Research Article **Open Access**

Seroprevalence of Bovine Brucellosis and its Risk Factors in Cattle in and around Gondar Town, North West Gondar, Ethiopia

Alehegn E, Tesfaye S* and Chane M

Veterinary Medicine, University of Gondar, Gondar, Ethiopia

*Corresponding author: Shimels Tesfaye, Faculty of Veterinary Medicine, University of Gondar, Gondar, Ethiopia, Tel: +251-911560651; E-mail: richshimels@yahoo.com

Abstract

Background: In Ethiopia sero-prevalence of bovine Brucellosis have been reported in different agro-ecology of the country intensively but very limited reports on disease dynamics in north Gondar zone compared to other locations of the country. This study was to determine the sero-prevalence of bovine Brucellosis and assess the potential risk factors in and around Gondar, south-western Ethiopia.

Methods: The total herds included in this study were 47, selected purposively based on the willingness of the owners. But an individual animal selection was using simple random sampling method except for those cows with history of abortion or active case of abortion which included purposively. All serum samples were initially screened by Rose-Bengal Plate Test and further tested by Complement Fixation Test (CFT) for confirmation.

Results: Overall sero-prevalences recorded were 4.9% and herds' level prevalence was 34%. Among the potential risk factors considered in the present study, breed of cattle, parity, production system, and breeding system had statistically significant association (p<0.05) with sero-positivity of bovine Brucellosis. Intensification of production system, introduction of exotic or cross-breeds and AI usage had found to have high risk of exposure for sero-positivity of bovine Brucellosis (ORs=7.11, 12.18, 4.63, P=0.029, 0.0023, 0.035) respectively. Using questionnaire survey, a prevalence of abortion were 22.4% and 4.9% after 5th month of pregnancy and before 5th month of pregnancy respectively. And abortion after 5th month of pregnancy was significantly associated with sero-positivity bovine Brucellosis (p<0.05).

Conclusion: In conclusion, there was an increase of sero-prevalence of bovine Brucellosis and the potential risk factors at both animal and herd level in the study area when compared with many reports before at the same study site as well as different regions of the country; therefore, further studies on isolation and strain characterization and appropriate control measures should be considered.

Keywords: Abortion; Brucellosis; Cattle; Rose bengal plate agglutination test; Sero-prevalence

Abbreviations: AI: Artificial Insemination; CFT: Compliment Fixation Test; EA: Elias Alehign; MC: Mersha Chane; OIE: Office International Des Epizootis; OR: Odds Ratio; RBPT: Rose Bengal Plate Agglutination Test; RFM: Retained Fetal Membrane; SPSS: Software Package used for Statistical Analysis; ST: Shimels Tesfaye

Introduction

Brucellosis is a highly contagious, zoonotic and economically important bacterial disease of animals worldwide. The economic and public health impact of Brucellosis remains of concern in developing countries [1]. Brucellosis results from infection by various species of *Brucella*; a Gram negative, facultative intracellular coccobacillus or short rod in the family Brucellaceae [2]. Brucellosis infection reduces milk production in aborting cows, include (*B. abortus, B. melitensis,* and rarely *B. suis*), increases the period between lactations in sheep (*B. ovis* and *B. melitensis*), goats (*B. melitensis*) and inter-calving period and loss of the calf crop that affects camels (*B. abortus* and *B. melitensis*) [3,4].

This disease is endemic in Ethiopian and researchers have established its prevalence rate in cattle in different regions of Ethiopia, though country wide prevalence in cattle is not yet determined [5]. In Ethiopia there is no documented information on how and when Brucellosis was introduced and established. However, in the last two decades several serological surveys have showed that bovine Brucellosis is an endemic and wide spread disease in the country. For instance, prevalence of 18.4% around Addis Ababa, 2.4% in Jimma zone, 11.6% in Sidama region, 4.2% in south east Ethiopia, 2.9% in Central Oromia, 11.2% in east Showa zone, 4.9% in Tigray region, 7.61% in Arsi region and 1.11% in Addis Ababa and Sululta abattoir were documented in different parts of Ethiopia [6].

In Ethiopia, so far higher sero-prevalence reports are 39% in western Ethiopia [7], 8.2% in Arsi area [8] in central part of the country, 22% in a dairy farm in Northeastern Ethiopia [9], 8.1% in dairy farms in and around Addis Ababa [10], 11%-15% in dairy farms and ranches in southwestern Ethiopia [11], and 7.7% in Tigray region [12]. Relatively low individual animal sero-prevalence in intensive farms were recorded in different part of the country [13], observed a prevalence of 0.14% in north Gondar zone [14] reported 0.77% in southwestern Ethiopia, and [15] documented 2.46% in Sidama zone of southern Ethiopia. Furthermore, a recent study conducted in pastoral

and agro-pastoral areas of East Shoa zones of Oromia Regional State of Hunduma and Regassa [16], reported the prevalence of bovine Brucellosis to be 15.2% for pastoral and 4.1% for agro-pastoral areas. Similar other studies on livestock Brucellosis were done in pastoral and agro pastoral areas of East Africa, sero-prevalence in Eritrea (8.2%) and in Sudan (5%) [17,18]. The reports also revealed that the major risk factors for animal Brucellosis are age, parity, retained fetal membrane (RFM), abortion, herd size and composition across different agro-ecologies and production system [5].

Even though, previous report of sero-prevalence of bovine Brucellosis in north Gondar zone was very small (0.14%). There is no any information on present status of sero-prevalence of bovine Brucellosis and the potential risk factors at both animal and herd level in the study area when compared with many reports of different regions of the country. The objective of the current study was to determine the sero-prevalence of bovine Brucellosis and assess the potential risk factors in and around Gondar, south-western Ethiopia.

Methods

Study area, study population and design

The present study was conducted in and around Gondar town, which is located 740 km away North of Addis Ababa, the capital of Ethiopia. The town of Gondar is situated at latitude of 12°4' North, longitude of 27°2' East with an altitude of 1800-2500 above sea level. The annual mean minimum and maximum temperature of the area vary between 12.3-17.7°C and 22-30°C respectively with an annual average temperature of 19.7°C. It receives a bimodal rainfall, the average annual precipitation being 1000 mm that comes from the long and short rainy seasons. The short rainy season occur during the months of March, April, and May while the long ones extend from June to September [19]. The agricultural practices observed in the area are cereal based crop production and livestock farming. The livestock population of North Gondar zone was estimated to be 2,771,701 cattle, 815,716 sheep, 1,251,867 goats, 27,248 horses, 9,695 mules, 376,841 donkeys, 3,628,832 poultry and 227,463 beehives [20].

The study subjects were indigenous, cross and exotic breeds of cowswhich consist of breeding females and replacement heifers kept under intensive, semi-intensive, and extensive husbandry system and also questionnaire survey amongst volunteer herd owners and attendants. Exotic breeds by definition for this study indicated as animals having >75% exotic blood level otherwise 100% poor blood level were not existed in the area.

A cross sectional study was done from October 2014 to May 2015, in and around Gondar town. Herds were regarded as the primary sampling units. The study herds were selected by purposive sampling method based on the willingness of the owners but individual animals with in the herds were selected using simple random sampling method except those cows with history of abortion or active case of abortion included purposively. Only herds with a minimum of 5 cattle that were older than 6 months at the time of sampling were included in the study. The sampling frame was taken from agriculture Office for Gondar towns and its surroundings, mainly the members of Lame Bora milk producers association and University Gondar dairy farm. Sample size for serum collection was determined using the groups was according to the result formula given by Thrusfield [21] expected prevalence of 50% at 95% confidence interval a sample size of 384 cows were sampled.

$$N = 1.96^2 \times P_{exp}\left(1 - P_{exp}\right) / d^2$$

Where,

n=Required sample size

Pexp=Expected prevalence

d=Desired absolute precision

$$N = 1.96^{2} {}^{*} 50/100\left(1 - 50/100\right) / \left(0.5\right)^{2} = 384$$

However, a total of 406 serum samples were sampled to increase the probability of positive result as the expected prevalence was found very low in the study area.

Sample and data collection

Semi-structured questionnaire survey with open and closed ended questions were used amongst the owners for herd level data collection including herd structure and size, production system, breeding system and management system. The questionnaires were also designed to collect information's on factors that are believed to be risk factors for *Brucella* infection at individual animal variables like age, sex, and history of abortion. These herd data were envisaged for further use in studying the herd-level risk factors for bovine Brucellosis.

Collection of serum samples and transportation

Approximately 10 mL of blood samples were collected from the jugular vein of each selected animals using plain vacutainer tube and needle. Identification of each animal was labeled on corresponding vacutainer tubes and kept over-night at room temperature to allow clotting. At the next day, sera were collected from the clot to another tube. Serum samples were kept at -20°C at University of Gondar, Faculty of veterinary medicine, Veterinary microbiology Laboratory until tested using Rose Bengal Plate Agglutination Test (RBPT).

Serology tests (RBPT and CFT)

RBPT: It was employed as a screening test on the serum samples for the presence of *Brucella* agglutinins. The protocol of RBPT as recommended by OIE was used as screening test for the presence of *Brucella* antibody in the sampled sera. This test is generally considered to be as a sensitive test which reported as 97.9% sensitive for RBPT [22]. The false positive results in the RBPT could be due to cross reactions with other bacteria such as *Yersinia enterocolitica*, *E. coli*, *Salmonella* spp. and *Pastuerella* spp. The test was performed according to manufacturer's manual. Before performing test, antigen and sera were brought to room temperature. 30 μL of serum was taken on a glass slide by micropipette and the antigen bottle was shaken well to ensure homogenous suspension and then one drop (30 μL) of Rose Bengal antigen was added. The antigen and serum were mixed thoroughly with the spreader and then the slide was rotated for 40 min. The result was read immediately after 4 min.

Complement fixation test (CFT): All sera which tested positive to RBPT were further tested using CFT at the National Veterinary Institute (NVI), Debre-Zeit, Ethiopia. The samples were reported as positive for Brucellosis, if they were found positive for both RBPT and CFT.

Data management and analysis

All data collected during the study period was checked, coded, and entered in to Microsoft Excel spreadsheet and analyzed using SPSS software version 16.0. Herd level prevalence for abortion and sero-positivity were calculated by dividing the number of herds with at least one positive for abortion or Brucellosis by the number of all herds in the study or tested. Risk factors association with abortion was determined by Pearson's chi-square (χ^2) test. Analyzing the effects of different potential risk factors on the seroprevalence of Brucellosis at both herd and individual level was performed by univariate logistic regression. Odds ratio (OR) was used to measure the degree of association between risk factors and seroprevalence of bovine Brucellosis. For statistical inference, P-value<0.05 (at 5% level of significance) was considered as statistically significant. Associations between sero-positivity and occurrences of abortion were also determined by univariate logistic regression (OR).

Results

Sero-prevalence of bovine Brucellosis in and around Gondar cattle herds

In the present study, a total 406 serum samples were collected from 47 herds with no history of vaccination for Brucellosis before. Of them, 22 (5.4%) were positive in a RBPT and out of those 22 *Brucella* positive reactors, 20 (4.9%) were confirmed to be sero-positive for CFT.

Therefore, an overall animal level sero-prevalence of bovine Brucellosis in the study area was 4.9%. Whereas from a total of 47 herds, 16 (34%) herds were found with at least one animal tested positive.

Factors affecting individual animal sero-positivity for *Brucella*

There was a significantly high sero-prevalence (7.5%, P=0.029) of bovine Brucellosis in intensive management system when compared to extensive management system (1.1%). The logistic regression result also revealed that those animals in the intensive management system were 7.11 times at higher risk than extensive management system. But, there was no statically significant variation (P>0.05) for intensive management system when compared with Semi-intensive management system. Sero-positivity in cross-breeds (8.3%) was significantly higher (P=0.0023) than that of local breeds (0.7). Cross-breeds were 12.18 times at higher risk than local breeds. For parity also, there was a significantly higher sero-prevalence (10.7%, P=0.023) in those older cows calved 5 and above calves when compared to younger cows calved ≤2 (2.3%). Older cows (≥5 calved) were 5.18 times at higher risk than younger cows (≤2 calved) (Table 1). Sero-positivity for AI used cows (9.5%) was significantly higher (P=0.035) than that natural mating (2.2%). AI used cows were 4.63 times at higher risk than natural mating cows for breeding. Besides, association between bovine Brucellosis and abortion history of the cows was statistically significant (P=0.000) (Table 2). There were no significant differences in sero-positivity between herd-sizes.

Factor	Group	N	Positive	Frequency (%)	Univariate		
					CI$^\#$ (95%)	OR$^+$	P-value
Management system	Intensive	214	16	7.5	1.067-301.50	7.11	0.029
	Semi-intensive	103	3	2.9	0.07-1.34	0.37	0.108
	Extensive	89	1	1.1	-	-	-
Herd size	≤10[a] animals	149	7	4.7	-	-	-
	10-20 animals	162	9	5.6	0.38-3.87	1.19	0.73
	≥20 animals	95	4	4.2	0.19-3.63	0.89	0.85
Breed	Local	135	1	0.7	-	-	-
	Cross-breeds	204	17	8.3	1.86-512	12.18	0.0023
	Exotic	67	2	3	0.21-244.99	4.12	0.214
Parity	≤2[a] calved	89	2	2.3	-	-	-
	2-5 calved	223	8	3.6	0.31-15.92	1.62	0.54
	≥5 calved	94	10	10.7	1.05-49.60	5.18	0.023
Breeding system	AI	126	12	9.5	-	-	-
	Natural mating	135	3	2.2	0.05-1.045	0.27	0.035
	Mixed	145	5	3.4	0.16-1.35	0.43	0.11

Table 1: Influence of common risk factors on sero-prevalence of bovine Brucellosis at individual animal level in and around Gondar town, North-west Ethiopia. [a]Others were computed in references to this category. $^\#$OR: Odds Ratio; $^+$CL: Confidence Interval.

Abortion history	N	Positive sera	Sero-prevalence	Univariate		
				CI (95%)	OR	P-value
Before 5th month	41	2	4.9	0.29-19.95	3.14	0.157
After 5th month	58	13	22.4	5.44-64.67	17.5	0
No history	307	5	1.6	-	-	-

Table 2: Sero-prevalence of bovine Brucellosis on the basis of abortion history.

Herd level sero-prevalence of bovine Brucellosis

There was a significantly high sero-prevalence (53.8%, P=0.026) herd level bovine Brucellosis in intensive management system when compared to extensive management system (11%). The logistic regression result also showed that intensive farms were 9.33 times at higher risk than extensive farms (Table 3).

Factor	Group	N	Positive	Frequency (%)	Univariate		
					CI (95%)	OR	P-value
Farm type	Extensive	9	1	11	-	-	-
	Semi-intensive	12	3	25	0.16-157	2.67	0.4225
	Intensive	26	14	53.8	0.95-445	9.33	0.026

Table 3: Herd level sero-prevalence of bovine Brucellosis in intensive, semi-inensive, and extensive productions in the study area assessed by logistic regression.

Prevalence of abortion at herd level

A total of 47 volunteer herd owners and attendants interviewed for the occurrences bovine abortion in their herds, the data revealed that there was an overall prevalence of 10.6% (5 herds) abortion record during the study year.

Variables	Groups	No of farms	Farms with abortion case	Prevalence (%)	χ2*	Df$^{¥}$	P-value
Breed	local	14	0	0	3.37	3	0.338
	Cross	22	4	18.2			
	Exotic	3	0	0			
	mixed	8	1	25			
Herd size	<10^{a}	26	3	11.5	1.3	2	0.523
	Oct-20	17	1	5.9			
	>20	4	1	25			
Production system	Intensive	26	4	19	3.3	2	0.192
	Semi-intensive	12	1	8.3			
	Extensive	9	0	0			
Breeding system	AI	11	3	27.3	5.07	2	0.081
	Natural mating	21	2	9.5			
	Mixed	15	0	0			

Table 4: Association of risk factors with prevalence of cattle abortion at herd level in and around Gondar town. ^{a}Others were computed in references to this category. *χ^{2}: Prison chi-square; $^{¥}$DF: Degree of freedom.

From this survey finding, breed of animals, herd size, production system and breeding system were investigated as a risk factor for the prevalence of abortion. All risk factors have no statistically significance (P>0.05) for the prevalence of abortion in this study (Table 4).

Association of sero-prevalence with abortion history

The sero-prevalence rate of bovine Brucellosis with the abortion history of cows was also investigated. The highest sero-prevalence was recorded in cows having abortion history after 5th month of pregnancy (22.40%) and the lowest in cows having no abortion history after 5th month of pregnancy (1.6%). The logistic regression result revealed that those cows with the history of abortion after 5th months were 17.5 times at higher risk than those cows without history of abortion.

Discussion

Bovine Brucellosis is mainly characterized by abortion and retained foetal membrane. A total of 47 volunteer herd owners and attendants interviewed for the occurrences bovine abortion in their herds, the data revealed that there was an overall prevalence of 10.6% (5 herds) abortion record during the study year. This finding is in agreement with the previous study reports in different parts of Ethiopia in which abortion reports ranging from 6.8% to 11.8% [13,23,24]. The slight variation among the reports may be due to different factors like husbandry system, agro-climatic condition of the study areas. According to Robert [25], incidence of abortion more than 2% to 5% should be viewed seriously.

The present study showed that the overall individual animal level seroprevalence of bovine Brucellosis determined with RBPT and CFT were 5.4% and 4.9% respectively. Since, CFT is the recommended confirmatory test for Brucellosis with high specificity [4], the overall seroprevalence of bovine Brucellosis in the study area is 4.9%. This study has higher prevalence rate than reports of Degefu [24] (1.84% RBPT and 1.38% CFT) in Jijiga zone of Somalia regional state, Asmare [26] (1.92%) in Sidama zone, Yohannes [6] (2.96% RBPT and 1.97% CFT) in Guto-Gida district of East Wollega Zone, Jergefa [27] (2.9%) in central Oromia and Magersa [28] (3.5%) in South Eastern Ethiopia and Berhe [29] (3.19) in Tigray region. On the contrary, it has also lower prevalence rate than the previous reports of Molla [30] (7.62%) in Arsi region, Shiferaw [31] (12.34%) in and around Reshid [32] (38.7%) in cattle owned by institute of agriculture research farm. The observed variation of the reports among different regions of Ethiopia could be attributed to various factors including agro-ecology, cattle rearing system, herd size, breeding system and breed types.

Among the potential risk factors considered in the present study, breed of cattle, parity, production system, and breeding system had statistically significant association (p<0.05) with sero-positivity of bovine Brucellosis. In breeds, almost all (95%) of the seropositive animals were cross-breeds and exotic breeds. This finding was supported by Jergefa [27], the sero-prevalence of (4.2%) cross-breeds and (0.2%) indigenous ones in central Oromia. On the contrary, Yohannes [33] reported that there is no statistically significant association (p>0.05) for bovine Brucellosis though the report still showed higher prevalence in cross breeds (3.64%) than in indigenous breeds (1.7%). This significant difference could be due to the compounded effects of management and mating methods. In the present study, farmers who owned crossbreed cattle tended to follow intensive management methods and prefer artificial insemination than natural mating for breeding.

The type of breeding system used by farmers was shown to significantly affect the prevalence of bovine Brucellosis (p<0.05). A higher prevalence was encountered on animals that breed using artificial insemination (9.5%). This report was in agreement with Jergefa [27] in central Oromia, who reported a higher sero-prevalence of bovine Brucellosis in herds that used artificial insemination (10.3%), but opposed to Adugna [34], who reported 2.6% from western Ethiopia.

Production system also had a strong association with sero-positivity. In this study, a higher prevalence was observed in cattle under intensive production systems (7.5%) than in those under extensive farming (1.1%). This finding agreed with previous reports of Jergefa [27] who reported (10.3%) and (2.7%) in intensive and extensive production systems respectively. The higher prevalence in intensive production systems could be explained by the fact that there is a greater chance of contact between infected and healthy animals in these systems, or between healthy animals and infectious materials, since most farmers do not follow hygienic practices.

The highest bovine Brucellosis sero-prevalence was observed within the older animals as measured by parity (>5 calved, 2.5%) and (<2 calved, 0.5%) with (p<0.05). This report is in agreement with Kebede [35], who reported 17.8% sero-prevalence for cows greater than 8 years old and 1.4% for cows greater than for cows less than 2 years old. This may be due to frequent exposure to risk factors like mating and infection due to contact.

Prevalence of abortion recorded were 22.4% and 4.9% after 5th month of pregnancy and before 5th month of pregnancy respectively. A previous history of abortion after 5th month of pregnancy was, as expected, significantly associated with sero-positivity (p<0.05). Many studies also reported supporting the above finding [26,29,34,36-39]. This could be explained by the fact that, erythritol sugar in the placenta and fetal fluid is elevated during gestation period. This stimulates the growth and multiplication of the bacteria in the reproductive organs [40]. The bacterial load often reduced in months following calving and abortion until the next pregnancy [4,41].

Conclusion and Recommendations

Based on this cross-section study, there was an increase of sero-prevalence of bovine Brucellosis in the study area when compared with many reports from different regions of Ethiopia. Potential risk factors considered in the present study, breed of cattle, parity, production system, and breeding system had statistically significant association (p<0.05) with sero-positivity of bovine Brucellosis. Additionally, use of Artificial insemination and presence of exogenous cattle potentially increase the risk of bovine Brucellosis. Besides, there was an overall prevalence of 10.6% abortion record, suggesting that incidence of abortion more than 2-5% should be viewed seriously. Therefore, considering the economic and public health importance of bovine Brucellosis, further studies on isolation and strain characterization and appropriate control measures should be considered.

Declarations

Acknowledgements

completing this study and to the farmers, for their invaluable input without which this work would not have been completed.

Availability of data and materials

Authors do not wish to share the dataset in current study, since our data is patented for university.

Authors' Contributions

ST carried out the conception of the research concept and designed the methodology, data analysis and interpretation and preparation of the manuscript for publication. EA carried out the laboratory work, sample collection, and revision of the manuscript. MC carried out funding and editing of the manuscript for publication. All authors read and approved the final manuscript.

Competing Interest

The authors declare that there is no financial or non-financial competing interest from anybody or institute. We also want to assure that we did not receive any technical assistant in developing the research concept or preparation of the manuscript.

Consent for publication

All the authors have agreed that it should be submitted to Journal of Animal science and technology. No part of this paper had been published elsewhere.

Ethics approval and consent to participate

There is no any organization still responsible in Ethiopia for ethics approval in animal participants but the authors followed international animal procedures for blood collection during sampling.

References

1. OIE (Office International Des Epizootis) (2000) Bovine Brucellosis. In: Manual of standards for diagnostic tests and vaccines (4th edn.) OIE, Paris pp: 328-345.

2. IOWA State University (2007) Brucellosis, College of Veterinary Medicine. Institute for International Cooperation in Animal Biologics.

3. Smith MC, Sherman DM (1994) Goat medicine, malvern pa. lea, febger. Brucella. In: Walker RL, Dwright CH, Chunge ZY (eds.) Veterinary Microbiology. Black Well Science, Massachusetts pp: 196-203.

4. Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007) Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats (10th edn.) Saunders Elsevier, London.

5. Yilma M, Mamo G, Mammo B (2016) Review on Brucellosis Sero-Prevalence, and ecology in Livestock and human population of Ethiopia. Achievements in the Life Sciences 10: 80-86.

6. Yohannes M, Degefu H, Tolosa T, Belihu K, Ronald C, et al. (2013) Brucellosis in Ethiopia. Afr J Microbiol Res 7: 1150-1157.

7. Mayer CE (1980) Report on Veterinary Activity. Institute of Agricultural Research, Ethiopia.

8. Bayleyegne M (1989) Seroepidemiological survey of bovine Brucellosis in Arssi Region. DVM Thesis, FVM, AAU, Debre Zeit, Ethiopia.

9. Tariku S (1994) The impact of Brucellosis on productivity in improved dairy herd of Chaffa State Farm. Ethiopia. Msc Thesis, Frei Universitate, Fachburg Veterinaemedizin, Berlin.

10. Yilkal A (1998) The epidemiology of Brucellosis in intra and peri urban dairy production system in and around Addis Ababa. MSc thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

11. Tekelye B, Kasali OB, Mugrewa M, Sholtens RG, Tamirat Y (2000) The prevalence of Brucellosis in indigenous cattle in central Ethiopia. Bull Anim Hlth Prod Afr 37: 97-98.

12. Mekonnen H, Kalayou S, Kyule M (2010) Serological survey of bovine Brucellosis in barka and arado breeds (Bos indicus) of western Tigray, Ethiopia. Prev Vet Med 94: 28-35.

13. Taddesse Y (2003) A survey of bovine Brucellosis in selected areas of North Gonder Zone. DVM Thesis, FVM, AAU, Debre Zeit, Ethiopia.

14. Tadele T (2004) Seroprevalence study of bovine Brucellosis and its public health significance in selected sites of jimma zone, western ethiopia. msc thesis, faculty of veterinary medicine, Addis Ababa University, Debre Zeit, Ethiopia.

15. Kassahun A, Shiv P, Yilkal A, Esayas G, Gelagaye A, et al. (2007) Seroprevalence of Brucellosis in cattle and high risk professionals in Sidama Zone, Southern Ethiopia. Ethiop Vet J 11: 69-84.

16. Hunduma D, Regassa C (2009) Seroprevalence study of bovine Brucellosis in pastoral and agro-pastoral areas of East Showa Zone, Oromia Regional State, Ethiopia. Am-Euras J Agric Environ Sci 6: 508-512.

17. Omer MK, Skjerve E, Woldehiwet Z, Holstad G (2000) Risk factors for Brucella spp. infection in dairy cattle farms in Asmara, State of Eritrea. Prev Vet Med 46: 257-265.

18. El-Ansary EH, Mohammed B, Hamad AAR, Karom AG (2001) Brucellosis among animals and human contacts in Eastern Sudan. Saudi Med J 22: 577-579.

19. CSA (Central Statistical Agency of Ethiopia) (2012) Annual surveillance report. Adiss Ababa, Ethiopia.

20. CSA (Central Statistical Agency of Ethiopia) (2012/13) Agricultural Sample Survey 2012/13 2012/13[2005E.C.]: A report on livestock and livestock characteristics, 570 statistical bulletin, Addis Ababa, 2: 37.

21. Thrusfield M (1995) Veterinary Epidemiology (2nd edn.) Back well scientific Ltd., UK pp: 182-198.

22. Dohoo IR, Wright PF, Ruckerbauer GM, Samagh BS, Robertson FJ, et al. (1986) A comparison of five serological tests for bovine Brucellosis. Can J Vet Res 50: 485-493.

23. Abraha T (2003) Brucellosis in cattle and small ruminants in selected sites of Tigray region, North Ethiopia. DVM Thesis, FVM, AAU, Debre Zeit, Ethiopia.

24. Degefa T, Duressa A, Duguma R (2011) Brucellosis and some reproductive problems of indigenous Arsi cattle in selected Arsi Zone's of Oromia Regional State, Ethiopia. Glob Vet 7: 45-53.

25. Robert JS (1971) Veterinary Obstetrics and Genital Disease (2 ed.) CBS Publisher and Distributors, India pp: 108-112.

26. Asmare K, Asfaw Y, Gelaye E, Ayelet G (2010) Brucellosis in extensive management system of Zebu cattle in Sida a Zone, Southern Ethiopia. Afr J Agric Res 5: 257-263.

27. Jergefa T, Kelay B, Bekana M, Teshale S, Gustafson H, et al. (2009) Epidemiological study of bovine Brucellosis in three agro-ecological areas of central Oromiya, Ethiopia. Rev Sci Tech 28: 933-943.

28. Megersa B, Biffa D, Niguse F, Rufael T, Asmare K, et al. (2011) Cattle Brucellosis in traditional livestock husbandry practice in Southern and Eastern Ethiopia, and its zoonotic implication. Acta Vet Scand 53: 24.

29. Berhe G, Belihu K, Asfaw Y (2007) Seroepidemiological investigation of bovine Brucellosis in the extensive cattle production system of Tigray region of Ethiopia. Int J Appl Res Vet Med 5: 65-71.

30. Molla B (1998) Sero epidemiological survey of bovine Brucellosis in Arsi region. DVM Thesis, FVM, AAU, Debre Zeit, Ethiopia.

31. Shiferaw A (1994) Sero-epidemiological study of bovine Brucellosis in and around Bahr Dar. DVM Thesis, Addis Ababa University, Debre Zeit, Ethiopia.

32. Reshid M (1993) Reproductive wastage in cattle due to bovine Brucellosis. Institute of Agriculture Research proceeding at the fourth national livestock improvement conference, held at Addis Ababa pp: 270-272.

33. Yohannes M, Mersha T, Degefu H, Tolosa T, Woyesa M (2012) Bovine Brucellosis: Serological Survey in Guto-Gida District, East Wollega Zone, Ethiopia. Glob Vet 8: 139-143.

34. Adugna KE, Agga GE, Zewde G (2013) Seroepidemiological survey of bovine Brucellosis in cattle under a traditional production system in western Ethiopia. Addis Ababa University, Faculty of Veterinary Medicine, Department of Microbiology, Immunology, Epidemiology and Public Health, Debre Zeit, Ethiopia. Rev Sci Tech Off Int Epiz p: 32.

35. Kebede T, Ejeta G, Ameni G (2008) Seroprevalence of bovine Brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue Méd Vét 159: 3-9.

36. Asfaw Y (1998) The Epidemiology of Brucellosis in Intra and Peri Urban Dairy Production System in and Around Addis Ababa. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.

37. Tolosa T, Regass F, Belihu K (2008) Seroprevalence study of bovine Brucellosis in extensive management system in selected sites of Jimma Zone, Western Ethiopia. Bull Anim Hlth Prod Afr 56: 25-37.

38. Ibrahim N, Belihu K, Lobago F, Bekana M (2010) Sero-prevalence of bovine Brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. Trop Anim Health Prod 42: 35-40.

39. Haileselassie M, Kalayou S, Kyule M, Asfaha M, Belihu K (2011) Effect of Brucella infection on reproduction conditions of female breeding cattle and its public health significance in Western tigray, northern ethiopia. Vet Med Int 2011: 354943.

40. Walker LR (1999) Brucella. In: Wight D, Hirsh C, Yuan Chung Z (eds.) Veterinary microbiology. Blackwell Science, Hoboken pp: 196-202.

41. Coetzer JW, Tustin RC (2004) Infectious disease of livestock (3rd edn.) South Africa Oxford University Press, Cape Town pp: 1507-1551.