ISSN: 2455 – 9938
PubMed- National Library of Medicine - ID: 101731611

EIJO: Journal of Science, Technology and Innovative Research (EIJO–JSTIR)
Einstein International Journal Organization (EIJO)
Available Online at: www.eijo.in
Volume – 3, Issue – 4, July – August - 2018, Page No. : 41 - 52

Seroprevalence and Associated Risk Factors for Seropositivity of Bovine Brucellosis at Wollega University Horro-Guduru Animal Breeding and Research Center

Jirata Shiferaw[1]*, Alemayehu Fikadu[2], Hika Waktole[3]

College of Medical and Health Sciences, School of Veterinary Medicine, Wollega University, Nekemte, Ethiopia

Correspondence Author: Jirata Shiferaw**,** College of Medical and Health Sciences, School of Veterinary Medicine, Wollega University, Nekemte, Ethiopia

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

A cross-sectional study was carried out from November 2011 to March 2012 to determine the seroprevalence and associated risk factors for seropositivity of bovine brucellosis at Wollega University Horro-Guduru Animal Breeding and Research Center (WUHGABRC) in Horro-GuduruWollega Oromia Regional State. The study population comprised local (Horro) and cross (Horro-Jersy) breed. A total of 415 blood sample were collected from cattle above 1 year of age and were screened for brucella antibodies by Rose Bengal Plate Test (RBPT) and positive reactors were further tested by Complement Fixation Test (CFT). Out of 415 sera 4 (1%) were positive using RBPT. The positive sera were further confirmed using CFT in which 2 (0.5%) were positive. All positive sera were from female cattle. The results of current study revealed that seropositivity to bovine brucellosis was significantly higher in pregnant animals than non- pregnant ($p<0.05$). In contrast there was no statistically significant difference ($p>0.05$) in seroprevalence of bovine brucellosis between breeds, among different age groups and between sexes of study animals. Even though the prevalence of bovine brucellosis in the current study is low, it shouldn't be ignored as nonexistent disease in the area where it may continue to become hazard to livestock industry and public consuming raw products of animal origin. Thus all peoples in general and high risk groups in particular need to be aware of zoonotic as well as economic importance of the disease, so as to adopt strategic control measures in order to reduce associated reproductive wastage and public health risk.

Keywords: *Bovine Brucellosis, CFT, RBPT, WUHGABRC, Seroprevalence.*

Introduction

Livestock rearing is the principal economic activity supporting livelihoods in the desert, arid grass lands and savannahs which cover about 14 million km^2, i.e. more than 50% of the sub Saharan Africa (SSA) land surface. In these areas, the harsh environmental conditions are unsuitable for any other form of agriculture. For the people living in these environments, livestock is the principal currency for social and commercial transactions (Mangen *et al.*, 2002).

Ethiopia has the largest livestock population in Africa which plays important role in the lives of its peoples. In addition to milk, meat, hide and dung that are major livestock products, animal draught power is also widely used for transportation and crop productions in most parts of Ethiopia. Livestock and livestock products also constitute one of the major export resources of the country. As a result, livestock play a vital role in country's economy (FAO, 2003).

Despite its huge resources and potentials the country is not self-efficient in animal product. Some of the factors contributing to food insecurity in Ethiopia have been reported to be old age production technologies, modern agricultural inputs such as research extension service rarity even by African standards and harvest failure often leads to loss of assets and fall in to poverty. Among technical causes of lowered productivity of livestock, wide spread prevalence of different animal diseases and parasites are common (Birhanu, 2002).

Within SSA, many of the known infectious disease occur commonly and are poorly controlled both in livestock and in human population. The presences of these diseases make the livestock industry less efficient and have negative impact on livestock export trade (Arimi and Mangen, 2002). In developed countries animal trade is ruled by strong veterinary regulation and do not allow importing any animal species from the country having transmissible disease or from unknown disease status countries. In most African and Asian countries the regulation is relatively low or totally absent. Therefore, to use these huge livestock resources, reducing the impact of major animal diseases that have economic and zoonotic importance such as brucellosis, tuberculosis etc is essential. In this study bovine brucellosis was taken as important problem to be investigated (Desalegn, 2008).

Brucellosis is infectious and contagious bacterial disease caused by member of genus brucella (OIE, 2004). The causal relationship between organisms and disease was first discovered in 1887 by Dr. David Bruce an English man who isolated the germ that caused abortion in goats at the islands of Malta. In 1897 Danish Veterinarian Bernhard Bang, isolated *Brucella abortus* as the cause of abortion in cattle and since then the disease has been known as Bang's disease. The disease has worldwide importance and affects a number of animal species including human but more common in countries having poorly standardized animal farming system and poor public health programs (Anonymous, 2006).

Bovine brucellosis in cattle is caused almost exclusively by *Brucella abortus*, less frequently by *Brucella melitensis* and rarely by *Brucella suis* (OIE, 2008). The disease widely spreads and highly infectious zoonoses with great economic impact on cattle. The infection caused by *Brucella abortus* in cattle is also known as "contagious abortion" or "Bang's disease" which is characterized by abortion or premature calving of recently infected animals, most often in the last trimester (between 5^{th} and 8^{th} months of pregnancy). In human the disease is called undulant fever, Malta fever, or Mediterranean fever (Berchovich, 1998). The main manifestation of bovine brucellosis is reproductive failure i.e. abortion, unthrifty new born and metritis in female, orchitits and epididymitis in male animals. Persistent infections with shedding of brucella in reproductive and mammary secretions are also common (Radostitis *et al.*, 2007).

Bovine brucellosis is widely prevalent in many countries causing significant economic losses including abortion, losses in milk production, low fertility rates, cost of replacements and impacts on export and has zoonotic importance except in those where it has been eradicated (Asfaw *et al.*, 1998). With high prevalence in dairy farms, it is one of the most important diseases of cattle in Africa, South America and Asia. Brucellosis in sheep and goats occur in Mediterranean basin of Europe and Africa, Southern Soviet Union, Middle East Asia; particularly with high prevalence in central and South America. However, Australia, Netherlands and North Europe are free from this disease (Brew *et al.*, 1999).

In Ethiopia there is no documented information before the last 3 decades on how and when brucellosis was introduced and established. It was first reported in 1970 by Veterinary section of the US Navy Medical Research unit (EMA, 1970). Since then several serological surveys have been carried out and found the prevalence of bovine brucellosis to range from 0.2%

in south western Ethiopia (Tadele, 2004) to 38.7% in western Ethiopia (Rashid, 1993). However, there was no investigation on bovine brucellosis in present study area. Therefore, the objectives of this study were: To determine seroprevalence of bovine brucellosis in Wollega University Horro-Guduru Animal Breeding and Research Center and to identify associated risk factors and conditions attributed for the occurrence of this disease.

Materials and Methods

Study Area

The study was conducted from November 2011 to March 2012 in Wollega University Horro-Guduru Animal Breeding and Research Center which is found in Guduru District, Horro-Guduru Wollega Zone, Oromia Regional State. It is located at about 282km west of Addis Ababa and 262km from Nekemte East Wollega Zone. The area is characterized by diverse agro ecological zones; with average elevation of 1500 to 2400 meters above sea level, between 09^{o}29' North and 37^{o}26'east longitudes. This area has maximum and minimum temperature of 32^{o}c to 18^{o}c respectively with mean annual rain fall of the area ranges from 1300 to 1500mm (CSA, 2011).

The center was established in 1988 E.C by Oromia Agricultural Development Beaurue with the aim of sheep breeding and improvement, but due to high mortality of sheep with undifferentiated disease, it was shifted to cattle rearing center in 1994 E.C. In 2002 E.C it was transferred to Wollega University and named as Wollega University Horro-Guduru Animal Breeding and Research Center. Currently, the center covers an area of 764.09 hectares land and has four station pool which are called; Botosho, Jara, Arbuderesa and Granchae. There were total of 1294 Pure Horro and Horro Jersey cattle in the ranch which are kept under semi-intensive and extensive management system. Except for calves, the housing system was traditional open air with fence only.

Mixed farming of crop and animal production is practiced in the area. Usually animals are kept together in the barn at night time and are allowed to freely mix at grazing pasture land and watering sites during the day, especially during the dry season. During cropping season the animals are kept in limited grazing land in order to protect them from damaging cultivated crop on available land. This results in overgrazing of the land which results in subsequent malnutrition.

Study animals

The target population for the study was cattle population of both sexes from Wollega University Horro-Guduru Animal Breeding and Research Center. The age of all study animals were from 1-12 years in which both Local and Horro-Jercy breeds were included. A total of 415 cattle were selected for taking blood serum samples and serologically tested by RBPT and CFT to determine seroprevalence of bovine Brucellosis in the center.

Table1: Summary of study animals categorized by sex, age, breeds and pregnancy status

Variables	**Number of animals sampled**
Sex	
Females	375
Male	40
Total	**415**
Age in years	

1-4	55
5-8	162
9-12	198
Total	**415**
Breed	
Local (Horro)	354
Cross (Horro-Jersy)	61
Total	**415**
Pregnancy status	
Pregnant	59
Non pregnant	356
Total	**415**

Study Design

A cross sectional study was carried out to determine the prevalence of bovine brucellosis on Horro and Horro-Jersey breeds in the study area. During the study period, a total of 415 sera samples were collected and examined using serological tests-RBPT and CFT for detection of antibody produced against brucella in the sera. Individual animal was selected by simple random sampling to encompass as much as possible both sexes, breeds, pregnancy status, age categories and different parties and all these information were recorded during sampling.

Sample Size Determination

Sample size for blood serum collection was estimated by first assuming a prevalence of 50% to get maximum number required by simple random sampling, because there was no previous work on prevalence of bovine brucellosis in the current study area. Accordingly, using the formula given by Thrusfield (1995) with expected prevalence of 50% at 95% confidence interval and 5% desired absolute precision a sample size was calculated as follows:

$N=\frac{a^2 (p)(1-p)}{d^2}$ where, N- required sample size

a- constant which is (1.96)

p- expected prevalence

d- desired absolute precision

Therefore, $N=\frac{(1.96)^2(0.5)(1-0.5)}{(0.05)^2}$

$=384$

Even if 384 cattle were aimed to be sampled, in order to increase precision a total of 415 study animals were used.

Study methodology

Blood sample collection

Samples of approximately 10ml whole blood were collected from the jagular vein of each of selected animal, using plain vacutainer tubes. After taking, the sample code/ labeling were given to the tubes, which contain the sample. The samples

were left at room temperature overnight in a slant position to allow clotting for serum separation. Serum was decanted to other vial and stored at -20^{o}c until serological tests should be performed.

Serological Tests

Rose Bengal Plate Test (RBPT): The collected sera were transported in icebox containing icepacks and screened for the presence of antibodies against brucella antigens by using RBPT at Sebeta National Animal Health Diagnostic and Investigation center (NAHDIC) Serology Laboratory room. In brief, 30µl of individual animal serum was mixed thoroughly with an equal volume of RBPT antigen suspension on a glass plate and rotated. After four minutes of shaking, any visible agglutination was considered as positive and those samples with no agglutination were recorded as negative (Morgan *et al.*, 1996).

Complement Fixation Test (CFT): Sera found positive for RBPT were retested for confirmation by CFT. The CFT was done at National Veterinary Institution (NVI), Debre Zeit, Ethiopia according to protocols recommended by OIE. As standard, CFT (veterinary laboratory agency, United Kingdom) was employed to detect the presence of antibodies against brucella in sera. The principle of CFT is that brucella antigen binds the brucella antibody in the test sera to form immune complex. This bound immune complex traps the complement. The complement is then unavailable to lyses the target cells in indicator system (SRBCs bound to hemolysin). In the absence of antibody, the complement remains unfixed and is available to lyses the target cells in the indicator system. The interpretation of the test, therefore, full hemolysis indicates negative result, while sedimentation of sheep RBC indicates positive reactions at different dilution concentration of the complement (OIE, 2004).

The protocol is in short as follows: The test is conducted on to a 96 U- shaped micro-titer plate at a dilution of 1:5. In one plate, four rows can be used for testing 48 sera samples and the other four rows for anti-complement (AC) control. Before testing, the sera were transferred into a micro plate wells, sealed by micro plate sealer and de-complemented in water bath at 58^{o}c for 30 minutes. Pre-diluted sera (1:5) were prepared by transferring 5µl of serum into 20µl of 1XVCM in a micro plate wells. Then 25µl of pre-diluted sera were transferred in duplicates for test sera and control. 25µl of antigen was added at working dilution in the test sera wells except the control wells. 25µl of 1XVCM was added into all control wells. Then the plates were covered by micro plate sealer to prevent evaporation and incubated at 37^{o}c for 30 minutes with constant agitation. 25µl of complement was added into all working dilution and incubated as above. After 30 minutes of incubation, 25µl of H.S (indicator) was added to all wells of the plate incubated as above. Finally, the micro plates were centrifuged at 2500rpm for 5minutes using sigma centrifuge. After 5 minutes of centrifugation, the sera with $\geq$50% hemolysis at a dilution of 1:5 and above were considered to be for brucellosis positive.

Data Analysis

The data collected from the field were entered into Microsoft (MS) excel spread sheet and analysis was done using SPSS standard software program. The seroprevalence rate of bovine brucellosis was calculated by dividing the number of RBPT and CFT positive animals by the total number of animals tested. Categorical variables (Age, Sex, Breed and Pregnancy status) were expressed in percentages. The degree of association between each of the above risk factors and bovine brucellosis were assessed using the Chi-square (X^2) test together with 95% confidence interval. For all analysis, a P-value of less than 0.05 was taken as statistically significant.

Results

All sera collected were subjected to RBPT for screening test and all RBPT positive sera were retested by CFT for confirmation. 4 out of 415 tested cattle sera (1%) were reacted positive for RBPT of which 2 (0.5%) were reacted positive for CFT from retested sera. The entire seropositive cases were from female animals. The overall seroprevalence of bovine brucellosis in this study area was found to be 0.5%. The potential risk factor associated with bovine brucellosis ($p<0.05$) was pregnancy status of animals. Age, Sex, Breed and management of system of animals were found to be statistically not significant.

Comparison was made on the seroprevalence of male and female animals to assess the existence of any association between the prevalence and sex. A total of 415 sera samples were tested for screening of bovine brucellosis. 375 sera samples were from female animals in which 4 (1%) were positive for RBPT. These 4 RBPT positive sera were further evaluated by CFT and 2 out of 415 (0.5%) were confirmed positive. 40 sera samples from male animals were tested for RBPT and all found to be negative. The difference in seropositivity between different sexes was not statistically significant ($p>0.05$) (Table 2).

Table 2: Seroprevalence of bovine brucellosis based on sex

Sex	**No. of animals Tested**	**RBPT Positive (%)**	**CFT Positive (%)**
Male	40	0	0
Female	375	1	0.5
Total	**415**	**1**	**0.5**

$X^2=0.214$, $p=0.512$ in which ($p>0.05$)

Similarly seroprevalence of bovine brucellosis in different age groups was analyzed. Accordingly, the seroprevalence was found to be 0%, 0.5% and 0.5% in age categories of 1-4 years, 5-8 years and 9-12 by RBPT and 0%, 0.25% and 0.25% by CFT respectively. (Table 3). The difference in seropositivity of cattle in different age groups was not found to be statistically significant ($p>0.05$).

Table 3: Seroprevalence of bovine brucellosis based on age

Age	**No. of animals tested**	**RBPT Positive (%)**	**CFT Positive (%)**
1-4 yrs	55	0	0
5-8 yrs	162	0.5	0.25
9-12 yrs	198	0.5	0.25
Total	**415**	**1**	**0.5**

$X^2=0.330$, $p=0.848$ in which ($p>0.05$)

Seroprevalence of bovine brucellosis by breed: Comparison of different breeds showed that, all seropositive animals for bovine brucellosis were found to be local (Horro) breeds. As a result a seroprevalence of, 4 (1%) and 2 (0.5%) by RBPT and CFT while it was 0 (0%) in cross (Horro-Jersy) by both RBPT and CFT (Table 3). Although the seropositivity was

higher in local (Horro) breed, the difference in prevalence of bovine brucellosis between the two breeds was not statistically significant (p>0.05).

Table 4: Seroprevalence of bovine brucellosis in different breeds

Breed	No. of animals tested	RBPT Positive (%)	CFT Positive (%)
Local(Horro)	354	1	0.5
Cross (Horro-Jersy)	61	0	0
Total	**415**	**1**	**0.5**

X^2=0.346, P=0.556 in which (p>0.05)

The seroprevalence of bovine brucellosis in groups of cows which are pregnant and non-pregnant was found to be statistically significant (p<0.05). The seroprevalence was higher in pregnant cows 0.72 % (3/415) and 0.5% (2/415) by RBPT and CFT respectively than in non-pregnant cows which are 0.25% (1/415) by RBPT that was negative by CFT (Table 5).

Table 5: Seroprevalence of bovine brucellosis based on pregnancy status

Pregnancy status	No. of animals tested	RBPT Positive (%)	CFT Positive (%)
Pregnant	59	0.72	0.5
Non pregnant	356	0.25	0
Total	**415**	**1**	**0.5**

X2=12.126, p=0.00 in which (P<0.05)

Discussion

The present study demonstrated that the overall seroprevalence of bovine brucellosis in the study area of Wollega University Horro-guduru Animal Breeding and Research Center in which animals partially intensified and reared extensively were 0.5%. In this study the seroprevalence of bovine brucellosis was slightly lower than previous finding in different parts of Ethiopia in which seropositivity of; 38.7% in cattle owned by institute of Agricultural Research Farm (Rashid, 1993), 18.4% in Dairy Farms around Addis Abeba (Gebremariam, 1985), 12.34% in and around Bahir Dar (Shiferaw, 1994), 11.2% in Pastoral and Agro-pastoral areas of East Shoa Zone, Oromia regional state (Hunduma and Regasa, 2009), 11.6% in Sidama Region (Endrias, 1996), 7.62% in Arsi Region (Molla, 1989), 2.9% in three Agro Ecological Areas of Central Oromia, Ethiopia (Tujuba *et al*, 2006). Slightly higher individual serological prevalence rate of 5.6% in Eritrea (Faye *et al*, 2005), 6.5% in sudan (Hellman *et al.*, 1984), 9.9% in Kenya (Kagumba and Nandokha, 1978) and 15.8% in Uganda (Omer *et al.*, 2000) was also reported. On the other hand it was slightly higher than the report of (Teferi *et al*., 2011) 0.05% in Merti district of Arsi Zone, Fikadu (1999) 0.2% in the Highland Agro Ecological Zone of Eastern Amhara Regional State, Tadese (2003) 0.14% in north Gonder. The overall seroprevalence of bovine Brucellosis in this study was closely in agreement with the finding of Tadele (2004) 0.77% in Jimma zone and Lidia (2008) 0.45 % in Central Highlands of Ethiopia.

All sera samples examined for the estimation of bovine brucellosis were subjected to RBPT as screening and the CFT as confirmatory test, in which a high degree of agreement between both tests normally expected (Dohoo *et al*., 1996). In the present study However, 4(1%) of the positive samples in RBPT could not be confirmed in CFT. A possible explanation

for this disagreement between RBPT and CFT could be that the RBPT antigen might be contaminated or expired. Antigen and sera may not have been brought up to room temperature before testing. Also an over estimation of the agglutination reaction by the individual investigator could be considered. Moreover, cross reaction with other bacteria such as *Yersinia enterocolitica, E.coli, Salmonella species* and *Pasteurella species* could have led to false positive reactions (Nielsen, 1990). However, according to Nakavuma (1994) the RBPT provides more likely false positive results than to miss brucellosis. Hence, the confirmatory test CFT was carried out to reduce number of false positive samples considerably.

Different seroprevalence results were reported in the last 30 years from different corners of Ethiopia due to difference in management, husbandry, climate, topography and other factors. Most of the studies so far were reported from South Eastern, Central Highland and few were reported from northern parts of the country (Desalegn, 2008). Generally, the occurrence of bovine brucellosis is described to cover large area of the country and rates of infection were varied from one region to another even between districts within the region. According to WHO (1986) the level of brucellosis infection tends to be relatively high in intensive farm than extensive farm. But, in the current study area, the prevalence of bovine brucellosis was low. This may be because of that, animals were not completely intensified, animals in the ranch do not have contact with animals of surrounding area and animals introduced to the ranch were quarantined and examined before mixing to animals in the ranch.

Sex has been one of the risk factors affecting susceptibility of cattle to *Brucella abortus* infection (Radostitis *et al.,* 2007). Even if the proportion of male animals tested were smaller as compared to female number, the positive reactor animals in the study area were all female. In the present study, prevalence of the disease based on sex was 0.5% in female and 0% in males. This idea has been supported by different investigators in the country such as Asfaw *et al.,* 1998, Bekele *et al.,* 2000, Desalegn, 2008. Different factors are probably involved in the variation of sex susceptibility including physiological and behavioral differences between males and female. Some of the reasons were that, males are kept for relatively shorter time of duration in breeding herd than females and thus, the chance of exposure is lower for males (Kebede *et al.,* 2008). Lower prevalence in male could be probably due to smaller number of males tested as compared to female and also due to the fact that serological response of male animal to brucella infection is limited because of confinement of bacteria in the testes and reticulo endothelial system (Crowford *et al.,* 1990). It is well known that cows are more susceptible to brucella organism because of preferential growth of *Brucella abortus* in the gravid uterus. It can inter the uterus as it disseminate from the principal site of carrier state (udder and supra mammary lymph node). In latently infected cows depending on the number of pregnancy events and presence of infection; this will give the organism sufficient contact with lymph node system to stimulate a significant immunity response (Lapraik, 1982).

Age is supposed to have some association with the occurrence of brucellosis, because sexual maturity is very important for the rapid multiplication of brucella organisms. In present study, the result suggested that cows older than 4 years of age were more likely to become seropositive to brucella. This finding was in agreement with reports of Lidia (2008). This high prevalence rate of bovine brucellosis among adult and older cows might be related to maturity and therefore, the organism propagates and produces either a latent infection or clinical manifestation. On the other hand, younger animal tends to be more resistant to infection and frequently clear infection although latent infection could occur (Radostitis *et al.,* 2007).

There is still controversy among different authors on the basis of breed susceptibility to brucellosis. In current study, the seroprevalence was found higher in local (Horro) breed animals (0.5%) than cross (Horro-Jersy) 0%. But this difference was not statistically significant which is in agreement with the reports of Lidia (2008) in central highlands of Ethiopia. However, in contrast to the finding of the present study there is no association between the breed of cattle and seroprevalence of bovine brucellosis (Radostitis *et al.*, 2007).

Pregnancy status is also one of the risk factors affecting the susceptibility of cows to brucella infection. In the present study, the seroprevalence of bovine brucellosis was found higher in pregnant animals (0.5%) than non-pregnant 0%. This association is highly statistically significant (p=0.00). The susceptibility of cattle to brucella infection is influenced by pregnancy status of animals in which pregnant animals were highly susceptible as in agreement with the previous finding of (Walker, 1999). It is clear that female cattle are more susceptible to brucella organism especially if it is pregnant due to the fact that sex hormones and erythritol sugar alcohol synthesized in placenta and in female reproductive tract which stimulate the growth and multiplication of brucella organism that tends to increase in concentration. There is also chance of getting the disease during mating from infected bull or during artificial insemination from contaminated semen (Robert, 1986).

Conclusion and Recommendations

The overall seroprevalence of bovine brucellosis in Wollega University Horro-Guduru Animal breeding and Research Center was 0.5% which was confirmed by CFT. This result was much lower than the figures of previous reports from different parts of Ethiopia. However, the result did not only suggest the presence of the disease in cattle population in the area, but also indicated the presence of foci of infection that could serve as sources of infection for the spread of the disease into non infected animals. The present study also revealed that bovine brucellosis was higher in pregnant cows which were statistically significant and higher in females than males, in adult and old than young, and in local than Horro-Jersy cross breed, even though these were not statistically significant. In conclusion, bovine brucellosis cannot be ignored as nonexistent disease in current study area where it may continue to become hazard to livestock industry and public consuming raw products of animal origin. Knowledge of prevalence of bovine brucellosis and herd level risk factor is essential for introduction of cost effective and efficient control program. Therefore, based on the current finding the following recommendations are forwarded:

- This study was the first to document the seroprevalence of bovine brucellosis in few randomly selected animals in the study. So, attention should be paid to know the exact prevalence in the ranch by testing all animals and efforts should be directed towards improving the animal health delivery system.
- A detail investigation should be conducted to characterize the isolates and determine the detail epidemiology of brucellosis in the study area to plan control and prevention accordingly.
- The public in general and high risk groups in particular should be aware of zoonotic importance and how to keep proper hygiene and disposal practice of fetal membrane and aborted fetus.

References

1. Anonymous, B. (2006): Brucellosis back ground prepared by *Amer Vet Assoc*, 44: 216-224.

2. Arimi,S. and Mangen,M. (2002): Brucellosis in Sub-Saharan Africa: Epidemiology, control and impact. Veterinary Microbiology, 90:111-132.
3. Asfaw, Y., Molla., B., Zessin ,K. and Tegegn, A. (1998): Across sectional study on bovine brucellosis and test performance in intra and peri-urban dairy production system in and around Addis Abeba. *Bull Animhlth and prod Africa*, 46: 217-224.
4. Bekele, A., Molaa, B., Asfaw, Y. and Yigezu, T. (2000): Bovine brucellosis in ranches and farms in south eastern Ethiopia. *Bull.Anim. Hlth. Prodn. Afr*.48: 13-17.
5. Berchovich, Z. (1998): The use of tests for diagnosis of brucellosis in cattle, 8th ed. Mosby, Elsevier, Pp 286-289.
6. Birhanu, A. (2002): Animal health and poverty Reduction Strategies in proceeding of the 16th Annual Conference of the Ethiopian veterinary association (EVA) held at Ghion Hotel Addis Abeba, Ethiopia, The president of Ethiopian Veterinary Assciation.
7. Brew, S.D., Perret, L.L., Stack, J.J., Macmillan, A. P. and Staunton, N.J. (1999): Human exposure to brucella recovered from a sea mammal, *vet rec*. 24: 483-490.
8. Crowford, R. p., Adams, L.G. and Williams, I.D. (1990): Relationship of fetal age at exposure of pregnant heifers and Brucellaabourtus, *Am. J. Vet.Res*. 48: 755-782.
9. CSA. (2011): The Federal Democratic Republic of Ethiopia. Central Statistics Agency, Ethiopia.
10. Desalegn, F.(2008): Seroprevalence study of bovine brucellosis in Asella Gevermental dairy farm, Asella, Ethiopia, DVM thesis, Jimma university college of agriculture and veterinary medicine Jimma Ethiopia.
11. Dohoo, I.R., Wright, P.F., Rucker, G.M., Semegh, B.S., Robertson, F.J. and Forbes, L.B. (1996): A comparison of five serological tests for bovine brucelosisis .canada.*J.Vet.Res*.50:485- 493.
12. EMA, (1970): Ethiopian ministry of Agriculture. A review on animal health and production factors. Sited from Dinka (995), Addis Abeba University, Faculity of Veterinary Medicine; Debrezeit, Ethiopia.
13. Endrias, Z. (1996): Seroprevalence study of bovine brucellosis in selected sites of sidama region, DVM thesis, FVM, Addis Abeba University Debrezeit, Ethiopia.
14. FAO. (2003): Guidelines for coordinated human and animal brucellosis surveillance, FAO animal production and health, Pp 156.
15. Fikadu, K. (1999): An epidemiological survey of bovine brucellosis in Amhara National regional state.DVM Thesis, FVM, AAU, Debre Zeit, Ethiopia.
16. Faye, B., Castel, B., Lesnoff, M., Rutabinda, D. and Dhalwa, S. (2005): Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda) .*prev.vet.Med*. 64(4): 267-281.
17. Gebremariam, K. (1985): The Prevalence of bovine brucellosis in four different farms around Addis Abeba, DVM thesis, FVM, AAU, DebreZeit, Ethiopai.
18. Hellman, E., Stack, C. and Baumann, M. (1984): Bovine brucellosis among two different populations in Bahr el Ghazal Province of Southern Sudan. *Trop.med.Parasitol*. 36(2): 123 -126.
19. Hunduma, D. and Regasa, C. (2009): Seroprevalence study of bovine brucellosis in pastoral and agro pastoral areas of east shoa zone, Oromia Rigional State Ethiopia.

20. Kagumba, M. and Nandokha, E. (1978): A survey of prevalence of bovine brucellosisin East Africa. *Bull.anim.hlth.prod*.26(3): 224-229.
21. Kebede, T., Ejeta, G. and Ameni, G. (2008): Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale- Jida district) *Revue Vet. Med*. 159(1): 3-9.
22. Lapraik, G. (1982): Latent bovine brucellosis. *Vet Rec*, 3(16-24): 578-580
23. Lidia, B. (2008): seroprevalence study of bovine brucellosis in central highland 0f Ethiopia, DVM thesis, Jimma University, college of Agriculture and Veterinary Medicine, Jimma, Ethiopia
24. Mangen, M., Otte, M., Pfeiffer, J. and Chilonda, P. (2002): Bovine brucellosis in Sub-Saharan Africa; estimation of seroprevalence and impact on meat and milk off take potential, Livestock Policy Discussion Paper No 8, Food and Agriculture Organization, Livestock information and policy branch AGAL.
25. Molla, B. (1989): Seroepidimiological survey of bovine brucellosis in Arsi Region. DVM thesis, FVM, Addis Abeba University Debrezeit, Ethiopia.
26. Morgan, W.J., MacKinnon, D.J., Lawson, J.R. and Cullen,G.A. (1996): The rose Bengal plate agglutination test in the diagnosis of brucellosis. *Vet. Record*, 85: 636-641.
27. Nakavuma, J. (1994): A serological survey of *Brucella abortus* in cattle and goats in the central and Southern Regions of Uganda, MSc. Thesis.Faculty of veterinary Medicine, Makerere University, Kampala, Uganda.
28. Neilsen, K. (1990): The serological response of cattle immunized with *Yersinia enterocolitica* O: 9 or O:16 to *Yersinia* and *Brucella abortus* antigens in enzyeme immunoassays. *Vet. Immunol.Immunopathol.*,24*: 73-382. Vet. and Animal Sci.*, 29: 1157-1162.
29. OIE (2004): world organization for animal health. Bovine brucellosis, in manual standard for diagnostic tests and vaccines 5thed, paris. Pp 242-262.
30. OIE, (2008): Bovine brucellosis manual of Diagnostic Tests and Vaccines for Terrisrerial animals, office International Des Epizooties,Pp 409-435.
31. Omer, M.K., Skjerve, E., Holstand, G., Woldehiwot, Z. and Macmillan,A.P.(2000): Prevalence of antibodies to Brucella species in cattle, sheep, goats horses and camels in the state of Eritrea, influence of husbandry system. *Epigemol. Infect*. 125(2): 447-453.
32. Radostits, M., Gay.,Hinchcliff, W. and Constable, D. (2007): Veterinary medicine. A text of disease of cattle, horses, sheep, goats and pigs, 10th ed. Saunders, Philadelphia, Pa, USA.
33. Rashid , M. (1993) : reproductive wastage in cattle due to bovine brucellosis proceeding of the 4thNational Livestock improvement conference, 13-15th November 1991, Institute of Agricultural research, Addis Abeba, Pp 270-272.
34. Robert, S.J. (1986): Veterinary obstetrics and Genital diseases. 2nded.CBS publisher and Distributers, Pp 107.
35. Shiferaw, A. (1994): Seroepidimiological study of bovine brucellosis in around Bahir Dar, DVM thesis, FVM, Addis Abeba ,Debrezeit, Ethiopia. Tadele, T. (2004): Seroprevalence of bovine brucellosis and its puplic health significance in selected Zone, western Ethiopia.
36. Taddese, Y. (2003): A survey of bovine in selected areas of North Gonder Zone, DVM Thesis, FVM, AAU, DebreZeit, Ethiopia.

37. Teferi, D., Asmamaw, D. and Reta, D. (2011): Brucellosis and Some reproductive problems of Indigenous Arsi Cattle in selected Arsi zones of Oromia Regional state, Ethiopia, *Glob Vet*, 7(1): 45-53.
38. Thrusfield, M. (1995): Sampling in veterinary Epidemiology, 2nd ed. B;lackwell science, Oxford, Pp251-281.
39. Tujuba, J., B. Kelay., M. Bekana., S. Teshale., H. Gustafason and H. Kindah. (2006): Epidimiological study of bovine brucellosis in three Agro-ecological areas of central Oromia, Ethiopia.
40. Walker, L. (1999): Brucella in dwright, C.H change, veterinary microbiology, Massachusetts, Black well science.
41. WHO. (1986): FAO-WHO expert committee on brucellosis. 6th report, world health organization Technical Report series 740, Geneva, Pp 27-66.