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Study on the Sero-Prevalence of Small Ruminant Brucellosis in and Around Bule Hora District of Western Guji Zone, Oromia Regional State and Southern Ethiopia

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Abstract

The small ruminant population of Ethiopia is estimated to be nearly 23.33 million goats and 23.62 million sheep. About 75% of the sheep population is reared in the high lands while over 76% of the goat population is found in the lowland pastoral areas of the country. Despite having a large number of small ruminants compared to other African countries, Ethiopia does not exploit small ruminant production to its full potential. This is because of small ruminant production is constrained by the compound effect of diseases, poor feeding, poor management and low genetic endowment. Among many factors that limit economic return from small ruminants production diseases stand in the front line. One of such disease that hampers the productivity of small ruminants is brucellosis. It is also one of the infectious diseases considered as most constraints for ovine and caprine productivity in Ethiopia.

Keywords: Economic; Diseases; Poor feeding; Mammal species; Milk production

Introduction

Worldwide, brucellosis is a disease that brings reproductive failure to livestock and serious health problems to humans [1]. The disease can cause significant loss of productivity through abortion, prolonged calving, kidding or lambing interval, low herd fertility and comparatively low milk production in farm animals Radostits M., and can cause chronic and febrile illness in humans. Even though brucellosis has been eradicated from developed countries, it remains endemic in many parts of the world, including Latin America, the Middle East, western Asia, some Mediterranean regions and Africa [2]. Brucellosis is an infectious disease caused by bacteria of the genus *Brucella*, characterized by abortion and infertility in several mammal species and considered to be one of the most important zoonosis worldwide [3]. Small ruminant brucellosis is mostly caused by *Brucella melitensis* [4].

In the world, the distribution of *B. melitensis* is more restricted than that of *B. abortus* and it is primary area of occurrences is in the Mediterranean region including southern Europe. According to AU-IBAR, *B. melitensis* was not reported from Ethiopia, although high prevalence rate of up to 13.6 by Wesinew, in small ruminants. However, the distribution of the disease in the Agro-pastoral production system has not been well studied particularly in Bule Hora district of Western Guji zone. Therefore, the objectives of this study was to estimate the seroprevalence of caprine and sheep brucellosis and its associated risk factors in selected areas of Bule Hora districts of Western Guji zone, Oromia regional states, Southern Ethiopia.

Materials and Methods

Study area

The study was conducted in and around Bule Hora from September 2020 to April 2021. Bule Hora is located at Southern part of Ethiopia and 470 km away from Addis Ababa. It is the capital city of Western Guji zone of Oromia regional state. The altitude of this town rages from 1300 to 2600 meter above sea level and temperature ranges from 15°C to 28°C. The area receives mean annual rainfall of 750 mm. The area is marked by two distinct seasons namely the dry season extending from December to May and the wet season which extends from June to September. The livelihood of major section of the population in the area depends on crop livestock mixed farming. According to data from Bule Hora livestock and fishery development office (Figure 1).

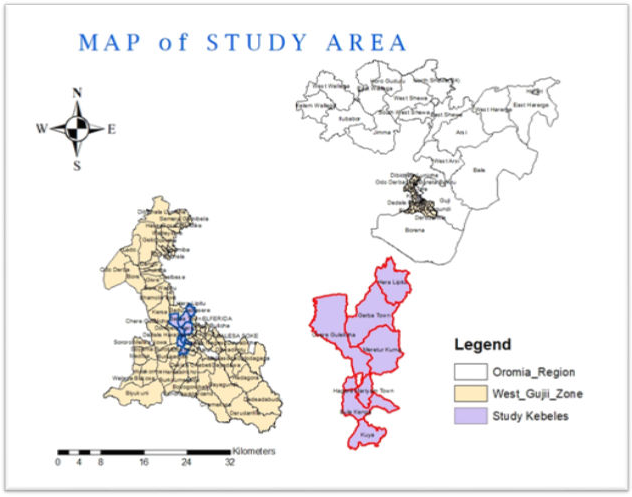

Figure 1: Map of study area.

Study design

A cross sectional (observational) study design was conducted to estimate the overall sero-prevalence of goat and sheep Brucellosis in Bule Hora district of Western Guji zone of Oromia regional state, Southern Ethiopia. After collecting the list of number of kebeles in Bule Hora district, the six kebeles (kuya, bule ano, chari gololcha, garba oda, muri turkum and hera liptu) were selected randomly based

on sheep and goat population, security, accessibility and availability of infrastructure. The sero-prevalence was estimated in respect to the number of risk factors such as host factors (age, gender and species of the animals) and the environmental factors included (kebeles, flock size, farming system and reproductive failure).

Study animals

The study animals were indigenous breeds of sheep and goats kept under both intensive and extensive management system. The sampling included all sheep and goats above 6 months of age with no history of previous vaccination against brucellosis. Then individual animal age, species, gender and origin of animals were recorded. The study animals consisted of 422 traditionally managed small ruminants of which 179 ovine and 243 caprine were obtained from six selected kebeles.

Sample size determination

The required sample size for the study animals was determined by the formula given by Thrusfield, assuming 95% of confidence interval and at 5% desired precision since there was no previous expected prevalence in the area, thus 50% expected prevalence of sheep and goat brucellosis in the study area.

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

Where: n=the required sample size,

Pexp=expected prevalence/previous prevalence,

d=desired absolute precision

Therefore, the sample size required for this study was 384 by using above formula. However, total number of sampled animals was increased to 423 for better accuracy.

Blood sample collection

Animals were restrained by owners and 8 ml of blood sample was collected from the jugular vein using disposable vacutainer tubes without anti-coagulant under aseptic condition. The samples were kept under the shade in a slant position for twenty-four hours. The sera sample was transferred to serum tubes (cryovial tube) and kept at -20°C until they will be tested. Corresponding to each sample, sample code, the age, species and gender of every animal's the information that registered on vacutainer tube, again registered on serum tubes (cryovial tube).

Questionnaire survey

Information on potential risk factors of small ruminant brucellosis was collected from 118 flock man's using close ended questionnaires. The potential risk factors considered during study period were flock size (small and medium), farming system (intensive, extensive and both), Presence of ram/buck (Yes, No), sources of breeding ram/buck (purchased, from other flock, born in flock), reproductive failure (Yes, No), types reproductive failure (retained placenta, abortion and still birth) and stage gestation period at reproductive failure occurred (0-3, 3-6 and 6-9 gestation period) were recorded. In addition to these, the awareness level of herd man about of brucellosis assessed were Level of education (illiterate, at least primary schools) and knowledge about brucellosis disease and it is transmission (yes, no) were recorded.

Serological testing

Screening test by Rose Bengal Plate Test (RBPT): All sera samples collected were initially screened by RBT using RBT antigen according to described procedures [4]. Then, sera and antigen were taken from refrigerator and left at room temperature for half an hour before the test to reach room temperature. For sheep and goats, in order to improve the sensitivity of RBT as previously recommended Nielsen, one volume of antigen and three volumes of serum (e.g. 25 ul with 75 ul) were used. The antigen and test serum were mixed thoroughly with a plastic applicator, shaken for 4 min and the result (presence of agglutination or not) was read immediately.

Confirmatory test by Indirect Enzyme Linked Immunosorbent Assay (I-ELISA): I-ELISA (BRUCELISA®) for detection of anti *Brucella* IgG antibodies was used as confirmation test. Accordingly, all RBPT positive sera were further tested using I-ELISA. The test was performed according to the manufacturer's instruction.

Data management and analysis

Data obtained from serological tests and questionnaires survey were entered in to Microsoft excel spreadsheet. A descriptive statically analysis was carried out using STATA version 11.0 for windows (stata corp. college station, TX, USA) to determine sero-prevalence small ruminant brucellosis. Pearson's *Chi-square* test (X^2) was used to study the association of potential risk factors with sero-prevalence of brucellosis. The degree of association between potential risk factors and sero-prevalence of brucellosis were determined using odd ratio. Statically significance was held at $p<0.05$. Animals tested positive to both RBPT and I-ELISA serially has been said to be sero-positive.

Results

Results of screening test by Rose Bengal Plate Test (RBPT)

Overall sero-prevalence in both ovine and caprine after screened by Rose Bengal Plate Test (RBPT) was 5.21% (22/422) (95% CI: 3.08-7.34%). In this study, the seroprevalence of brucellosis in studied Kebeles were higher in Kuya (8%) followed by Muri Turkuma (6.5%), Cari Gololcha (5%), Hire Lipichu (4.8%), Garba Oda (3.2%) and Bule Ano (2.6%) as shown. However, this difference had not statistically significant effect on seropositivity ($P>0.05$) as shown. Also the seroprevalence of brucellosis in female and male was 4.9% (n=16) and (6.3%) (n=6). However, this difference in seroprevalence was not statistically significant ($p>0.05$) as shown. On a species level, the prevalence of brucellosis in caprines was 9 (3.7%) from 243 tested caprine and 13 (7.3%) in ovine from 179 tested ovine shown. On Pearson's *Chi-square* test (x^2) analysis revealed that there was no association between prevalence brucellosis among species of animal as shown. On a gender level, the prevalence of brucellosis in female was 16 (4.9%) from 325 tested female animals and 6 (6.3%) from 95 tested male animals. However, this difference in seroprevalence was not statistically significant ($p>0.05$).

Results of confirmatory test by Indirect Enzyme Linked Immunosorbent Assay (I-ELISA)

Up on further testing the RBPT positive sera by an I-ELISA, was 0.71% (3/422) (95% CI: -0.094-1.52) were found seropositive. Thus, overall number of seropositive in both ovine and caprine in selected areas of West Guji zone was 0.71% (3/422) after RBPT and I-ELISA. On a species level, the prevalence of brucellosis in caprines was 2 (0.82%) from 243 tested caprine and 1 (0.56%) in ovine from 179 tested ovine shown. On Pearson's *Chi-square* test (x^2) analysis revealed that there was no association between prevalence brucellosis among species of animal as shown. The results revealed that among the risk factors considered in the analysis, species, gender, age, flock size and origin of animals spp (kebeles) had not statistically significant effect on seropositivity (P>0.05) as indicated (Figure 2).

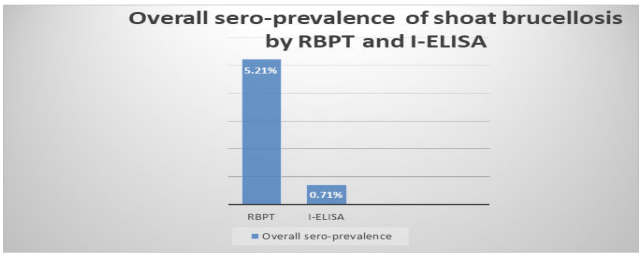

Figure 2: Overall sero-prevalence of sheep and goat brucellosis by RBPT and I-ELISA.

Results of questionnaires survey

A questionnaire survey was administered to 118 sheep and goat herders/owners for the purpose of evaluating the general communities awareness about the Brucellosis in the study districts. About 92% of the respondents were illiterate while 8% of them attended at least primary schools. All respondent (100%) interviewed had no knowledge about brucellosis disease and its transmission. Additionally, none of the respondents were separated animal species from others and they did not have knowledge of disease transmission from one species to other. About 96 % of interviewed agro pastoralists were responded there was abortion history in their herd and only 4% of respondent were responded no history of abortion in their herd one year ago. About (68%), 24% and 8% of respondents were responded the abortion mostly occurred at 3-6, 0-3 and 6-9 months of gestation period respectively (Tables 1-3 and Figure 3).

Question	Categories	No of respondents'	Percent (%)
Level of education	Illiterate	109	92%
	At least primary schools	9	8%
Knowledge about brucellosis	Yes	0	0%
	No	118	100%
Farming system	Intensive	17	14%
	Extensive	87	74%
	Both	14	12%
Separation animal species	Yes	0	0%
	No	118	100%
Presence of ram/buck	Yes	77	65%
	No	41	35%
Sources of breeding ram/buck	Purchased	24	20%
	From other flock	30	25%
	Born in flock	64	55%
Reproductive failure	Yes	53	45%
	No	65	55%
Types reproductive failure	Retained placenta	0	0%
	Abortion	113	96%

	Still birth	5	4%
Stage gestation period at reproductive failure occurred	0-3 gestation period	28	24%
	3-6 gestation period	80	68%
	6-9 gestation period	10	8%

Table 1: A questionnaire survey results regarding possible risk factors for occurrence of shoat brucellosis in and around Bule Hora district of Western Guji zone.

Risk factors	Category	No. of animals tested	RBPT positive%	I-ELISA positive	Prevalence
Kebeles	Kuya	75	6 (8%)	1	1.30%
	Muri Turkuma	77	5 (6.5%)	0	0
	Bule Ano	76	2 (2.6%)	0	0
	Cari Gololcha	100	5 (5%)	2	2%
	Garba Oda	31	1 (3.2%)	0	0
	Hire Lipichu	63	3 (4.8%)	0	0
Species	Caprine	243	9 (3.7%)	2	0.82%
	Ovine	179	13 (7.3%)	1	0.56%
Gender	Female	325	16 (4.9%)	2	0.62%
	Male	95	6 (6.3%)	1	1.05%
Age	Young (<3 years)	104	5 (4.8%)	0	0
	Adult (3-7 years)	318	17 (5.3%)	3	0.94%
Flock size	Small	370	21 (5.6%)	3	0.81%
	Medium	52	1 (1.92%)	0	0

Table 2: The result of sero prevalence of small ruminant brucellosis in Bule Hora woreda.

Risk factors	Category	No. of animals tested	Positive for RBPT	I-ELISA	X^2	P- value
kebeles	Kuya	75	6 (8%)	1 (1.3%)	4.5523	0.473
	Muri Turkuma	77	5 (6.5%)	0		
	Bule Ano	76	2 (2.6%)	0		
	Cari Gololcha	100	5 (5%)	2 (2%)		
	Garba Oda	31	1 (3.2%)	0		
	Hire Lipichu	63	3 (4.8%)	0		
Species	Caprine	243	9 (3.7%)	2 (0.82%)	0.1021	0.749
	Ovine	179	13 (7.3%)	1 (0.56%)		
Gender	Female	325	16 (4.9%)	2 (0.62%)	0.1982	0.656
	Male	95	6 (6.3%)	1 (1.05%)		

Age	Young (<3 years)	104	5 (4.8%)	0	0.9882	0.32
	Adult (3-7 years)	318	17 (5.3%)	3 (0.94%)		
Flock size	Small	370	21 (5.6%)	3 (0.81%)	0.4235	0.515
	Medium	52	1 (1.92%)	0		

Table 3: *Chi-square* analysis association between potential risk factor and prevalence of brucellosis.

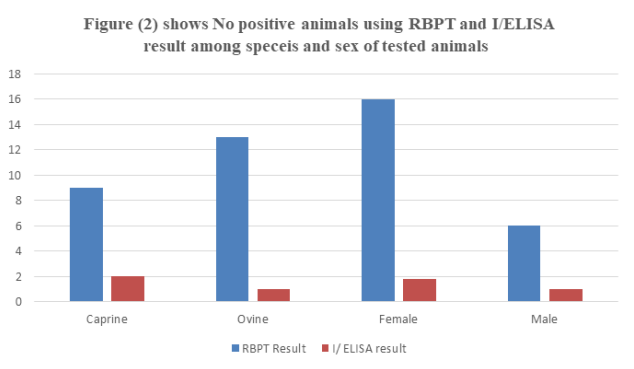

Figure 3: Result of RBPT and I/ELISA among species and gender.

Discussion

This study demonstrated that the overall seroprevalence of small ruminant brucellosis in and around Bule Hora district of western Guji Zone was 5.21% (22/422) (95% CI: 3.08-7.34%). By the RBPT and 0.71% (3/422) (95% CI: -0.094-1.52) by an I-ELISA. More than threefold of the sera which tested positive for RBPT, tested negative for an I-ELISA. This could be due to cross reactions between *Brucella* and other bacteria which share similar epitopes. Thus, the overall number of seropositive in both ovine and caprine in selected areas of West Guji zone during current study was 0.71% (3/422) after RBPT and I-ELISA. This prevalence is lower than prevalences recorded in previous studies carried out in different parts of the country; 16% in Afar region Muhie Yibeltal 13.7% in Afar region [5] 12.35% in Chifra and Ewa district of Afar region Tegegn, 9.7% in Afar and Somali Teshale Sori, 8.1% in Yabello districts Wubishet, 6.2% in Liban district of Guji zone Wubishet, 5% in sheep and 1.3% in goats in central highlands of Ethiopia Tekeleye Bekele and Kassali and 3.37%, 3.94% from Afar region and Borena zone respectively Melesse Balcha. The difference in the prevalence of brucellosis between the current and previous studies might be attributed to the differences in geographical location, sample size and management systems. In Afar, Borana zone and Somali Pastoralists, large numbers of different species of animals are raised on communal pastures under limited watering areas, where as the livestock management in the Bule Hora district Western Guji zone is characterized by mixed farming, in which fewer animals are raised separately, tied with rope around their own farm and no mixing of one herd with other. The other possible reason might be associated with breeding of small ruminants by healthy rams that as most respondent (55%) breeding rams that are born in flock they considered free from disease and mating of one ewe or due to only one ram in the herd for breeding purpose.

The present study is coinciding with that of Tewodros and Dawit who found 0.7% prevalence of small ruminant brucellosis in and Around Kombolcha district of Amhara region. The current study prevalences is relatively higher than prevalence recorded in previous studies carried out in Somali region (0.11%) and south Omo zone (0.49%) by Melesse Balcha. The difference in the prevalence of brucellosis between the current and previous studies might be attributed to the differences in geographical location, sample size and test used. It is possible that this might be due to variation in animal management and production systems.

The study demonstrated that from a total of 422 sera tested, only 1 ovine and 2 caprine were found sero-positive for I-ELISA. This relatively higher prevalence of brucellosis in caprine as compared to ovine might be mainly due to the difference in the pattern of grazing between both species and the greater susceptibility of caprine to *Brucella* infection than ovine [6]. The difference in sero-positivity between sheep and goats might also be because of the difference in the number of the animals which were sampled and tested for brucellosis. Partly it could be due to the fact that sheep are not excreting the organism for longer period of time unlike goats. This can reduce the potential of the spread of the disease among sheep population [7]. However, the analysis revealed that there was no statistically significant ($p>0.05$) difference among two species. Which agree with founding of Tewodros and Dawit and Tadeg.

The study demonstrated that from a total of 422 sera tested, only 1 male and 2 females were found sero-positive for I-ELISA. Female sero reactor was high relatively than male. It could probably due to the small number of males (n=95) sampled and tested as compared to the large number of females (n=325) however, females are more susceptible than males [8]. In *B. melitensis* infection, males of sheep and goats are less susceptible than females [9]. Hirsh et al, have also reported that male animals are less susceptible to *Brucella* infection due to the absence of erythritol the sugar that found in uterus of pregnant female. In addition to these, some studies also reported that serological response of male animals is limited and thus infected animals are usually observed to be non-reactors or show low antibody titter [10,11]. However, the analysis revealed that there was no statistically significant ($p>0.05$) difference among two categories.

From the questionnaire survey, almost all small ruminant owners residing in the study area were able to recognize the occurrence of abortion in their flocks. About 96 % of interviewed agro pastoralists were responded there was abortion history in their herd and only 4% of respondent were responded no history of abortion in their herd one year ago.

Conclusion

The overall prevalence of brucellosis in small ruminants in Bule Hora district was 0.71% (3/422). Such findings in the absence of any vaccination against brucellosis indicate the occurrence of natural infection. In conclusion, even though the overall prevalence that was found during the study period is low, the findings still have the power

to indicate the presence of the problem in the area. The existence of the disease in the study area has possible risk of spread in the future.

Recommendations

Based on above conclusion, the following recommendation is forwarded:

- Elimination of positive reactors will provide better considerable success in the control of brucellosis in small ruminant.
- Quarantine of imported small ruminant and also movement control newly purchased animals.
- Disposal of aborted foetuses, fetal membrane and discharges with subsequent disinfection of contaminated area.
- All placentas and dead foetuses should be buried as a routine practice.
- Vaccination of herds/flock. The experience from all over the world, that vaccination is in most situations the only practical method of control of brucellosis in sheep and goats.

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