International Journal of Medical Science in Clinical Research and Review
Online ISSN: 2581-8945
Available Online at http://www.ijmscrr.in Volume 3|Issue 04 (July-August) |2020 Page: 147-156
Original Research Paper

Prevalence and associated risk factors of brucellosis in sheep and goat from South Omo Zone, Ethiopia

Authors:
Feyera Gemeda Dima[1,2*], Youcef Shahali[3], Maryam Dadar [3]
[1,2]*Cellphone: +251944184915; ORCID: 0000-0002-6631-653X*
[1] *College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia.*
[2]*Addis Ababa University, Aklilu Lemma Institute of Pathobiology, Addis Ababa, Ethiopia.* [3]*Razi Vaccine and Serum Research Institute (RVSRI); Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.*
Corresponding Author:
Feyera Gemeda Dima
Article Received 21-05-2020, Accepted 22-06-2020, Published 27-07-2020

ABSTRACT:
Brucellosis is known as a highly contagious zoonotic bacterial disease, with a significant effect on the livestock industry. It is caused by several species of cocco-bacilli Gram-negative bacteria from the Genus *Brucella*, and distributed worldwide including Ethiopia. However, there was a scarcity of epidemiological data on its occurrence in pastoral areas. A cross-sectional investigation was performed from September 2018 to June 2019, to estimate the seroprevalence of brucellosis and to characterized main *Brucella* isolates infecting small ruminants in two randomly selected pastoral districts of the South Omo Zone, Ethiopia. A pre-tested questionnaire was used and collected data were subjected to statistical analyses (multivariate logistic regression). For the serological test, blood samples were drawn from a total of 124 small ruminants with a history of abortion. Subsequently, 30 vaginal swabs were sampled from seropositive animals for *Brucella* isolation. All sera were first analyzed serologically by a modified Rose Bengal Plate Test (mRBPT) and *Brucella* seropositivity was further confirmed by Complement Fixation Test (CFT). The brucellosis seroprevalence among small ruminants with a history of abortion was 21% (26/124; 95% CI: 0.14 - 0.28). Multivariable logistic regression analysis showed that the main risk factors related to *Brucella* spp. infections were history of abortion (OR: 0.28, 95% CI: 0.18 - 0.43) and parity numbers (OR: 0.20, 95% CI:- 0.72). *Brucella* spp. were also isolated from 5 (16.7%) of the 30 vaginal swabs cultured on *Brucella* Selective Agar. The isolates were identified as *B. melitensis* based on biochemical and bacteriological culture results. In conclusion, this study revealed the high prelance of brucellosis in small ruminants in the studied area. Therefore, regular testing of breeding animals is necessary to reduce brucellosis and its economic impact in the region.

Keywords: Abortion; Risk factor; B. melitensis, seroprevalence, small ruminants

INTRODUCTION:

Brucellosis is a multi-species infectious and contagious bacterial disease causing economic losses for livestock production industry in many developing countries worldwide (Corbel 1997, Nielsen 2018). It is the most common zoonosis worldwide with over 500,000 cases every year, often considered as a neglected hazard for the public health (Dadar et al. 2019b, Pal et al. 2017). Despite efforts made to establish brucellosis control program in different countries, it still represents an endemic disease in several regions worldwide, including the Central Asia, Middle East, Mediterranean region, and parts of Africa, Latin America (Franc et al. 2018). Brucellosis is responsible for reproductive losses in livestock animals, that are commonly caused by *Brucella melitensis* or *B. ovis* in small ruminant, *B. abortus* in cattle, *B. suis* in pigs, and *B. canis* in dogs. Small ruminant brucellosis is the most common bacterial zoonosis in low-income countries including Ethiopia (Tewodros and Dawit 2015). *Brucella melitensis* is the main species infecting small ruminants. Despite the economic losses incurred and the wide spread distribution of small ruminant brucellosis in Ethiopia, especially in South Omo Zone, less attention has been paid to the spread of the disease in pastoral areas. The accuracy of the diagnostic tests of brucellosis is an

© Feyera Gemeda Dima**et al.**

essential component in the success of test, eradication and control strategies. The isolation and identification of *Brucella* species in small ruminants are essential in these areas where livestock and pastoralists have close contact in their daily life. This could help authorities and decision makers to plan disease control and appropriate prevention strategies (Tewodros and Dawit 2015). This represents an important challenge as brucellosis is endemic in Ethiopia and commonly causes retained fetal membrane, abortion, as well as infection of the accessory sex gland and orchitis in males. It is a widely distributed neglected zoonotic disease, with poor awareness among the community, and cause serious economic losses in small ruminants' production industry (Ashagrie et al. 2011, Bugeza et al. 2019, Corbel 1997). The incidence of brucellosis is generally considered higher in pastoral settings of Africa. However, because of the difficulty to access pastoral communities, the occurrence and the control of brucellosis is poorly understood both in humans and their animals in the pastoral settings of the sub-Saharan Africa where the burden of the disease could be high (McDermott and Arimi 2002). In Ethiopia, small ruminants are the main source of livelihood for small holders under extensive pastoral production system (Ashagrie, Deneke and Tolosa 2011). However, Ethiopia fails to optimally utilize this resource and brucellosis significantly affects livestock productivity. In Ethiopia, several studies showed individual seroprevalence ranging from 0.1–15.2% in different parts of the country (Ashagrie, Deneke and Tolosa 2011, Asmare et al. 2010, Haileselassie et al. 2010, Mohammed et al. 2017) and most of them are largely confined to serological surveys. Although, isolation of *Brucella* species is the gold standard for the identification and confirmation of animal brucellosis, there is little research done to isolate and identify causative agents in Ethiopia. A recent study showed poor community's knowledge about brucellosis and high risk for *Brucella* infection among pastoral communities of South Omo Zone (Ashagrie, Deneke and Tolosa 2011). Therefore, the aim of the current study was to evaluate, the prevailing *Brucella* species and the assessment of potential risk factors of *Brucella* infections among small ruminants in South Omo Zone.

MATERIALS AND METHODS:

Study area and sampling methods:

Two districts (Nyangatom and Dasenech) of South Omo Zone, in the Ethiopian were selected for this study (Figure 1). These areas were selected because of several factors like: the absence of enough data on the status of brucellosis, large population of small ruminants in the areas and source of the export animals, poor livestock management practices (no constructed houses for small ruminants), seasonal mixing of flocks of different origins (Ethiopia 2006). The target animals for this study were small ruminants of the Nyangatom and Dassenech districts in South Omo Zone. The sample size for serological study was estimated in accordance with previous reports on the seroprevalence of *Brucella* infection in aborted small ruminants (Ashagrie, Deneke and Tolosa 2011). A total of 124 small ruminants (sixty-two small ruminants from each district) were considered for this study, while parallel to this, a number of milk and vaginal swabs were sampled from sheep and goats for bacteriological culture. From each of the two districts, three sub districts were included in this study. These sub districts were selected using simple random.

Sampling Technique:

Blood, milk and vaginal swab samples collection

Blood samples (86 goats and 38 sheep), milk (8 specimens) and vaginal swab (24 specimens) were collected from selected districts in South Omo zone. The serum samples were collected and tested for *Brucella* spp. infectious. Swab samples were achieved with sterile applicator stick in Ames with Charcol Transport Medium (HiMedia, Mumbai, India). Similarly, milk sample were collected aseptically after washing, drying and disinfecting the whole udder and teats. Informed consent for questionnaire administration on the risk factors was handled to 124 interviewees for 124 small ruminants (86 goats and 38 sheep).

Questionnaire survey:

A pre-tested questionnaire on the risk factors was handled by 124 interviewees for 124 small ruminants (86 goats and 38 sheep). The potential risk factors for brucellosis considered in this study included animal species, age, body condition, abortion history, frequency of abortion and parity number. Furthermore, the awareness and the way of prevention and control of the diseases were interviewed among animal owners.

Laboratory analysis

Serological analysis:

All serum samples were screened by modified Rose Bengal Plate Test (mRBPT) and Complement Fixation Test (CFT) following standard procedures(Alton et al. 1988b). For mRBPT, the agglutinations were recorded as 0, +, ++ and +++, according to the agglutination degree (Nielsen 2018). Then, confirmation test by CFT was done on positive sera by standard *B. abortus* antigen S99 according to the guideline of the World Organization for Animal Health. (https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdf)

.

Bacteriological Analysis:

Bacteriological tests were carried out under Biosafety

level three (BSL3) with high personal protections at the Brucellosis laboratory. All individual milk and vaginal samples from serologically positive animals were subjected to bacterial culture using *Brucella* selective supplement (HiMedia, Mumbai, India) with selective antibiotics supplement (FD005) (HiMedia, Mumbai, India) and inactivated 5% horse serum in *Brucella* agar (Himedia, Mumbai, India). Bacterial culture was carried out with 10% CO2 in 37°C for 10 days. The centrifuge of milk samples was done at 6000 rpm for 15 minutes and afterwards, the upper layer of cream and sediments were smeared separately on *Brucella* agar plates (Dadar et al. 2019a). After 10 days of incubation, the bacterial cultures were discarded if no growth was visible. Typical *Brucella*- suspected colonies were subjected to further biochemical analysis.

Classical Biotyping:
Classical biotyping such as growth in media containing basic fuchsin and thionin, CO2 dependence, H2S production, agglutination by acriflavin, lysis by specific phages, and agglutination with specific *Brucella* antisera monospecific antisera A and M, were done according to Alton et al by a panel of biotyping tests, (Alton et al. 1988a). The results of biotyping were reported according to the OIE guideline (http://www.oie.int/en/animal-health-in- the-world/animal-diseases/Brucellosis/).

Biochemical tests:
Further biochemical characterization of the organism included oxidase test, catalase test, nitrate reduction test, urea hydrolysis, hydrogen sulphide (H2S) production and hemolyisis on blood agar. The growth of *Brucella* isolates were also evaluated in the presence of thionin and basic fuchsin dyes (incorporated at 20 to 40 µg/ml) and carbon dioxide (Dadar, Alamian, Behrozikhah, Yazdani, Kalantari, Etemadi and Whatmore 2019a, Garin-Bastuji et al. 2006).

Ethical Consideration:
Ethical clearance was achieved from Institutional Review Board of Addis Ababa University, Aklilu Lemma Institute of pathobiology (ALIPB IRB/019/2011/2019). The protocol for field studies and collection of samples from animals was approved by Nyangatom and Dassenech District's agricultural and veterinary authorities of South Omo Zone and informed consents from livestock owners were obtained following ethical requirements.

Data Analysis:
Statistical analysis (multivariate logistic regression) was done using Stata version 14. Prevalence was computed by dividing the number of positive cases by the total sample size multiplied by 100. The Chi-square ($\chi 2$) and logistic regression tests was performed to identify the association between different risk factors and *Brucella* infection in animals. The degree of association was considered as significant when a *P-value* of less than 0.05 is obtained or according to the 95% confidence intervals of the odds ratio in the multivariable logistic regression analysis (Thrusfield 2018).

Results

Seroprevalence:
The seroprevalence was 24% (95% CI: 0.17- 0.32) using mRBPT while it was 21% (95% CI: 0.14 - 0.28) by CFT tests. Thus, the overall seroprevalence of *Brucella* infection in aborted small ruminants in Nyangatom and Dassenech districts of South Omo Zone was 21 % by the combined mRBPT and CFT tests.

Potential risk factors of small animal *Brucella* infection
The analysis of the association between environmental factor and *Brucella* infection, on the basis of the combined mRBPT and CFT results, was done using Pearon's chi-square and Fisher's exact tests (Table 1). There was a significant association between the locality of the kebeles (pastoral areas) and *Brucella* seroreactivity ($P < 0.05$). Among kebeles, a high percentage of seropositivity was observed in charrii (32%), while lobot (0.2%) and nikiya (0.2%) were affected at lesser extent. Age, body conditions, frequency of abortion and parity status were significantly associated to *Brucella* seropositivity, but no association was shown with animal species and gender ($P <0.05$). The difference in seropositivity in chi square and logistic regression among the variable indicates, there were confounding factors (age, body condition, and frequency of abortion) in this study, as they were only significant with chi square, but not when compared with Multivariate logistic regression analysis (confidence interval of their Odds ratio included 1).

Table 1: Association of risk factors with *Brucella* seropositivity in small ruminants, Nyangatom and Dassenech districts of south Omo Zone.

Variables	N/Tested	Seropositive	Prevalence (%)	χ^2- value	*P-Value*
Past Assoc (Kebeles)					
Kakuta	22	6	27.3	1.298 [b]	0.255
Lorekacho	20	2	10	0.782 [a]	0.676
Charrii	22	7	32	13.367[b]	0.000
Lobot	20	4	20	5.971[b]	0.015
Nikiya	20	4	20	3.856[b]	0.050
Trongole	20	3	15	2.599 [b]	0.107
Species				1.152 [b]	0.283
Caprine	86	20	23.3		
Ovine	38	6	16		
Age (Years)				3.19[b]	0.026*
≤ 3	18	3	17		
>3-5	38	3	7.9		
> 5	68	18	26.5		
Body condition				14.410 [b]	0.001*
Good	28	11	39.3		
Medium	33	1	3		
Poor	63	14	22.22		
Frequency of abortion				1.115[b]	0.003
Once	86	2	2.33		
> One times	38	24	63.2		
Parity no				6.15[b]	0.046*
Monoparous	7	4	57.14		
Pluriparous	117	22	19		
Total	**124**	**26**	**21**		

From the above table (Table 1); χ^2: Chi-Square; [a]Fishe's exact value; [b]Pearson's chi-square value; *Significant; N/tested: number of animals tested; Past Assoc: pastoral association

According to the multivariable logistic regression model fitted (Table: 2) many of the putative risk factors considered: district (animal location), species, age, body condition, frequency of abortion and parity status appeared to be significantly related to *Brucella* seropositivity. Small ruminants in age group ≤3 years (OR=0.06, 95% CI: 0.07-0.44) were likely to be at higher risk for *Brucella* infection than animals in >3-5 and >5 years' age groups. The multivariate analysis also revealed that increased parity of sheep and goats was more likely to be associated with an increasing risk of getting *Brucella* infection when evaluated collectively with other factors. Thus, animal with multiple parturition were at higher risk of encountering *Brucella* infection (OR=0.50, 95% CI: 0.399–0.63) than monoparous animals.

Table2: Multivariable logistic regression analysis of risk factors associated with *Brucella* Seropositivity

Variables	N/Tested	Infected	Crude OR (CI 95%)	Adjusted OR(CI 95%)	*P-Value*
PastAssoc (Kebeles)					
Kakuta	22	6(27.2%)	1[a]	1.5(0.67- 3.34)	0.321
Lorekacho	20	2(0.1%)	1[a]	0.999(0.45 -2.23)	1.000
Charrii	22	7(32%)	1[a]	0.087(0.02- 0.037)*	0.001*
Lobot	20	4(0.2%)	1[a]	0.062(008 - 0.471)*	0.007*
Nikiya	20	4(0.2%)	1[a]	0.067(0.009-0.50)*	0.009*
Trongole	20	3(0.15%)	1[a]	0.07(0.009 - 0.54)*	0.011*
Species				**0.52(0.15-1.74)**	**0.287**
Caprine	86	20(23.3)	1[a]	0.32(0.198-0.528) *	0.000*
Ovine	38	6(16)	1[a]	0.43 (0.28–0.67)*	0.000*
Age (Years)				**2.01(0.97-4.17)**	
≤ 3	46	3(17%)	1[a]	0.06(0.07-0.44)*	0.006*
> 3-5	10	3(7.9%)	1[a]	0.52 (0.35–0.76)*	0.001 *
> 5	68	18(26.5%)	1[a]	0.69(0.58-0.83)	0.000*
Body condition				**0.63(0.36-1.12)**	

© Feyera Gemeda Dima**et al.**

Good	28	11(39.3%)	1[a]	0.75(0.35 - 1.56)	0.451
Medium	33	1(3%)	1[a]	0.17 (0.06 - 0.47)*	0.001*
Poor	63	14(22.2%)	1[a]	0.66(0.54 - 0.81)*	0.000*
Frequency of abortion				**0.67(0.22-2.01)**	
Once	86	2(2.33%)	1[a]	0.32 (0.19 - 0.52)*	0.000*
> One times	38	24(63.2%)	1[a]	0.45(0.29 - 0.69)*	0.000*
Parity no				**0.2(0.059-0.72)**	
Monoparous	7	4(57.14%)	1[a]	1.33 (0.298–5.96)	0.706
Pluriparous	117	22(19%)	1[a]	0.50(0.399 - 0.63)*	0.000*
Total	**124**	**26(21%)**	1[a]	0.50(0.399 - 0.63)*	0.000*

***Significant; OR: Odds Ratio; CI: Confidence Interval, aReference. N/tested: number of animals tested; Past Assoc: pastoral association.**

Isolation and identification of *Brucella* spp.

From a total of 124 seropositive clinical samples, 8 milk and 22 vaginal swabs were subjected to bacteria culture and further characterized using conventional biochemical methods. The result showed that 16.7% (5/30) of samples were positive for the *Brucella* spp.. All isolates, were from vaginal swabs of 4 goats (13. 3 3 %) and 1 sheep (3. 33 %), while no isolates were found from milk samples (Table 3). The isolates were initially recognized on the basis of colony morphology which was characteristic of *Brucella* spp. with very small, smooth, glistening, pin-point and round like colonies with honey like appearance. Microscopic examination showed small Gram- negative coccobacilli arranged singly and in pairs. The Modified Ziehl-Neelsen (MZN) stain showed the *Brucella* organisms as red on a blue background. The identified isolated bacteria showed phenotypic features of *Brucella* spp. All isolates grew in 10 % carbon dioxide (CO2) after 5- day incubation at 37°C. The isolates were Gram negative with very small, smooth, glistening, pin-point and round like colonies with honey like appearance. The isolates were further differentiated by biochemical analysis which led to the characterization of *B. melitensis* in vaginal swap samples of sheep and goat. *Brucella* i solates were found to be positive for urea hydrolysis, nitrate reduction tests, oxidase, and catalase.

Table 3: *Brucella* isolates recovered from seropositive sheep and goats' vaginal swabs and milk.

Type of sample	No of sample cultured	No. of isolates	Percentage (%)
Vaginal swab	22	5	22.7
Milk	8	0	0
Total	**30**	**5**	**16.7**

DISCUSSION:

Small ruminants are considered as an important reservoir of *Brucella* spp. in many areas worldwide. Long-term serological screening programs and culling of infected animals along with mass vaccination campaigns represent an effective strategy to control brucellosis among small ruminant (McDermott and Arimi 2002, Musallam et al. 2015). Given the important role of small ruminants in the economy of many countries, different risk factors related to sheep and goat brucellosis should be carefully identified at regional level (Ashagrie, Deneke and Tolosa 2011, Bekele et al. 2013, Bugeza, Muwonge, Munyeme, Lasuba, Godfroid and Kankya 2019, Igawe et al. 2020, Tsegay et al. 2015). Among them, animal species, age, body condition, frequency of abortion, and parity are of critical importance (Kebede et al. 2008, Tsegay, Tuli, Kassa and Kebede 2015). This study showed that the seroprevalence of brucellosis in small ruminants having abortion is high, reaching 21% (n=26) in South Omo Zone of Ethiopia. Previous reports in Ethiopia reported the overall seroprevalence of brucellosis in small ruminant as 7.52% in Afar Region (Tekle 2016), 9.6% in Yabello pastoral Area (Yohannes et al. 2013) and 9.11% in Dire Dawa(Tekle 2016). Various factors influence the spread of the disease in livestock including animal husbandry, communal grazing of range lands and watering areas as well as the influence of climatic conditions (Dadar et al. 2020, Teshale et al. 2006). The prevalence reported in this study using CFT was among animals that had history of recent abortion, thereby increasing the prevalence rate for brucellosis, since *Brucella* is among the major causes of abortion in small ruminants (Asmare, Asfaw, Gelaye and Ayelet 2010, Bugeza, Muwonge, Munyeme, Lasuba, Godfroid and Kankya 2019, Franc, Krecek, Häsler and Arenas- Gamboa 2018). In the same study area, the overall prevalence of b r u c e l l o s i s in small ruminants was reported around 4% (Ashagrie, Deneke and Tolosa 2011). In Somali pastoral areas, Teshale et al. reported a seroprevalence of 1.7% in goat and 1.6% in sheep (Teshale, Muhie, Dagne and Kidanemariam 2006). Another study also reported seroprevalences of 1.3% in goat and 1.5% in sheep in central highlands of Ethiopia (Tekle 2016). However, most of these prevalence studies used standard Rose Bengal Plate Test (RBPT) for screening, while our study used mRBPT. This simple modification is achieved by increasing the amount of sera for the test dose from 25 to 75 µl, while maintaining the antigen volume at 25 µl. This may significantly increase the sensitivity of the test without affecting the specificity (Blasco et al. 1994, Ferreira et al. 2003). All other risk factors considered in this study including agroecological location, age groups, body conditions, frequency of abortion and parity numbers were found to be associated with *Brucella* infection in small ruminants. Brucellosis is a disease mainly affecting sexually matured animals. Our results also showed that the age categories above three years were more likely to be seropositive in comparison with young animals (less than one year). This study also showed that the frequency of abortions and parity was significantly associated with seropositivity for *Brucella* infection in the studied animals. This indicates that abortions or stillbirths and retained placenta are typical outcomes of brucellosis in the region (Mugizi et al. 2015, Tekle 2016, Tsegay, Tuli, Kassa and Kebede 2015). The prevalence of brucellosis among small ruminant with a history of abortion revealed a significant difference ($P<0.05$) in different pastoral *kebeles*. The highest seroprevalence was reported for the Charrii followed by Kakuta, Lobot, Nikiya, Trongole and Lorekacho. Furthermore, age, frequency of abortion and parity status showed significant relevance with *Brucella* seropositivity in multivariable logistic regression analysis. Seropositivity for *Brucella* infection increases approximately 6 times in animals with age > 5 years old when compared to ≤ 3 and >3–5 years old. This is in accordance with several previous studies showing an elevated seroprevalence of brucellosis in adult groups of small ruminants (Ashenafi et al. 2007, Bekele, Tessema and Melaku 2013). The multivariate analysis also revealed that increased parity of sheep and goats was associated with an increasing risk of *Brucella* infection. Thus, animals with multiple parturition were at higher risk for encountering *Brucella* infection when compared to monoparous animals (OR=0.50, 95% CI: 0.399-0.63). Interestingly, multivariable logistic regression revealed that the risk of seropositivity was approximately 5.5 times higher in pluriparous animals when compared to monoparous animals. This result also is in accordance with the findings of Ashagrie that reported that higher parity played an effective role in the spread of brucellosis (Ashagrie, Deneke and Tolosa 2011). On the other hand, isolation of *Brucella* species is the gold standard for identification and confirmation of animal brucellosis. However, most surveys of brucellosis in Ethiopia rely on serological tests only, and there is little evidence for bacteriological isolation of *Brucella* species. To the best of our knowledge, only one study reported the isolation of *B. meltensis* from aborted goats in Afar Region (Tekle 2016). In the present study, all isolates were obtained from vaginal swabs, while no isolates were recovered from milk. Shedding of *Brucella* organisms through body secretion was an important source of infection in humans (Dadar, Shahali and Whatmore 2019b). The isolation of *Brucella* spp. form vaginal swabs is very important from an epidemiological standpoint since farmers have no personal protection to pull out the retained placenta and they have direct contact with the placenta fluids. This stresses the need to coordinated

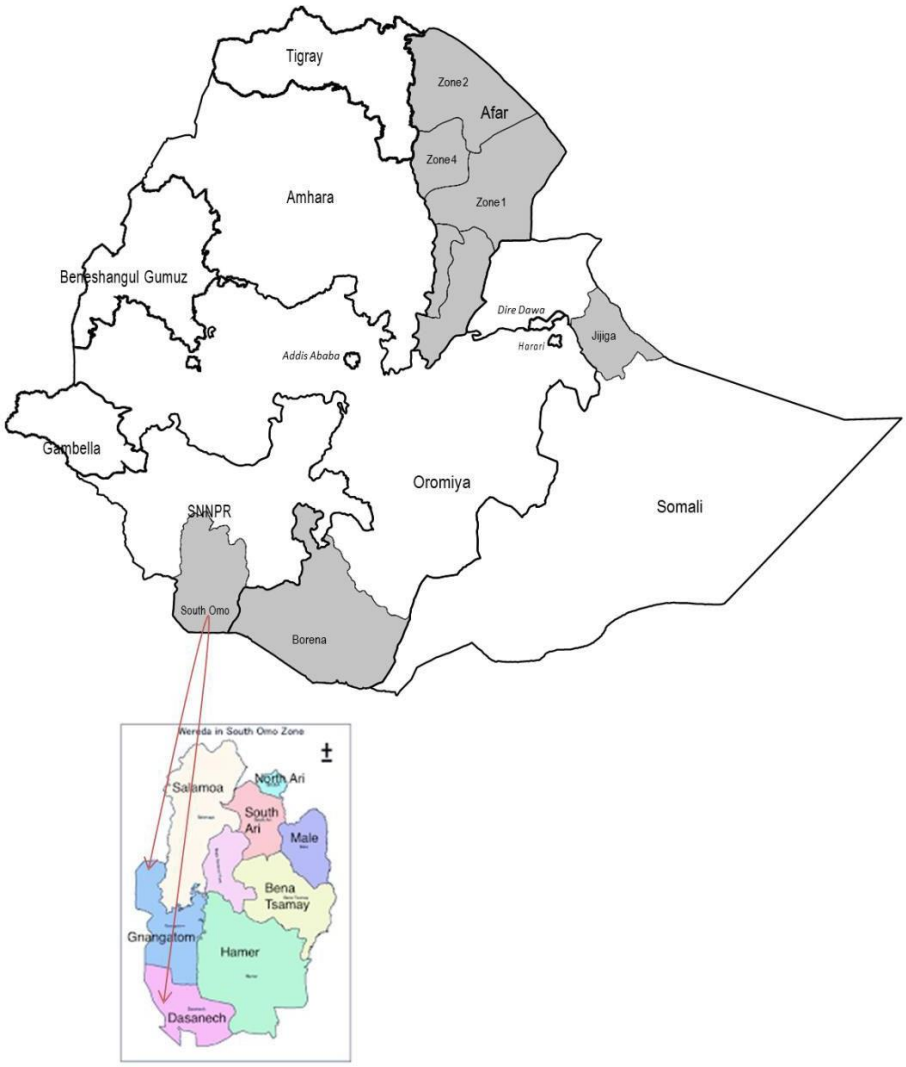
brucellosis prevention strategies for human and animals (Habtamu et al. 2015). Based on biochemical characterization, *B. melitensis* was recovered from 22.7% (5/22) of vaginal swabs. This is in accordance with results obtained by Tekle et al. (2016), in Afar Region (Ethiopia), where 6 out 28 (21.43%) vaginal swabs were infected by *B. melitensis* (Tekle 2016).

CONCLUSION:

This study revealed that the history of abortion and parity numbers were remarkably associated to *Brucella* infection in pastoral areas of Ethiopia. Animal owners have poor awareness on brucellosis and proper management of the animals could considerably help to control the spread of the disease in these regions. Among, key features that may aid to improve farmer safety and reduce the transmission of the disease, regular cleaning of the housings and the safe disposal of aborted materials appeared to be essential. In order to prevent brucellosis transmission within the flock or to the other healthy flocks, the regular screening of small ruminants for brucellosis along with the vaccination of animals after birth should be implemented in pastoral areas.

Acknowledgements:

The authors wish to thank their respective institutions as well as Dr. Youcef Shahali, Dr. Maryam Dadar, Prof Gobena Ameni, Dr. Abebe Animut and Dr. Tadesse Eguale for their precious scientific and technical supports to this study.



REFERENCES:

1. Alton, G.G., Jones, L.M., Angus, R., Verger, J., 1988. Techniques for the brucellosis laboratory.
2. National Institute for agronomic Research (INRA).
3. Ashagrie, T., Deneke, Y., Tolosa, T., 2011. Seroprevalence of caprine brucellosis and associated risk factors in South Omo Zone of Southern Ethiopia. Afr J Microbiol Res5, 1682-1685.
4. Ashenafi, F., Teshale, S., Ejeta, G., Fikru, R., Laikemariam, Y., 2007. Distribution of brucellosis among small ruminants in the pastoral region of Afar, eastern Ethiopia. Revue scientifique et technique26, 731.

5. Asmare, K., Asfaw, Y., Gelaye, E., Ayelet, G., 2010. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. African Journal of Agricultural Research5, 257-263.
6. Bekele, W.A., Tessema, T.S., Melaku, S.K., 2013. Camelus dromedarius brucellosis and its public health associated risks in the Afar National Regional State in northeastern Ethiopia. Acta veterinaria scandinavica55, 89.
7. Berhe, G., Belihu, K., Asfaw, Y., 2007. Seroepidemiological investigation of bovine brucellosis in the extensive cattle production system of Tigray region of Ethiopia. International Journal of Applied Research in Veterinary Medicine5, 65.
8. Blasco, J., Garin-Bastuji, B., Marin, C., Gerbier, G., Fanlo, J., Jiménez de Bagués, M., Cau, C., 1994. Efficacy of different Rose Bengal and complement fixation antigens for the diagnosis of Brucella melitensis infection in sheep and goats. Veterinary Record134, 415- 420.
9. Bugeza, J., Muwonge, A., Munyeme, M., Lasuba, P., Godfroid, J., Kankya, C., 2019. Seroprevalence of bovine brucellosis and associated risk factors in Nakasongola district,Uganda. Tropical animal health and production51, 2073-2076.
10. Corbel, M.J., 1997. Brucellosis: an overview. Emerging infectious diseases3, 213.
11. Dadar, M., Alamian, S., Behrozikhah, A.M., Yazdani, F., Kalantari, A., Etemadi, A., Whatmore, A.M., 2019a. Molecular identification of Brucella species and biovars associated with animal and human infection in Iran. In, Veterinary Research Forum, 315-320.
12. Dadar, M., Shahali, Y., Whatmore, A.M., 2019b. Human brucellosis caused by raw dairy products: A review on the occurrence, major risk factors and prevention. International journal of food microbiology292, 39-47.
13. Ethiopia, C., 2006. Agricultural Sample Survey, 2004/2005 (1998 EC): Report on Livestock and Livestock Characteristics. Statistical Bulletin361.
14. Fensterbank, R., 1987. Some aspects of experimental bovine brucellosis.
15. Ferreira, A., Cardoso, R., Dias, I., Mariano, I., Belo, A., Preto, I., Manteigas, A., Fonseca, A., De Sá, M.I.C., 2003. Evaluation of a modified Rose Bengal test and an indirect Enzyme- Linked Immunosorbent Assay for the diagnosis of Brucella melitensis infection in sheep.
16. Franc, K., Krecek, R., Häsler, B., Arenas-Gamboa, A., 2018. Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action. BMC public health18, 125.
17. Garin-Bastuji, B., Blasco, J., Marin, C., Albert, D., 2006. The diagnosis of brucellosis in sheep and goats, old and new tools. Small Ruminant Research62, 63-70.
18. Habtamu, T., Richard, B., Dana, H., Kassaw, A., 2015. Camel brucellosis: its public health and economic impact in pastoralists, Mehoni district, Southeastern Tigray, Ethiopia. Journal Microbiol Res5, 149-156.
19. Haileselassie, M., Shewit, K., Moses, K., 2010. Serological survey of bovine brucellosis in barka and arado breeds (Bos indicus) of western Tigray, Ethiopia. Preventive Veterinary Medicine94, 28-35.
20. Kebede, T., Ejeta, G., Ameni, G., 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue de Médecine Vétérinaire159, 3.
21. McDermott, J.J., Arimi, S., 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Veterinary microbiology90, 111-134.
22. Mohammed, M., Mindaye, S., Hailemariam, Z., Tamerat, N., Muktar, Y., 2017. Sero-Prevalence of Small Ruminant Brucellosis in Three Selected Districts of Somali Region, Eastern Ethiopia. J Vet Sci Anim Husb5, 105.
23. Mugizi, D.R., Boqvist, S., Nasinyama, G.W., Waiswa, C., Ikwap, K., Rock, K., Lindahl, E., Magnusson, U., Erume, J., 2015. Prevalence of and factors associated with Brucella sero- positivity in cattle in urban and peri-urban Gulu and Soroti towns of Uganda. Journal of Veterinary Medical Science, 14-0452.
24. Nielsen, K., 2018. Animal brucellosis. CRC press.
25. Pal, M., Gizaw, F., Fekadu, G., Alemayehu, G., Kandi, V., 2017. Public health and economic importance of bovine Brucellosis: an overview. American Journal of Epidemiology5, 27- 34.
26. Tekle, M., 2016. Seroprevalence of Brucellosis and Isolation of Brucella from Small Ruminants That

Had History of Recent Bbortion in Selected Kebeles of Amibara District, Afar region, Ethiopia. Addis Ababauniversity.
27. Teshale, S., Muhie, Y., Dagne, A., Kidanemariam, A., 2006. Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia: the impact of husbandry practice. Revue de médecine vétérinaire157, 557.
28. Tewodros, A., Dawit, A., 2015. Sero-Prevalence of Small Ruminant Brucellosis in and around Kombolcha, Amhara Regional State, North-Eastern Ethiopia. J. Vet. Sci. Med. Diagn4.
29. Thrusfield, M., 2018. Veterinary epidemiology. John Wiley & Sons.
30. Tsegay, A., Tuli, G., Kassa, T., Kebede, N., 2015. Seroprevalence and risk factors of Brucellosis in small ruminants slaughtered at Debre Ziet and Modjo export abattoirs, Ethiopia. The Journal of Infection in Developing Countries9, 373-380.
31. Yohannes, M., Degefu, H., Tolosa, T., Belihu, K., Cutler, R.R., Cutler, S., 2013. Brucellosis in Ethiopia. African Journal of Microbiology Research7, 1150-1157.