Libyan Agriculture Research Center Journal International 3 (2): 53-59, 2012
ISSN 2219-4304

DOI: 10.5829/idosi.larcji.2012.3.2.62119

Survey of Bovine Brucellosis in Pastoral and Agro-pastoral Production Systems of Borana Zone, Southern Ethiopia and its Public Health Implications

[1]Bedane Adane, [2]Kamil Kemal, [2]Feyissa Begna,
[3]Fanos Tadesse, [4]Demelash Biffa and [5]Asseged Bogale

[1]Oromia Agricultural Research Institute,
Yabello Pastoral and Dry land Agriculture Research Center, Yabello, Ethiopia
[2]Jimma University, College of Agriculture and Veterinary Medicine, Jimma, Ethiopia
[3]Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia
[4]University of Arizona, College of Medicine 1656 E. Mabel, Tucson, Arizona 85719, USA
[5]College of Veterinary Medicine, Nursing and Allied Health,
Tuskegee University, Williams-Bowie Bldg 114, Tuskegee, AL 36088, USA

Abstract: A cross-sectional study was carried out to determine the sero-prevalence of bovine brucellosis and associated risk factors as well as to depict its public health implications employing serological and questionnaire surveys. Study subjects were selected by three stage cluster sampling followed by simple random sampling, where individual animals were considered as primary units. A total of 290 blood samples were collected from animals aged 6 months and above comprising 176 females and 114 males. All sera samples were initially screened by Rose Bengal Plate Test (RBPT) and those found positive (n = 17) were re-tested by Compliment Fixation Test (CFT) for further confirmation. Thus, the overall sero-prevalence of brucellosis was 5.9% at 95% CI = [3.2, 8.6] and it ranged from 5.4% (n = 92) in agro-pastoral production system to 6.1% (n = 198) in pastoral production system. The difference between the two production systems revealed no statistically significant difference ($p>0.05$) although the difference in the prevalence between female 7.4% (n = 176) and male 3.5% (n = 114) subjects was found to be statistically significant ($p<0.05$). Similarly, statistically significant difference was observed ($p<0.05$) among age groups, indicating that brucellosis may be associated with an advancing age. Increasing herd size also revealed a corresponding highly significant ($p<0.05$) association with brucellosis prevalence, implicating that high stocking density may favor the spread of brucellosis in the herd. On the other hand, a history of abortion ($p<0.001$) and parity ($p = 0.05$) showed highly significant association with brucellosis prevalence although still birth was not found to be significantly associated with the occurrence of brucellosis. This study has revealed the zoonotic significance of bovine brucellosis in pastoral and agro-pastoral livestock production systems, which implicated that livestock owners are at a high risk of acquiring the infection and thus improving management and hygienic practices, particularly when handling animals and raising community awareness of the study settings are recommended as critical in curbing the spread of the infection.

Key words: Pastoral · Agro-Pastoral · Complement Fixation Test · Public Health Rose Bengal Plate Test · Borana Zone, Ethiopia

INTRODUCTION

Brucellosis, an endemic disease in most African countries [1], is an important zoonosis and a significant cause of reproductive losses in animals. However, with the exception of South Africa [2], surveillance and control of brucellosis is rarely implemented in sub-Saharan Africa (SSA). Within SSA, multitudes of infectious diseases occur commonly and are poorly controlled both in livestock and in human populations. Despite their social

Corresponding Author: Bedane Adane, Oromia Agricultural Research Institute,
Yabello Pastoral and Dry land Agriculture Research Center, P.O. Box 85, Yabello, Ethiopia.
Tel: +251912127212/46446756.

and economic importance, public funds raised for the control of infectious diseases such as brucellosis for example, progressively decreased over the last several decades [1, 2].

Ethiopia hosts large number of cattle that are raised under extensive pastoral production system or in adjunct to crop production. The cattle population of the country is estimated to be about 49 million and ranked first in Africa. Although there is no documented information regarding when brucellosis was introduced, numerous surveys conducted in intensive livestock management systems in Ethiopia have indicated that bovine brucellosis gradually established over the last two decades. Specific reports from different parts of the country documented varying prevalence levels, including 7.6% in Arsi [3], 15.6% [4] and 1.7% [5] in Sidama zone, 22.0% in Chafa state farm, Wello [6], 8.1% in and around Addis Ababa [7] and as high as 19.0% in Abernossa ranch [8]. A latest report [9] also depicted a sero-prevalence of 10.6% in Borana pastoral and agro-pastoral herds of cattle. On the other hand, Genene *et al*. [10] reported high prevalence (34.9%) of human brucellosis in Yabello district of Borana pastoral and agro-pastoral settings which indicates public health importance of the disease in this area. These all information constituted the basis for this study with the objectives to investigate the current prevalence of bovine brucellosis and risk factors associated with its occurrence in pastoral and agro-pastoral cattle herds in Yabello District of Borana Zone, Southern Ethiopia and to depict its public health implication.

MATERIALS AND METHODS

Study Area: The study was conducted from October 2010 to March 2011 in Yabello district, Borana zone, Ethiopia. The Yabello district comprises about 23 pastoral associations (PAs), in which 48% (11 PAs) and 52% (12 PAs) of the peoples dwelling in and around the district practice pastoral and agro pastoral activities, respectively [11]. Pastoral societies mainly rear and derive most of their income from livestock, where as agro-pastoralists are segments of pastoral society who promote opportunistic crop farming integrated to their livestock husbandry practices [12].

Study Population: According to Borana zone agricultural office in charge, the total cattle population of Yabello district during the study period was estimated to be about 232,949. The total cattle population of selected study pastoral associations (Pas) comprised 52,579 cattle and this was considered as study population.

Table 1: Total study population and production systems of selected PAs'

PA/Villages	Cattle population	Production system	No. animal sampled
Dida-Yabello	4622	Agro-pastoral	26
Elwayou	11694	Agro-pastoral	66
Hara-bake	4953	Pastoral	26
Hara-weyu	11660	Pastoral	64
Surupha	19650	Pastoral	108
Total	52,579		290

The study involved a three multi-stage random sampling technique to select study pastoral associations, villages, households and animals, randomly 5 pastoral associations of which 60% (3 PAs') from pastoral and the remaining 40% (2 Pas') from agro-pastoral were selected.

Study Design: A cross-sectional sero-epidemiological study was carried out to determine the current prevalence of bovine brucellosis and risk factors associated with its occurrence in pastoral and agro-pastoral cattle herds in Yabello District. The study subjects were selected by 3-stage sampling. The primary units were villages in the agro-pastoral or crop-livestock mixed production system and pastoral communities in pastoral production system. The secondary units were cattle herds in selected villages. Individual samples were selected from the previously identified herds by simple random sampling method. Thus, the primary sampling unit was an animal in the selected herds. The sample size for this study was determined as described by Thrusfield [13].

Blood Sample: Approximately about 7-10 ml of blood sample was drawn from the jugular vein of 290 adult animals using plain vacutainer tubes. The blood sample was allowed to clot over night at room temperature. The sera was separated and transported in an ice box to Yabello Regional Veterinary Laboratory (YRVL) and stored in deep freeze (-20°C) until tested.

Data Collection: Relevant data on the animals such as sex, age and potential risk factors associated with brucellosis were recorded during each sample collection. Furthermore, a structured questionnaire was administered to thirty seven randomly selected cattle owners within the pastoral association's (PA's) of study settings to collect information regarding history and period of abortion, retention of fetal membranes and presence of testicular and joint swellings.

Serological Tests

Rose Bengal Plate Test (RBPT): All sera samples were initially screened by RBPT, using RBPT *Brucella* antigen (Institute Pourquier, Montpellier, France). Before running the test, test sera and antigen were thawed at room temperature for half an hour.

Complement Fixation Test (CFT): The entire sera reacted positively to RBPT were further tested with CFT. The CFT test was conducted at the National Veterinary Institute Laboratory, Debre-Zeit, Ethiopia, according to the protocols recommended by [14], where the test was regarded as positive (4+, 3+, 2+, or +) when the reading was as complete fixation or partial hemolysis. The test was regarded as negative (0) when there was complete hemolysis.

Data Analysis: Statistical analysis was performed using 'Statistical Package for the Social Sciences' (SPSS) version 16.0. Categorical variables such as (sex, age, herd size and breeding history) were expressed in percentages. The prevalence was calculated as the number of animals testing positive by the RBPT/CFT, divided by the total number of animals tested. The association between each risk factor and the outcome variable was assessed using Chi-square (χ^2) statistics, with Fisher's exact test. For all analyses, a p-value of less than 0.05 was taken as significant.

RESULTS

Serological Results: From the total of 290 sera samples tested for brucellosis, 5.9% (n = 17) turned positive by RBPT. Confirmatory test using CFT, similarly gave a positive reaction for *Brucella* antibodies in these samples. Therefore, the overall prevalence of bovine brucellosis in this study was 5.9% at 95% CI = [3.2, 8.6].

Potential Risk Factors: The sex was assumed to be a risk factor for the prevalence of the disease. Accordingly, the study showed the prevalence of 7.4 and 3.5% in female and male, respectively. Statistical analysis revealed that there was no significant difference in sero-positivity to *Brucella* antibodies between male and female (P>0.05) (Table 3).

Table 2: Sero-prevalence of bovine brucellosis in pastoral and agro-pastoral PAs

		RBPT result	
PA/Village	No. of animal tested	No. positive	Prevalence (%)
Did-Yabello	26	2	7.7
Elwayou	66	3	4.5
Hara-bake	26	2	7.7
Hara-weyu	64	5	7.8
Surupha	108	5	4.6
Total	290	17	5.9

Fisher Exact: (χ^2= 1.26 df = 4 p>0.05)

Table 3: Sero-positivity of bovine brucellosis on the basis of different risk factors

		RBPT result		Fisher exact result
Risk factors	No. of animal tested	No. positive	Prevalence (%)	χ^2(P_value)
Sex				
Male	114	4	3.5	1.3(0.264)
Female	176	13	7.4	
Age				
>4 years	220	17	7.7	4.4(0.008)
<4 years	70	0	0.0	
Herd size				
<25	87	1	1.2	
25-40	102	4	3.9	
41-55	64	6	9.4	12.8(0.005)
>55	37	6	16.2	
Agro-ecology				
Pastoral	198	12	6.1	0.0(0.954)
Agro-pastoral	92	5	5.4	
Parity no.				
No parity	45	0	0.0	
Mono parity	11	0	0.0	6.6(0.0378)
2 and above parities	120	13	10.8	

Table 3: Continued

Risk factors	No. of animal tested	RBPT result		Fisher exact result
		No. positive	Prevalence (%)	χ^2(P_value)
Abortion				
Present	45	11	24.4	22.5(0.00001)
Absent	131	2	1.5	
Still birth				
Present	34	1	2.9	0.6(0.241)
Absent	142	12	8.5	

Sera were sampled from 220 and 70 animals of (>4 years) and (<4 years) respectively and prevalence of bovine brucellosis in young animals within the above-mentioned age category of this study was 0% while that of adult was 7.7%. The analyzed result revealed that significantly higher infection rate was recorded in adult animals as statistically significant effect of age witness (P<0.05) (Table 3). The herd size was also assumed as a risk factor of the disease prevalence and the study revealed that as the herd size was increased, the infectious rate also increased (Table 3) and it was statistically observed to be highly significant and sound risk factor for brucellosis occurrence in the study population (p<0.05). The two production systems, pastoral and agro-pastoral practiced in the study area were also considered as a risk factor for the occurrence of the disease. Accordingly, the prevalence for pastoral production system was 6.1% where that of agro-pastoral production system was 5.4%. Statistically, agro-ecology was observed to be not of significant difference (P>0.05) (Table 3). Parity, abortion and still birth were supposed as risk factors for occurrence and distribution of the disease for females. From a total of 176 sampled female animals, about 25.6% (n = 45) were with no parity, 6.3% (n = 11) have mono parity where as 68.2% (n = 120) have 2 and more than 2 parities. Data analysis revealed high infectious rate in the later groups (100%) while the formers two groups were not infected. For this study, parity was observed statistically significant (p<0.05) (Table 3). The other risk factor considered was abortion as indicator for the clinical manifestation of brucellosis in the herds. In the current study, out of 176 females sampled about 25.6% (n = 45) females have previous history of abortion and 24.4% prevalence was recorded for these animals. From a total of 13 females positive for both RBPT and CFT, 84.6% (n = 11) were found associated with abortion, which was statistically observed to be very highly significant (p = 0.00001) for female animals and also considered as the major risk factor for brucellosis occurrence in the current study (p<0.05) (Table 3). In the same way, still birth is one risk factor which was considered for the occurrence of the disease. From 176 sampled females, about 19.3% (n = 34) have the history of still birth. The infectious rate recorded for these animals was 2.9% (n = 1). Still birth was not observed to be statistically significant for this study (p>0.05) (Table 3).

DISCUSSION

This study demonstrated that the overall sero-prevalence of bovine brucellosis in pastoral and agro-pastoral settings of Yabello district, Borana Zone, Oromia Regional State, was 5.9% at 95% CI = [3.2, 8.6]. This rate is lower than the overall bovine brucellosis sero-prevalence of 10.6% reported from the same area previously [9]. Similarly, sero-prevalence rate of 8.1% [7] was observed in and around Addis Ababa and Bekele *et al.* [8] also reported a rather high prevalence rate of 10.8% at Agarfa state farm. The prevalence of present study is lower than the previous investigators this might be attributable to the majority of herds sero sampled for this study have bull of their own which have resulted in the current figure, which had cross mating among herds been in place attributable to communality of bulls within the study settings, would have resulted in higher prevalence than this. The discrepancy might be attributed to severe drought that was attacking the Borana lowlands during the study period. During drought, the cattle herds are often split into groups and translocated to mitigate pasture and water shortages. However, our finding is within a range of 4.2% [8] and 7.6% [3] prevalence reported from central Ethiopia. Rahman *et al.* [15] previously reported bovine brucellosis prevalence range of 2.4%-18.4% in cattle in Bangladesh.

In this study, statistically insignificant difference in prevalence was recorded in pastoral and agro-pastoral production systems (Table 2). Another study in Ethiopia by Hunduma and Regassa [16] which is in agreement with

this finding had reported infectious rate of bovine brucellosis as 15.2% and 4.1% in pastoral and agro-pastoral production system, respectively in east Shewa zone, Oromia regional state. According to their report, pastoral animals are more than three times more likely of being exposed to *Brucella* infection when compared to animals in the agro-pastoral farming system. The highest prevalence observed in pastoral production than agro-pastoral systems is in agreement with other reports [7,8,17,18], which may be attributable to communality of feeds and water to animals within pastoral than agro-pastoral study settings. The fact that prevalence was higher in large size herds compared to small size herds is in line with other reports [16, 19] which claim that large herd size enhances exposure to *Brucella* organisms, especially following abortions through increased contact and common feeding and watering points. It was also explained that mobile herds have greater opportunity to come into contact with other potentially infected herds during their movement into different areas [20]. This explanation holds true for Borana pastoral herds where animals move from place to place during feed and water scarcity (Personal observation). Berhe *et al.* [17] states that the reasons for low prevalence in agro-pastoral herds than that of pastoral cattle herds in this system are smaller herd size and relatively sedentary life style. As a result agro-pastoral cattle herds have relatively little possibility of contact with other infected herds; thus, there is less risk of acquiring the disease. In this study, brucellosis sero-prevalence increased with age and parity (Table 3), which is in agreement with several other reports [7, 8, 17, 21]. Kazi *et al.* [22] also reported similar finding in animals more than 4 years of age compared to younger animals. The high prevalence of brucellosis among older cows might be related to maturity and with advancing age, since susceptibility of cattle to *Brucella* infection is influenced by age of the animal. Thus, sexually mature and pregnant cattle are more susceptible to infection with *Brucella* organisms than sexually immature animals of either sex [23]. On the other hand, younger animals tend to be more resistant to infection and frequently clear infections, although latent infections could occur [24]. This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity [25]. In accordance to several reports [7, 18, 21, 25], higher prevalence rate was recorded in females than in males. According to Kebede *et al.* [21], males are kept for a relatively short duration in the breeding herd than females and thus the risk of exposure during their lifetime is lower. This may also be related to the level of erythritol, which stimulates the growth of *Brucella* organism although brucellae have been found in the reproductive tract of animals with no detectable levels of erythritol. Erythritol, a sugar alcohol, is synthesized in the ungulate placenta and has been credited with the preferential localization of this bacterium within the placenta of ruminants [26]. This study also revealed statistically highly significant ($p<0.05$) infection rate in animals with larger herd sizes. The report of Asmare *et al.* [5] is in agreement with this finding. Bekele *et al.* [8] also reported similar fact in south east Ethiopia. This may be accounted to the fact that, transmission of contagious diseases such as *brucellosis* is increased in advanced population. In this finding, contribution of *Brucella* for abortion and still birth in female animal was also assessed and statistically significant infectious rate 24.4% and insignificant infectious rate of 2.9% of abortion and still birth, respectively was recorded. Megersa *et al.* [9] previously suggested that *Brucella* infection is the likely cause of abortion in cattle thereby, contributing to reproductive losses. Regarding prevalence rate, similar report is recorded by Gebreyohannis [27] who reported prevalence of 4.4% in Addis Ababa dairy farms. Adane [28] also reported 11.8% prevalence rate in Jersey cows at Wolaita Sodo state dairy farm. Abreha [29], 7.4% in Tigray and Taddese [30] 6.7% in north Gonder zone, also reported the indicated prevalence. These differences in prevalence might be attributed to the variation in cattle husbandry and management systems. As was previously described by Seifert [31], abortion that occurs during the last trimester is the most obvious manifestations of bovine brucellosis, which may also cause stillbirth or yield weak calves. Based on the questionnaire survey, it seems that the community is well aware of associating the occurrence of abortion in the herds with the stage of pregnancy. Accordingly, greater proportions of the respondents confirmed the presence of abortion, locally labeled as *Sallessa,* in the last trimester. The respondents were also asked about methods of handling and ways of discarding of the fetal membrane, aborted fetus and uterine fluid. The greater proportions of the respondents never use protective gloves and the after birth (fetal membrane) is thrown into the open field. On the other hand, several activities, such as the habit of consuming raw and untreated milk obtained from aborted cows and those with retained placenta, among others, may predispose the community to zoonotic brucellosis. In line with this, Genene *et al.* [10], previously reported high

prevalence of human brucellosis in this vicinity. It has also been described [12, 32] that brucellosis can be a health hazard to human, particularly to pastoral households, who in many ways are exposed to the disease.

This study has demonstrated the significance of bovine brucellosis in pastoral and agro-pastoral livestock production systems. This implies that livestock owners are at a high risk of acquiring the infection. This is further compounded by the lack of awareness, the habit of consuming untreated milk, poor hygienic practices regarding the handling of aborted fetuses and the afterbirth and close contact between people and their animals. Therefore, improving management and hygienic practices, particularly when handling animals and raising community awareness are critical in curbing the spread of the infection.

ACKNOWLEDGMENTS

The authors acknowledge Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM) for financing the project during the study period. Our gratitude also goes to Yabello Pastoral and Dry land Agriculture Research Center and Yabello Regional Veterinary Laboratory for allowing us to use their facilities. Pastoral and agro-pastoral communities for their co-operation to sample their animals during the study period.

REFERENCES

1. Mangen, M., M. Otte, J. Pfeiffer and P. Chilonda, 2002. Bovine brucellosis in sub-Saharan Africa: Estimation of sero-prevalence and impact on meat and milk off take Potential. Food and Agriculture Organization Livestock Information and Policy Branch, AGAL December.
2. Mc Dermot, S., J. John and S.M. Arimi, 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Vet. Microbiol., 90: 111-134.
3. Molla, B., 1989. Sero-epidemiological survey of bovine brucellosis in Arsi region, DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
4. Zewdu, E., 1989. Sero-prevalence Study of Bovine Brucellosis in Selected Sites of Sidamo Region. Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit DVM thesis.
5. Asmare, K., Y. Asfaw, G. Esayas and G. Ayelet, 2010. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. Afr. J. Agr. Research, 5: 257-263.
6. Sintaro, T., 1994. The impact of brucellosis on productivity in an improved dairy herd of Chaffa state farm, Ethiopia. Fachburg veterinae medizin, Msc. thesis, Frei Unversitate, Berlin.
7. Asfaw, Y., 1998. The epidemiology of bovine brucellosis in intra and peri-urban dairy production system in and around Addis Ababa, Ethiopia. http.//Library;//Library.Vet.Med. FUBerlin, de/diss-abstracts/113428. html.
8. Bekele, A., B. Molla, T. Asfaw and T. Yigezu, 2000. Bovine brucellosis sero-epidemiological study in selected farms and ranches in southeastern Ethiopia. Bull. Anim. Hlth. Prod. Afr., 48: 13-17.
9. Megersa, B., D. Biffa, F. Abunna, A. Regassa, J. Godfroid and E. Skjerve, 2010. Sero-prevalence of brucellosis and its contribution to abortion in cattle, camel and goats kept under pastoral management in Borana, Ethiopia. Bull. Anim. Hlth. and Prod. Afr., 43(3): 651-656.
10. Genene, R., M. Desalew, Y. Lawrence, T. Hiwot, G. Teshome, G. Asfawesen, A. Abraham, H. Theresia, Abdoel and L. Henk Smits, 2009. Human Brucellosis in Traditional Pastoral Communities in Ethiopia. Intern. J. Trop. Med 4(2): 59-64.
11. Coppock, D.L., 1994. The Borana Plateau of Southern Ethiopia Synthesis of Pastoral Research, Development and Change, 1980-91. ILCA (International Livestock Center for Africa), Addis Ababa, Ethiopia, pp: 418.
12. Habtamu, T., 2004. Pastoralism and agro-pastoralism systems. In the Proceedings of the 18th Annual Conference of the Ethiopian Veterinary Association (EVA), held in Addis Ababa, June 9-10, 2004, Ethiopia.
13. Thrusfield, M.V., 2005. Veterinary Epidemiology, 3rd edition. Blackwell Science, Oxford, pp: 234-238.
14. OIE, 2004. Bovine brucellosis. In: Manual of Standard for Diagnostic Tests and Vaccines, 5th edn., OIE, Paris, pp: 246-262.
15. Rahman, M.S., J.C. Han, J. Park, J.H. Lee, S.K. Eo and J.S. Chae, 2006. Prevalence of brucellosis and its association with reproductive problems in cows in Bangladesh. Veterinary Record, 159: 180-182.

16. Hunduma, D. and C. Regassa, 2009. Sero-prevalence Study of Bovine Brucellosis in Pastoral and Agro-pastoral Areas of East Showa Zone, Oromia Regional State, Ethiopia. American-Eurasian J. Agric. and Environ. Sci., 6(5): 508-512.
17. Berhe, G., K. Belihu and Y. Asfaw, 2007. Sero-epidemiological Investigation of Bovine Brucellosis in the Extensive Cattle Production System of Tigray Region of Ethiopia. Intern. J. Appl. Research in Veterinary Medicine, 5(2): 65-71.
18. Tolosa, T., F. Regassa and K. Belihu, 2008. Sero-prevalence Study of Bovine Brucellosis in Extensive Management System in Selected Sites of Jimma Zone, Western Ethiopia. Bull. Anim. Hlth. and Prod. Afr., 56(1): 25-37.
19. Hellmann, E., C. Staak and M. Baumann, 1984. Bovine brucellosis among two different cattle populations in Bahr el Ghazal Province of Southern populations in Bahrel Ghazal Province of Southern Sudan. Trop. Med. Parasitol., 35: 123-126.
20. Omer, K., G. Holstand, E. Skjerve, Z. Woldehiwet and G. MacMillian, 2000. Prevalence of antibodies to Brucella species in cattle, sheep, goats, horses and camels in the State of Eritrea, influence of husbandry system. Epidemiol. Infect, 125: 447-453.
21. Kebede, T., G. Ejeta and G. Ameni, 2008. Sero-prevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue de Médecine Vétérinaire, 159(1): 3-9.
22. Kazi, M., R. Amin, M.B. Rahman, M.S. Rahman, J. Han, J. Park and J. Chae, 2005. Prevalence of Brucella antibodies in sera of cows in Bangladesh. J. Vet. Sci., 6: 223-226.
23. Walker, R.L., 1999. Brucella. In: C. Dwight, Z. Hirsh and Z. Yuang, (eds.): Veterinary microbiology. USA: Blackwell Science Inc., pp: 196-203.
24. Radostits, M., C. Gay, W. Hinchcliff and D. Constable, 2007. Veterinary Medicine, A text book of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. Grafos, S.A. Arte sobre papel, Spain, pp: 719-722.
25. Ahmed, R. and M.A. Munir, 1995a. Epidemiological investigations of brucellosis in Pakistan. Pakistan Vet. J., 15: 169-172.
26. Smith, H., A.E. Williams, J.H. Pearce, J. Keppie, P.W. Harris-Smith, R.B. Fitz-George and K. Witt, 1962. Foetal erythritol: a cause of the localization of Brucella abortus in bovine contagious abortion. Nature, 193: 47-49.
27. Gebreyohannes, T., 2004. Survey of bovine brucellosis in Addis Ababa Dairy farm DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
28. Adane, B., 1998. Abortion and Reproductive Performance of Jersey Cows at Wolaita Sodo State Dairy Farm. Faculty of Veterinary Medicine, Addis Ababa University, DVM thesis, Debre Zeit, Ethiopia.
29. Abreha, T., 2003. Brucellosis in cattle and small ruminants in selected sites of Tigray Region, North Ethiopia. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia
30. Taddese, Y., 2003. A survey of brucellosis in selected areas of North Gonder Zone, DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
31. Seifert, H.S., 1996. Brucellosis in: Trop. Anim. Hlth., 2nd edn, Dordrecht: Kluwer Academic Publishers group, pp: 358-362.
32. Abbas, B. and H. Agab, 2002. A review of camel brucellosis. Preventive Veterinary Medicine, 55: 47-56.