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

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## Abstract

Across sectional study was conducted with objective to determine sero-prevalence bovine brucellosis occurrence from Dec 2009 to Feb 2010 at Chench district of Gamo Gofa zone. Total of 384 serum samples were collected from cattle and tested for the presence of brucella 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; Chench; Ethiopia; GamaGofa; Seroprevalence

## 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 mature 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].

*Brucella* 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 endemicity 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 Chench 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<sup>2</sup> and support 232,432 residents whose main occupations subsistence farming. Chench 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 Chench include Chench, Dorze, Dokko and Ezo. Chench 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 vaccutainer 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 Chench 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 <sup>2</sup>
<b>Age</b>				
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 <sup>2</sup> )
	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 <sup>2</sup> )
	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.

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