			
Disease	53	10 (18.9)	43 (81.1)
Delivery assistance (bare handling)	53	8 (17.7)	45 (82.3)

Sero-prevalence of small-ruminant brucellosis

From the total of 985 animals (490 sheep and 495 goats) sera samples examined, 34 sera were found positive to CFT

Table 2 Flock- and animal-level sero-prevalence of small-ruminant brucellosis in different districts of Sothern Zone of Tigray, Northern Ethiopia, between October 2011 and April 2012

District	Animal-level sero-prevalence		Flock-level sero-prevalence	
	Number of animals tested	Positive animals (%)	Number of flocks tested	Positive (%)
Alamata	396	23 (5.8)	20	10 (50)
Raya azebo	199	9 (4.5)	9	4 (44.4)
Ofla	160	2 (1.3)	12	1 (6.25)
Endamohoni	230	0 (0)	12	0 (0)
Total	985	3.5	53	15 (28.3)

giving an overall individual animal level sero-prevalence of 3.5 % (95 % CI, 2.4–4.7). Species level sero-prevalence recorded was 5.5 % in goats and 1.4 % in sheep. The overall sero-prevalence of small-ruminant brucellosis at animal and flock level among the districts of the zone is summarized (Table 2). The highest individual animal level sero-prevalence (5.8 %) was recorded in Alamata district and none (0 %) in Endamohoni. From the total of 53 flocks studied, 15 flocks were found positive giving an overall flock-level sero-prevalence of 28.3 % (95 % CI, 16–40). The within-flock sero-prevalence varied between 0 % and 22.2 % (Table 2); the largest flock prevalence was seen in Alamata district and there were no sero-positives in Endamohoni.

Potential risk factors

The univariable logistic regression analysis on individual animal-level risk factors showed that the type of species has a statistically significant effect on sero-prevalence ($P<0.05$). Sero-prevalence in goats (5.5 %) was significantly ($P<0.05$) higher as compared to the sero-prevalence of sheep (1.4 %). Goats were more likely to be positive to brucellosis by 3.9 times than sheep (Table 3).

The flock-level risk factors considered during the study were altitude/agro-ecology, flock size, presence of introduced animal within flocks, abortion management, and dog presence, and their associations are summarized in Table 4. Higher sero-prevalence was observed in the lowland (50 %) and mid-highland (30.8 %) than in highland (5 %). Larger flocks had higher sero-prevalence (36.8 %) than smaller ones (6.1 %). Flocks with history of abortion had higher sero-prevalence (41.2 %) than flocks with no history of abortion (12.5 %), and flocks with newly introduced animal in their composition also have higher sero-prevalence as compared to flocks with no newly introduced animals. The univariable logistic regression analysis showed that there was a statistically significant ($P<0.05$) association of flock size, flocks

Table 3 Univariable logistic regression analysis of individual animal-level sero-prevalence of small-ruminant brucellosis and risk factors using CFT test

Factors	Animal tested	RBPT+/CFT+	OR	95 % CI	*P* value
Species					
Caprine	495	27 (5.5)	3.9	1.7–9.2	0.001
Ovine	490	7 (1.4)	1		
Sex					
Female	876	31 (3.5)	1.3	0.4–4.3	0.672
Male	109	3 (2.8)	1		
Age in months					
6–18	218	5 (2.3)	–		
≥18	767	29 (3.8)	1.8	0.6–4.0	0.22
Parity status					
Null	69	0 (0.0)			
1–2	367	14 (3.8)	6.4	–	0.99
>2	415	15 (3.6)	6	–	0.99

Table 4 Flock-level sero-prevalence of small ruminant brucellosis and associated potential risk factors by univariable logistic regression

Risk factor	*N*	Positive to CFT of RBPT positives (%)	OR	OR (95 % CI)	*P* value
Agro-ecology[a]					
Highland (>2,400)	20	1 (5)	1	–	–
Mid-highland (1,600–2,300)	13	4 (30.8)	8	1.7–70	0.07
Lowland (<1,500)	20	10 (50)	19	1.0–86	0.009
Flock size[a]					
<150	15	1 (6.7)	1	–	–
≥150	38	14 (36.8)	8.1	1–68	0.05
Introduced animals					
No	23	7 (30.4)	–		
Yes	30	8 (26.7)	1.2	0.4–4.0	0.76
Dog presence with flocks					
No	11	2 (18)			0.4
Yes	42	13 (31)	0.5	0.1–2	
Abortion in the flock[a]					
No	24	3 (12.5)			
yes	29	12 (41.2)	4.9	1.2–20	0.027
Abortion management					
Burying and drying on trees	7	1 (16.7)			
Throwing on fields	47	14 (29.8)	2	0.2–19	0.5

N number of flocks

[a] Considered to be included in multivariate logistic regression analysis

with history of abortion, and altitude to flock-level sero-prevalence of small-ruminant brucellosis (Table 4). In the multivariable logistic regression analysis after Spearman's correlation test, risk factors that showed significant effect in the univariable logistic regression were fitted. Flock size and abortion history in flocks were significantly associated with flock-level sero-positivity of small-ruminant brucellosis (Table 5).

Discussion

Farm characteristics

In this study, improper abortion managements, common grazing lands, watering points, keeping of animals together, and sharing of breeding ram/buck are the common practices observed in the area. The situation is further aggravated by poor farmers' perception and awareness on disease transmission and abortion management. This agrees with findings of Kaoud et al. (2010) in Egypt and Bekele et al. (2011) in Somali region, Ethiopia. Community awareness is a cornerstone for coordinated control of small-ruminant brucellosis especially in developing countries where resource is limited (Zinsstag et al. 2007; Amenu et al. 2010). Community awareness is also a guarantee for application of the sanitary procedures and measures in rearing, raising, and breeding places to prevent introduction of diseased animals to disease-free flocks (Kaoud et al. 2010).

Sero-prevalence of small-ruminant brucellosis

In the present study, the overall individual animal sero-prevalence of small-ruminant brucellosis was 3.5 % (95 % CI, 2.4–4.7). Ashenafi et al. (2007) in Afar region, Ethiopia, reported a similar finding. The similarity of the results in different studies might be due to similar husbandry system, type of production system, and agro-ecology. In this study, 28.3 % (95 % CI, 16–40) flock-level sero-prevalence was recorded similar to other findings in other countries (Ozgur and Ibrahim 2009 in Turkey and Kaoud et al. 2010 in Egypt). In Ethiopia, a recent report has confirmed this fact in which 32.5 % (95 % CI, 21.9–43.0) of pastoral flocks had at least one sero-positive goat per flock (Asmare et al. 2013).

Table 5 Multivariable analysis of potential risk factors associated with flock-level sero-positivity in small-ruminant brucellosis using CFT

Risk factor	β[a]	SE[b]	OR[c]	CI (95 %)		*P* value
				Lower	Upper	
Constant	−3.8	1.2				
Flock size	2.2	1.1	9	1.0	84	0.04
Abortion	1.7	0.8	5	1.2	24	0.022

[a] Estimate of the change in dependent variable attributed to a change of one unit in independent variable

[b] Standard error

[c] Odds ratio log-likelihood ($\ln L$)=45, P<0.05

Potential risk factors

The univariable logistic regression analysis showed that sero-positivity to *Brucella* infection was significantly higher in goats (5.8 %) in comparison to sheep (1.4 %) in which goats are 3.9 times more likely to be infected than sheep. The observed differences in sero-positivity between species might be due to the preference of the host for the *Brucella* species, in which goats are more susceptible for *Brucella melitensis*. Additionally, goats excrete the bacteria for longer period of time that could create favorite condition for further spread of the infection within and other contact flocks (Radostits et al. 2008). This is consistent with the finding of Teshale et al. (2006) who reported 11.76 times more risk in goats than sheep. Even though statistically insignificant, females were 1.3 times more likely to be positive than males in this study. Males are less susceptible due to low erythritol presence in reproductive glands (Hirsh and Zee 1999). This is consistent with the report of Bekele et al. (2011) and Ashenafi et al. (2007) who reported more *Brucella*-positive reactors in females than males. In this study, there was insignificant difference among age groups. This could be due to the endemicity of the disease in small ruminants that animals are affected early in their age and all the age groups could have positive reactors where there is no control and prevention measure undertaken (Megersa et al. 2011). This was consistent with the finding of Ashagrie et al. (2011) who found insignificant differences among age categories in a study done on caprine brucellosis in southern Omo Zone, Southern Ethiopia.

In this study, a statistically significant difference (P<0.05) in sero-prevalence across different agro-ecological zones was observed; small ruminants in the mid-highland and lowland more likely to be affected than the highland ones. In lowland, more goat population was observed than in highland, and this could contribute indirectly to the high sero-prevalence of the disease. A higher sero-prevalence in pastoral production systems has been reported where high goat population present and prevalence lowered as flock size decreases and vice versa (Coelho et al. 2007; Mekonnen et al. 2010; Asmare et al. 2013). In this study, the larger the flock sizes, the more likely they are at risk than the smaller ones. This is comparable to the finding of Solorio-Rivera et al. (2007) and Asmare et al. (2013) who reported higher sero-prevalence in larger flocks than the smaller ones. This could be due to the higher exposure, contact of animals during watering, and feeding which favor easy spread of the disease among animals. Larger herds are usually associated with more animal movements, and this practice increased the risk of introducing an infected animal into the herd. Larger herds might be expected to be associated with intensive management practices that are typically more difficult to control and allow for closer contact between animals and their environment which increase the potential for exposure to infectious excretions (Solorio-Rivera et al. 2007; Coelho et al. 2007; Coelho et al. 2008).

In this study, the presence of abortion in flocks was one of the risk factors for small-ruminant sero-positivity. Reproductive loss due to abortion, birth of weak offspring, and infertility are recorded as the common clinical signs of brucellosis in natural hosts (Martin et al. 2009; Samadi et al. 2010; Sonawane et al. 2011). About 55 % of the respondents ascertained that abortion in their flocks is one of the production problems listed. Flocks with history of abortion were more likely to be positive than flocks with no previous abortions history. Kabagambi et al. (2001) from Uganda has reported that herds with history of abortion were 3.5 times likely to have positive reactors than herds with no history of abortion. Similarly, Megersa et al. (2011) in Ethiopia has reported more positive reactors in animal with history of abortion than animals with no history of abortion.

Conclusions

This study showed that small ruminant brucellosis prevalent in the study area. Moreover, previous abortions history of flocks and larger flock size were the major risk factors identified for sero-positivity of small ruminants. The characteristic of the husbandry system and low awareness of the community could spread the disease and consequently could affect other disease-free or with low disease prevalence populations.

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Conflict of interests None.

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