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SERO-PREVALENCE OF BOVINE BRUCELLOSIS IN AND AROUND CHENCHA DISTRICT, GOMOGOFA ZONE, SOUTH EASTERN ETHIOPIA

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ABSTRACT

Brucellosis is an infectious bacterial zoonotic disease caused by member of the genus Brucella. The disease affects both animals and human beings resulting in a serious economic loss in animal production sector and deterioration of public health. Cattle brucellosis has significant economic and zoonotic implication for the rural communities in Ethiopia in consequence of their traditional life styles, feeding habits and disease patterns. Hence, knowledge of brucellosis occurrence in traditional livestock husbandry practice has considerable importance in reducing the economic and public health impacts of the disease. A cross sectional study was conducted with objective of determining prevalence and associated risk factors for bovine brucellosis occurrence from November 2009 to March 2010 at Chencha district of Gamo Gofa zone. During the survey a total of 384 serum samples were collected from cattle 2-12 years age and were tested for the presence of brucell antibodies using Rose Bengal Plate Test (RBPT). In this study an overall seroprevalence of 4 (1.04%) were positive using RBPT recorded in the study area. Among the risk factors, Abortion and Retain Fetal membrane (RFM), were found significantly associated with serop-ositivity ($p<0.05$). The present study in general showed that low sero-prevalence of bovine brucellosis in the study area and it was clinically associated with abortion and retained fetal fetal membrane.

KEYWORDS: Abortion, Bovine, Brucellosis, Chencha, Ethiopia, Gamo Gofa, Seroprevalence.

INTRODUCTION

Ethiopia is a country with different agro-ecological zones where considerable populations of livestock are raised were reported by Tegegne, et al.[35] Ethiopia has one of the largest livestock resources in Africa, with a total cattle population of 47.6 million[15] 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.[14]

Bovine brucellosis is one of these infectious diseases and has been reported from several parts of the country.[9]

It is one of the oldest and most widespread zoonotic diseases, affecting food production in the tropics and subtropics.[22] It is an economically important disease of livestock causing reproductive wastage through infertility, delayed heat, loss of calves, reduced meat and milk production, culling and economic losses from international trade bans.[22] Clinical sign of brucellosis is characterized by abortion and retained fetal membrane (RFM) in cows and orchitis and epididymitis in bulls.[5]

Animal disease is one of the most important constraints to increase the productivity of food animals in sub Sahara Africa.[21] Brucellosis is an important bacterial disease of animals and humans caused by Brucella abortus, *B.melitensis*, *B.ovis* and *B.suis* with their host preference in order being cattle, goats, sheep and swine.[19]

Although Brucella species have a wide host range they are not readily transmitted from preferential to dissimilar hosts and when this occurs, they usually localized in the mammary gland and reticuoendthelial system rather than in the uterus and fetal membrane.[28]

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. In bulls the disease usually causes orchitis, epididymitis, seminal vesiculitis and sterility. It is an important zoonosis, causing undulant fever in humans characterized by an acute septicemic phase followed by chronic stage, which may extend over many years.[13,7,28] Brucellosis in cattle is caused almost exclusively by Brucella abortus, less frequently by Brucella melitensis and rarely by Brucella suis.[25]

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.[28] Allantoic factors stimulate the growth of most brucella. These factors include erythritol, possibly steroid hormones and other substances. Shared antigens occur between the brucella and other gram negative bacteria such as Francisella, Campylobacter, Salmonella, Pasteurella and Yersinina enterocolitica involving the somatic antigens. In agglutination test cross reactions have also been recorded in cattle, dog and other species.[5,27]

Burcella species possesses tow 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.[27]

The reported prevalence of bovine brucellosis in the different countries was as follows: Djibouti (4%), Somali (11.9%), Kenya (19%), Rwanda (34.9%), Sudan (6.5-22.5%) and Uganda (1.8%). In Ethiopia several investigator have established the endemcity of bovine brucellosis in different part of the country.[11,1,2,39,33,3]

In Ethiopia the existence of small ruminant brucellosis has been reported by few studies carried out on central Shoa, Afar and Somali with sero prevalence ranging from 7.4%[30] and 16%.[38] Most of these works have been carried out at field level with aim of assessing the magnitude of brucellosis in small and large ruminant. A survey of 226 animals in Gobe ranch by Bayelyegn[11] 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, 137 (14.2%) were infected.[34] Other studies were also carried out in different parts of the country and revealed the following prevalences: 38.7% (57/147) at Baka Research center at Western Ethiopia and 16.9% around Bahir Dar[2], 0.1% from 1447 cattle at North Gondar[33], 0.7% prevalence from 430 cattle in Tigray[3] and 2.1% of 1985 animals tested in shoa were positive for brucellosis.[10] Indicating the disease was widespread in both indigenous and exotic crosses in the country.[3]

In Ethiopia brucellosis was first reported in 1970 by Veterinary section of the US Navy Medical Research unit.[16] Since then several serological surveys have been reported and found the prevalence of bovine brucellosis to range from 0.2% in south western Ethiopia[32] to 38.7% in western Ethiopia.[29] However, there was no investigation on bovine brucellosis in the present study area.

In the study area with the intervention of Artificial Insemination service delivery for genetic important and frequent report of the case for abortion and retain fetal membrane take our 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 small house holder in local/indigenous breeds in the study area to generate baseline information on the level of bovine brucellosis at small household in order to forward precaution measures disease exposure. For the study area the work report is the first. Therefore, the objectives of this study were: To determine the seroprevalence of bovine brucellosis in small holder dairy cows and bulls in chencha Gamo Gofa, SNNPR, Ethiopia, to identify risk factors and quantify their degree of association with brucellosis in cattle, to assess risk factors associated with human brucellosis through questionnaire survey and to forward appropriate recommendations which are important to control the disease in both humans and animals.

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 Arbaminch respectively. According to the CSA (2004) the district has a total surface area of 1.182.73 km^2 and support, 232, 432 residents whose main occupations subsistence farming. Woreda's agricultural planning office classified the study area into agro climatic zones "Kolla" (low land) "Weynadega" (Midland) and Dega (highlands) accounting to 34%, 20% and 46% respectively. Altitude ranges from 1100-2700 meters above sea level with irregular topography of mountain, marshy areas, steep slope and water covered parts. Arbaminch 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 934-1000mm and mean annual temperature of 15-28^oC.

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 population was cattle, which consist of in calf, heifers, breeding females and available bulls. A total of 384 animals of 2-12 years of age, were sampled of which 33 males and 347 were females. In general indigenous breed types in the study area none of the animals tested were vaccinated against brucellosis.

Study design

A cross sectional study was carried out on bovine brucellosis with in selected Peasant Associations (PAs) in and around Chencha, Gamo Gofa, SNNPR, Ethiopia during the period covering from November 2009 to March 2010 using Rose Bengal plate Test (RBPT) and sero survey methods. In all selected PA purposively cattle population with the history of abortion and retain fetal membrane were sampled and randomly non clinical case animal were also selected from the household cattle hared.

Sample size determination

The study was across sectional type PAs were selected by convectional sampling method based on the willingness of the "Gotimeri" and animal owners with in the PAs and animals with in the PAs were selected using simple random sampling. 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 computed with the expected precision of 5% and at 95% confidence interval. The sample size was 48 from each Peasant associations (PAs) and Chencha Veterinary open air clinic, however, a total of 384 animals were sampled. Thus for sample size estimation, the formula described by Thursfield[36] was used.

$$N= \frac{1.96^{2*} P^{*}(1-P)}{d^{2}}$$

CI= 95%, (p=0.5, q=0.5), d= 0.05 n=384

CI = Confidence Interval n: Sample size

P: Prevalence d: Precission

$$\text{Therefore, } N= \frac{1.96^{2*} P^{*}(1-P)}{d^{2}} = 384$$

Collection of blood Sample

Random sampling from all accessible cattle were used approximately 7-10ml of blood was collected from the jugular vein of 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 coded sample was shipped to Wolaita Soddo Veterinary Regional Laboratory in Ice box the tubes were left tilted over night at room temperature and1500rpm for 10 minute centrifugation to allow clot retraction and sera were separated from the clot by siphoning using plastic pipette, in to the other sterile test tubes. Then the Sera sample stored at – 20^0C until serological tests was undertaken.

Serology Diagnosis

RBPR, the procedure described by Alton et al.[8] was followed with little modification by Blasco et al.[12] Reaction were categorized as 0, +, ++, +++, according to Nielson and Dunkan[24] where: 0 = means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing, and ++ = clumping, definite clearing. Those sample identified with no agglutination (0) were regarded as negative, where as those with +, ++ and +++ were considered as positive. This was performed following the procedure described by Blasco et al.[12] mixing 1.30 µL serum mixed with equal volume of antigen. The plates were shaken for4minutes and any agglutination that appeared within this time was recorded as a positive reaction.

Data Storage and analysis

The raw data generated from both serological testes and questionnaire survey were systematically arranged and stored in MS excel spread sheet and statistical analysis was performed by using SPSS program for window (version 13.0, 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 (x^2) test and differences was recorded statistically significant if p-value is less than 0.05.

RESULT

Overall Seroprevalence in cattle

A cross sectional study was conducted to determine the prevalence of bovine brucellosis and risk factors associated with the occurrence of the disease in Chencha district Gamo Gofa zone, SNNPR, Ethiopia from November 2009 to March 2010. Serological test was used to determine the prevalence of bovine brucellosis and some risk factors associated with the occurrence of the disease were also investigated.

The 384 serum collected were subjected to RBPT and 1.04% (4/384) 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% (4/384).

Sero prevalence in Age

The seroprevalence in different age groups was calculated (Table 1) and age groups 5-8 years had the highest seroprevalence. This age group was found epidemiologically very important in transmission of disease however no significant (p >0.05) was recorded in the study area in our finding.

Table - 1. Prevalence of bovine brucellosis according to Age.

Variables: Age	Number of animals tested	RBPT Positive	P-value	X^2
2years - 4years	224	2(0.9%)		
5years - 8years	144	21(4%)	0.825	0.385NS
>8 years	16	0(0.0%)		

***P=0.001, *P=0.01, NS= P>0.05

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

Table. 2: Prevalence of abortion and Retain Fetal membrane.

Total number of cows	Abortion	Still birth
384	49 (12.76%)	30 (7.81%)

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 Retain Fetal membrane (Table 3 and 4).

Table. 3. Association of brucellosis with abortion.

Result of test	History of abortion		Total	Chi-square (X^2)
	Aborted cows	Non-aborted cows		
$RBPT^+$	2(6.25%)	2(0.6%)	4	
$RBPT^-$	30	350	380	11.43^{***}
Total	32	352	384	

***P=0.001, *P=0.01, NS= P>0.05

Table. 4: Association of brucellosis with stillbirth.

Result of test	History of still birth		Total	Chi-square (X^2)
	Cows which have history of still birth	Cows which have no history of still birth		
$RBPT^+$	2(7.4%)	2(0.6%)	4	
$RBPT^-$	30	350	380	11.43^{***}
Total	32	352	384	

***P=0.001, *P= 0.01, NS=P>0.05

For cows with the history abortion and retain fetal membrane statistically risk factor for sero reactivity that cow with history of abortion 2 (4.1%) were investigated and statistical significan test result was recorded. The risk of aborted was 0.27 times higher in sero positive cow (OR=0.27, CI=[0.1454435 – 0.501151], P value=0.000). Similarity cow with the history of Retain Fetal membrane 2(6.25%) were investigated and statistical significant test result was recorded. The risk of Retain Fetal membrane was 1.76 times higher in sero positive cow (OR=1.763951, CI=[1.091255 – 2.851325], P value=0.021).

DISCUSSION

Individual level brucellosis infection rate observed in extensive management involving indigenous animals was low. In this system, as well there are reports that agree with this finding. Shiferaw[31] in shoa, Wondimu[37] in the central high lands of Ethiopia, Kebede[20] in eastern Amhara National Regional state observed infection rates of 1.5%, 3% and 1.8% respectively, in local breeds kept under extensive management. The lower prevalence report of tropical high land (2%) Omer et al.[26] showed similarity among traditional management systems in this regard. According to Bayleyegn[11] survey of 226 animals in Gobe ranch showed 16.8% of the animals were sero positive to brucellosis.

A study recorded at the Abernosa ranch indicated that of 963 animals, which had been tested for brucellosis 14.2% was infected.[34]

A study around Sidama region of Ethiopia revealed a prevalence rate of 11.6% reactors in cattle reported by Endrias.[17] 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[2], 0.1% at North Gonder[33], 0.7% prevalence from 430 cattle in Tigray[3] and 2.1% of 1985 animals tested in Shoa were positive for brucellosis.[10] Indicating the disease was widespread in both indigenous and exotic crosses in the country.[4] 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 attest and slaughter policy.[18,23]

Herd sero-prevalence of brucellosis was higher in herds that had a history of abortion (4.1%) and RFM (6.25%) in 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 Adugna et al.[6] 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.[3]

CONCLUSION AND RECOMMENDATIONS

A cross- sectional study of bovine brucellosis in Chencha showed a low prevalence of infection (1.04%). Therefore, the positive animals can be a susceptible animals and humans in the study area. Abortion and retain fetal membrane were found important risk factors associated for the occurrence of brucella reactor cattle. The main clinical signs of brucellosis are abortion and retain fetal membrane were significantly associated with seroprevalence of brucellosis. Based on the above findings and conclusion, the following recommendations forwarded

- The study was the first to document the seroprevalence of bovine brucellosis in few randomly selected animals in the study area. Thus, attention should be taken to know the exact prevalence in the region by testing all animals and improve the animal health delivery system.
- Control strategy should be designed to reduce the impact of the disease in the livestock sector of the country and public awareness should be increased through education on cause, transmission and risk factors.
- Extensive extension service including health education must be launched to make the farm owners, cattle attendant and the consumers aware of the disease and pasteurization/boiling of milk before consumption.
- For the overall reduction of sero reactor cattle and minimization of the associated risk factors, proper hygienic practices and continuous monitoring of the dairy animals for brucellosis.

- Strict movement control of animal from one area to another in order to prevent the spread and transmission of the disease from infected cattle to the non-infected ones.
- Further studies should be under taken to confirm Brucella biotypes occurring in the area through isolation and characterization form the clinical material to support prevention and eradication Brucella.

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