**Research Article**      **Open Access**

# Rose Bengal Plate Test (RBPT) Based Sero-prevalence of Bovine Brucellosis in and around Chench, Gama Goffa, Southern Ethiopia

**Melese Yilma***

*Areka Agricultural research center, Southern Agricultural Research Institute, Areka, Ethiopia*

**\*Corresponding author:** Melese Yilma, Areka Agricultural research center, Areka, Ethiopia, Tel: +251 910 75 51 90; E-mail: melese.yilma@yahoo.com

## Abstract

Across sectional study was conducted with objective to determine sero-prevalence bovine brucellosis occurrence from Dec 2009 to Feb 2010 at Chencha district of Gamo Gofa zone. Total of 384 serum samples were collected from cattle and tested for the presence of brucell antibodies using Rose Bengal Plate Test (RBPT). Overall sero-prevalence of 4 (1.04%) was recorded in the area. Among the risk factors Abortion and RFM (Retain Fetal membrane) were found significantly associated with sero-positivity (p<0.05). In conclusion the present work generally showed low sero prevalence of bovine brucellosis in the study area and brucellosis was clinically associated with abortion and retained fetal membrane.

**Keywords:** Abortion; Bovine; Brucellosis; Chencha; Ethiopia; GamoGofa; Seroprevalenc

## Introduction

Ethiopia is a country with different agro-ecological zones where considerable populations of livestock are raised [1]. Current estimates of Ethiopia livestock population indicate that the country owns 44.4 million cattle, 23.6 million sheep and 23.3 million goats. Majority of the livestock are reared in the low land of Somali, Afar and Borena pastoral areas [2].

Animal disease is one of the most important constraints to increase the productivity of food animals in sub-Sahara Africa [3]. Brucellosis is an important bacterial disease of animals and humans caused by *B. abortus, B. melitensis, B. ovis* and *B. suis* with their host preference in order being cattle, goats, sheep and swine [4]. Although Brucella species have a wide host range [5]. Brucellosis in cattle is characterized primarily by abortion in late pregnancy, frequently followed by fetal membrane retention and endometritis, which may be the cause of infertility in subsequent pregnancies [5-7].

Brucellosis is essentially a disease of the sexually nature animal, the predilection sites being the reproductive tracts of males and females, especially the pregnant uterus [5]. Allantoic factors stimulate the growth of most brucella. These factors include Erythritol, possibly steroid hormones and other substances [8,9].

*Burcella* species possesses two heat-stable surface antigens, designated A and M that are responsible for agglutination reactions. *B. abortus* contains large amounts of A and small amounts of M antigens [9].

The reported prevalence of bovine brucellosis in East Africa was ranges 1.8%-34.9% Uganda and Rwanda respectively [10]. In Ethiopia several investigator have established the endemcity of bovine brucellosis in different part of the country [11]. Most of these works have been carried out at field level with aim of assessing the magnitude of brucellosis in ruminants [12,13]. In particular study area with the intervention of Artificial Insemination (AI) service delivery for genetic improvement and frequent report of the case for abortion and RFM (Retain Fetal membrane) take my pine point to conduct the work in the particular area. As the disease has both Veterinary and public health importance it is necessary to assess the current status of bovine brucellosis in smallholder level in the area to generate baseline information on bovine brucellosis to forward precaution measures disease exposure. For the particular study area the work report is the first.

## Materials and Methods

### Description of study area

The study was conducted in Chencha district, Gamo Gofa zone, South Eastern Ethiopia, which is located at about 537 km and 37 km from Addis Ababa and Arba Minch respectively. According to the [2] the district has a total surface area of 1.182.73 km$^2$ and support 232,432 residents whose main occupations subsistence farming. Chencha is bordered on the south by Arba Minch Zuria, on the west by Dita, on the north by Kucha and Boreda, and on the east by Mirab Abaya. Towns in Chencha include Chencha, Dorze, Dokko and Ezo. Chencha have area coordination of 6°15'0"N    37°34'12"E, with irregular topography of mountain, marshy areas, steep slope and water covered parts. Arba Minch state farm meteorological station recorded two rainy seasons in the area the long rainy season that extends from February to April with mean annual rain fall of 934 mm-1000 mm and mean annual temperature of 15°C-28°C.

The vegetation cover of the area includes various bush formation and deciduous forest. The soil type of the area is also classified as sandy and clay sandy soil. According to the record by Woreda agricultural planning office the livestock population of the area is estimated about 52,666 head of cattle; 55,239 head of sheep; 60, 171 head of goats; 6608 equine; 12, 725 chickens and 5,982 beehives are also registered in the district. Almost all people in the area engaged in mixed crop livestock production with the exception of Dorze community.

### Study animals

The target populations were smallholder cattle breed under extensive management system, which consist of breeding caws. None of the animals were vaccinated against brucellosis.

### Study design

Across sectional study was conduct, during the period of Dec 2009 to Feb 2010 using Rose Bengal Plate Test (RBPT) and sero survey methods. Within selected PAs purposively cattle population with the history of abortion & RFM were sampled and randomly non clinical case animal were selected from the household cattle hared.

### Sample size determination

The sample size was calculated on the basis of 50% prevalence of bovine brucellosis was considered for there was no previous work done in the area, with the expected precision of 5% and at 95% confidence interval. A total of 384 animals were sampled. Thus for sample size estimation, the formula described by [14] was used.

### Collection of serum sample

From accessible cattle approximately 7mL-10 mL of blood was collected from the jugular vein of clinically case and apparently healthy adult animals for serological examination using plain vaccutainar tubes and needles. After the identity of each animal was labeled on the corresponding vacutainer tube the sample was shipped to Wolaita Soddo Veterinary Regional Laboratory (WSVRL) in Ice box the tubes were left tilted over night at room temperature and 1500rpm for 10min, centrifugation to allow clot retraction and sera were separated from the clot by siphoning using plastic pipette, in to the other sterile test tubes.

### Serology Diagnosis

RBPT, the procedure described by [15] was followed with little modification by [16]. Reactions were categorized as 0, +, ++, +++, according to [17] where: 0= means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing, and +++ = clumping, definite clearing. Those samples identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were regarded as positive. This test was performed following the procedure described by [16] mixing 1.30 μL serum mixed with equal volume of antigen. The plates were shaken for 4 min and any agglutination that appeared within this time was recorded as a positive reaction.

### Data storage and analysis

The raw data generated from serological testes and sero-survey were systematically arranged and stored in MS excel spread sheet and statistical analysis was performed by using SPSS program for window (version 16.0, 2003). Descriptive program statistics such as percentage was used to determine categorical variables (Age, sex, Abortion and stillbirth). The prevalence rate was calculated as the number of categorical variables/ RBPT positive animals divided by the total number of animals tested. The association between each risk factors and the outcome variable was assessed using person's chi-square test and differences was recorded statistically significant if P-value is less than 0.05.

## Result

Serological test was used to determine the prevalence of bovine brucellosis and risk factors associated with the occurrence of the disease were also investigated. 384 serum collected were subjected to RBPT and 4 (1.04%) serum samples were found positive. Therefore, all the subsequent RBPT test analysis for prevalence were based on the sera (n=4) that were positive to RBPT. The overall prevalence of bovine brucellosis in Chencha district was found 1.04%. The sero prevalence in different age groups was calculated (Table 1) and age groups 5-8 years had the highest sero-prevalence. This age group was found epidemiologically very important in transmission of disease but no significant was recorded in my finding.

| Variable | Number of animals tested | RBPT positive | P-value | $X^2$ |
|---|---|---|---|---|
| **Age** 2 years-4 years | 224 | 2 (0.9 %) | | |
| 5 years-8 years | 144 | 2 1(4%) | 0.825 | 0.385NS |
| >8 years | 16 | 0 (0.0%) | | |

**Table 1:** Prevalence of bovine brucellosis according to Age;***P=0.001; **P=0.01; NS-P>0.05.

During collection of samples history of abortion and RFM (Retain Fetal membrane) were recorded among sampled cows. Out of the tested animals above 3 years age (N=384), there were 49 (12.76%) and 30 (7.8%) cows with the history of abortion and RFM respectively (Table 2).

| Total number of animals | Abortion | RFM (Retain Fetal membrane) |
|---|---|---|
| 384 | 49 (12.76%) | 30 (7.81%) |

**Table 2:** Prevalence of abortion and retain fetal membrane.

The association of brucellosis with abortion and retain fetal membrane was tested using chi-square. It was found that brucellosis was highly associated with abortion and retained fetal membrane (Tables 3 and 4).

| Result of test | History of abortion | | Total | Chi-square ($X^2$) |
|---|---|---|---|---|
| | **Aborted cows** | **Non-aborted cows** | | |
| RBPT+ | 2 (4.1%) | 2 (0.6%) | 4 | |
| RBPT- | 47 | 333 | 380 | 26.97*** |
| | | | 384 | |

**Table 3:** Association of brucellosis with abortion; ***P=0.001; **P=0.01; NS-P>0.05.

| Result of test | History of RFM | | Total | Chi-square ($X^2$) |
|---|---|---|---|---|
| | **Cows which have history of RFM** | **Cows which have no history of RFM** | | |

| | | | |
|---|---|---|---|
| **RBPT+** | 2 (6.25%) | 2 (0.57%) | 4 | |
| **RBPT-** | 30 | 350 | 380 | 11.43*** |
| **Total** | 32 | 352 | 384 | |

**Table 4:** Association of brucellosis with retain fetal membrane (RFM);***P=0.001; **P=0.01; NS-P>0.05.

For caws with the history abortion and RFM statistically significant risk factor for sero reactivity that caw with the history of abortion 2 (4.1%) were investigated and statistical significant test result was recorded. The risk of aborted was 0.27 times higher in sero-positive caw (OR=0.27, CI=[0.1454435 - 0.501151], p value=0.000). Similarly caw with the history of RFM 2(6.25%) were investigated and statistical significant test result was recorded. The risk of RFM was 1.76 times higher in sero-positive caw (OR=1.763951, CI= [1.091255 - 2.851325], p value=0.021).

## Discussion

This prevalence report agree with the findings of [18] 1.5% in shoa, 3.0% [19] in the central high lands of Ethiopia, 1.8% [20] in eastern Amhara National Regional State for cattle kept under extensive management system. The lower prevalence report of tropical high land 2% [21] showed similarity among traditional management systems in this regard. A survey of 226 animals in Gobe ranch by [22] showed 16.8% of the animals to be Sero-positive to brucellosis. A study carried out at the Abernosa ranch showed that of 963 animals, which had been tested for brucellosis 14.2% were infected [23]. A study around Sidamo region of Ethiopia revealed a prevalence rate of 11.6% reactors in cattle [24]. Other studies were also carried out in different parts of the country and revealed the following prevalence: 38.7% at Baka Research center at Western Ethiopia, 16.9% around Bahir Dar [25], 0.1% at North Gondar [26], 0.7% prevalence from 430 cattle in Tigray [27] and 2.1% of 1985 animals tested in shoa were positive for brucellosis [28]. Indicating the disease was widespread in both indigenous and exotic crosses in the country [29]. Control and eradication of brucellosis can be achieved through reduction of the disease to the possible lowest level by widespread of use of vaccination and then application of a test and slaughter policy [30,31].

Herd sero-prevalence of brucellosis was higher in herds that had a history of abortion (4.1%) and RFM (6.25%) compared with herds with no history of abortion (0.6%) and RFM (0.57%). Significant difference in prevalence of brucellosis was observed between aborted and non-aborted cows and the prevalence report is in agreement with [32]. The prevalence in the study area was agreement with the other investigators such as prevalence of 4.5% in Addis Ababa, 3.2% in Kombolcha, 11.8% in Jersey cows at Wolaita Sodo dairy farm, 6.7% in North Tigray [33]. Therefore to conclude that brucellosis was clinically associated with abortion and retained fetal membrane. So further studies should be conduct to confirm Brucella biotypes circulating in the area through isolation and characterization form the clinical material to suggest prevention and control option.

## Acknowledgement

## References

1. TegegneA, Mengistie T, Desalew T, Teka W, Dejen E (2009) Transhumance cattle production system in North Gondar, Amhara Region, Ethiopia In: IPMS of Ethiopian Farmers Project. Working Paper 14 ILRI Nairobi Kenya 73.

2. CSA (2004) The 2001/02 Ethiopian Agricultural sample enumeration, executive summary. Central Statistical Authority, Addis Ababa, Ethiopia.

3. Lemma M, Kassa T, Tegene A (2001) Clinically manifested major health problems of cross breed dairy herds in urban and peri urban production systems in the central highland of Ethiopia. Trop Anim Hlth Prod 33: 85-93.

4. Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, et al. (2011) Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Prev Vet Med 2954:14.

5. Radostits OM, Gray CC, Blood DC, Hinchciff KW (2000) Brucellosis in Veterinary Medicine, text book of the diseases of cattle, sheep, pig, goats and horses, 9th edition W.B. Saunders Ltd Oxford: 867-891.

6. Buxton A, Fraser G (1977) Animal Microbiology. Vol. 1, London, Edinburgh, North Balawyn: Black well scientific published.1:133-140.

7. Aiello S (1998) The Merck Veterinary Manual, 8thedition Merck and Co. Inc, White house station NJ USA 999-1002.

8. Acha NP, Szyfres B (2001) Brucellosis In: Zoonosis and Communicable Diseases to man and animals. 3rd edtion, Pan American Health Organization Washington DC USA 1: 40-62.

9. Quinn PJ, Carter ME, Markey B, Carter GR (2002) clinical Veterinary Microbiology microbial disease, Black well sciences. Publishing Wolf Spain 2: 261-267.

10. Yilkal A, Bayleyegn M, Zessin KH, Azage T (1998) A cross- sectional study of bovine brucellosis and test performance in per urban and urban production systems in and around Addis Ababa, Ethiopia. J Afr Ani Hea Produ 46: 217-224.

11. Yohannes M, Degefu H, Tolosa T, Belihu K, Cutler R (2013) Brucellosis in Ethiopia. Afr J Microbiol Res 7:1150-1157.

12. Sara T (2008) Study on sero-prevalence of small ruminant Brucellosis at Modjo, Luna, Elfora and Helmex abattoirs, Ethiopia. DVM Thesis FVM AAU Debre Zeit Ethiopia.

13. Yibeltal M (2005) A sero-prevalence study of small ruminant brucellosis in selected sites of Afar and Somali region Ethiopia. DVM Thesis FVM AAU Debre Zeit Ethiopia.

14. Thursfield M (1995) Veterinary epidemiology (2ndedn), London: Black Well Science Ltd., pp. 178-179.

15. Alton GG, Jones LM, Angus RD, Verger JM (1988) Techniques for the Brucellosis Laboratory. Institute National de la Recherché Agronomique, Paris, France.

16. Blasco JM, Garin-Bastuji B, Marin CM, Gerbier G, Fanlo de Bagues et al. (1994) Efficiency of different rose Bengal and complement fixation agents for the diagnosis of Brucella melitensis infection in sheep and goats. Vet Res 134: 415-420.

17. Nielson K, Dunkan R (1990) Animal brucellosis: In Bovine brucellosis. Manual of standards for diagnostic tests and vaccines (3rdedn) pp. 252-265.

18. Shiferaw A (1987) The prevalence of bovine brucellosis under different management system around shoa. DVM Thesis FVM AAU Debre Zeit Ethiopia.

19. Wondimu A (1989) The epidemiology and economics of bovine brucellosis in the central highlands of Ethiopia. Veru: Reading University MSc Thesis FVM, Ethiopia.

20. Kebede F (2000) An Epidemiological survey of bovine brucellosis in Amare National Regional state. Proceeding of the 4th annual Ethiopian Veterinary Association Conference, Addis Ababa.

21. Omer MK, Skejerve E, Holstd G, Woldehiwot Z, Mackmilan AP (2000) Prevalence of antibodies to Brucella species in cattle, sheep, goats, horses and camels in the state of Eritrea, influence of husbandry, system. Epid & inf 12: 447 -453.

22. Bayleyegn M (1989) Sero epidemiological survey of bovine brucellosis in Arsi region. DVM Thesis FVM AAU Debre Zeit Ethiopia.

23. Taye Y (1991) Sero prevalence of bovine brucellosis at Abernosaranch. Ethiopia DVM Thesis FVM AAU Debre Zeit Ethiopia.

24. Endrias Z (1989) Sero epidemiological study of bovine Brucellosis in selected sites of Sidamo region DVM Thesis FVM AAU Debre Zeit Ethiopia.

25. Abeje S (1994) Sero epidemiological study of bovine brucellosis in and around Bahir Dar. DVM Thesis FVM AAU Debre Zeit Ethiopia.

26. Tadesse Y (2003) A survey of bovine brucellosis in selected areas of North Gondar Zone DVM Thesis FVM AAU Debre Zeit Ethiopia.

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

28. Assegid S (1987) The prevalence of bovine brucellosis under different management systems around Shoa based on serological test DVM Thesis FVM AAU Debre Zeit Ethiopia.

29. Abrha B (2007) Seroprevalence of Bovine Brucellosis in dairy farms DVM Thesis FVM AAU Debre Zeit Ethiopia.

30. FAO (1986) Brucellosis (Expert Committee) World Health Organization Technical Report sixth Series 740 Geneva, pp.66-77.

31. Nicoletti P (1984) The control of brucellosis in tropical and sub-tropical region. Prev Vete Med 2:193-196.

32. Adugna KE, Agga GE, Zewde G (2013) Seroepidemiological survey of bovine brucellosis in cattle under a traditional production system in western Ethiopia. Rev Sci tech off Int Epiz 32: 1-20.

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