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Sero-prevalence of Bovine Brucellosis in and Around Kombolcha, Amhara Regional State, Ethiopia

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Abstract

A cross-sectional study was carried out on bovine brucellosis in and around kombolcha form November 2006 to April 2007, in the Amhara Regional State. A total of 240 blood samples were collected from semi-intensively and extensively managed cattle. The Rose Bengal Plate Test (RBPT) was used as a screening test. Those serum samples reacting positively to (RBPT) detected 9 of 240 (3.75%) of the samples as brucellosis positive. The positive sera when further retested using CFT 5 out of 9 RBPT positive sera were confirmed to be positive. The prevalence of brucellosis based on CFT in and around kombolcha was 2.08%, and all positive sera were from old aged female cattle. An attempt was also made to investigate the prevalence rate of abortion and fetal membrane retention in both extensive and semi-intensively management systems. A higher prevalence rate of abortion was recorded in extensively managed cows (10.8) than semi-intensively managed cows (2.08%).

The difference in prevalence rate was statistically significant (P<0.05). A relatively higher prevalence rate of retained fetal membrane was found in extensive managed cattle (13.8%) than semi-intensively managed cattle (4.1%). The difference in prevalence rate was statistically significant (p<0.05). In conclusion, the prevalence rate of brucellosis is low in and around kombolcha. However, this low infection of bovine brucellosis may be spreaded in the study area and may cause economic loss and human infection unless control strategy should be conducted.

Keywords: Abortion; Bovine brucellosis; Prevalent; Rose bengal plate test; Kombolcha; Amhara

Introduction

Ethiopia has the largest number of livestock in Africa, and the most diversified domestic animal genetic resources [1]. Livestock contribute to over 40% of the value of annual agricultural production and no less 15% the gross national product (GNP) [2]. These sectors cover also 19% of the export earnings [3]. However, the performance of livestock as food producer in Ethiopia is poor. Meat and milk production annual growth is 1.9% and 2.8% respectively which is below the recommended average of 4%that is needed to feed the growing population [2].

This recommended average increase can only be achieved, if good progress is made in increasing feed supply, in genetic improvement, in improving technology transfer, and in improving reproductive efficiency and controlling diseases [4]. One major constraint to low productivity and mortality of animals in Ethiopia is the presence of different wide spread animal disease in the country devastating epizootic diseases like CBPP (contagious ovine pleuropneumonia), lumpy skin disease (LSD) and endemic bacterial disease like pasteurellosis, anthrax and blackleg causes high mortality in animals but are to some extent in a better control by prophylactic vaccinations however, other diseases like brucellosis and tuberculosis, which have serious economic and public health impact but low mortality, have not received the attention they deserve [4,5].

Brucellosis is essentially a disease of sexually mature animals, the predilection sites being the reproductive tracts of males and female. If the animal is not pregnant, a chronic disease results without symptom and perhaps negative serology. However, if such an animal becomes pregnant the production of simple carbohydrate eryhritol in the fetus and its membranes cases enormous multiplication of bacteria in the uterus and this are likely to end in abortion [6].

The infected pregnant cow or heifer is the most important source of *B. abortus*. The fetus, fetal membranes and vaginal discharges from an aborting animal contain 1012 to 1014 organisms, which contaminate the vulva, tail and legs of the animal and the surrounding environment [7]. To a lesser extent farm are can be contaminated by fecal matter or calves fed on contaminated matter or calves fed on contaminate milk, since not all organisms are destroyed in gastrointestinal tract [8].

Infection usually occurs from ingestion of contaminated pasture, water, or y licking the discharge of an animal, newborn calf or retained fetal membrane. Transmission can also occur via penetration of intact skin, the conjunctive or by inhalation. Calves can be infected inutero or by sulking of infected dams [9,10]. Occurrence of brucellosis is increasing in tropical and subtropical regions because of practices such as nomadism; communities grazing by livestock, and modern changes to wards larger animal populations and increased commerce [11]. Importation of high producing livestock due to demands for additional animal protein and trend towards intensification of animal production favors the spreas and transmission of the infection [12].

Brucellosis has a considerable impact on animal and human health as well as wide socio economic impacts; especially in countries in which rural income relies largely on livestock breeding and dairy products. In cattle, the disease cause losses due to abortion, low milk production, condemnation of animals falling to breed, affects the animal export trade of a nation and losses in financial investment and government cost on research and eradication schemes [13].

Many countries have made considerable progress with their eradication programs but in countries like Africa brucellosis is considered to be one of the most serious disease problems facing the veterinary profession [7]. In Ethiopia, different individuals have reported bovine brucellosis. Some of the prevalence rates reported were: 7.62% [4]; 16.92% [14]; 8.11% [4]; 4.9% [15], 1.8% [16] however information on the extent of brucellosis, in and around Kombollcha is not well known. Therefore, the present study was undertaken to establish the seroprevalence of bovine brucellosis in and around Kombolcha.

Materials and Methods

Study area

The study was conducted from December 2006 may 2007 in and around Kombollcha 375 km North East of Addis Ababa. Kombollcha is found in South Wollo administrative zone of Amhara People Regional State. It is geographically categorized as 47% highland (Dega), 45% mid altitude (Weyna Dega) and 8% mountainous (Wurch) areas with an altitude rage of 1700-3800 meters above sea level. The north and south wollo zone s experience bimodal rainfall: the short (March to April and long (July to September) rains with 39.63 mm and 100mm respectively.

The average maximum and minimum daily temperature is 23.9°C to 11.7°C respectively, and the relative humidity of the area varies from 23.9% to 79%. The farming system is mixed cop-livestock production type and extensive management system with few semi-intensive private dairy farms. Oxen are generally used for traction to plough land. The main livestock grazing land includes swampy and water logged area, forest margins, hilly crop ad mountainside, stony and infertile lands, and roadsides.

Study animals

The target population was cattle, which consists of female and male under extensively and semi-intensively reared. A total of 240 animals were selected of which 131 cattle were from semi-intensive dairy farms and small holders in the study area and 109 cattle were from extensive traditional cattle rearing areas of the districts. Breeds of cattle in the areas were local zebu and crossbred. None of the animals tested were vaccinated against brucellosis.

Study design

Sampling methodology

The study was a cross-sectional type. Animals were selected depending on the availability of animal and the willingness of animal owners. A total of 240 cattle were selected. The sample size of the cattle was calculated by using the formula of Thursfield [17] and on the basis of the expected prevalence rate of 1.85% which was reported by Fekadu [16] in East Amhara Region, and computed with worst acceptable error of 5% and at 95% confidence interval. It was 186. However, a total of 240 animals were selected.

Questionnaire survey

Recorded of disease-associated events was handled by questionnaire format. The individual animal detail such as identification No, of the animals, age, sex and breed of the animals was recorded. In addition, the clinical indicators including history of abortion and fetal membrane retention were recorded. In addition the clinical indicators including history of abortion and fetal membrane retention were recorded.

Collection of blood samples

Approximately 10 ml of blood sample was obtained from the jugular vein of each animal using plain vaccutainer tubes and needle. After the identity of each animal was labeled on the corresponding vaccutainer tubes were set tilted at room temperature to allow clotting, then era were removed from the clot by siphoning in to another sterile test tube to which the animals identity was labeled finally, serum samples were kept at 200°C in Kombolcha veterinary laboratory until tested for positivity.

Serology

The Rose Bengal plate Test (RBPT) was used as a screening test of serum samples for the presence of Brucella agglutinins and was carried out at the Faculty of veterinary medicine, Debre-zeit. Positive reactor sera were retested by CFT at the Natinal veterinary institute (NVI), Debre-zeit According to WHO (1996) [18] and OIE [19]. RBPT is recommended as a screening test with samples being retested by the CFT. In this study the RBPT was used as screening test and the CFT as confirmatory test. The RBPT was reported to have a sensitivity of 98.3% and specificity of 68.8% CFT has also as specificity of 100% in non-vaccinated cattle [20] and sensitivity of 95.2% [21].

Rose bengal plate test (RBPT)

Procedure

The RBPT technique was according to the procedure described by Nielsen et al. [19] briefly as follows.

- The sera and antigen removed from the refrigerator and left at room temperature for at least 30 minutes before the test was commenced.
- 30 ul of test serum was dispensed on each of the 22circles of the plate.
- After the antigen bottle was gently shaked, drop (30 ul) of RBPT antigen was dropped alongside the serum.
- For each plate negative and positive controls were included in the remaining circles of the plate.
- With an applicator stick, the antigen and serum were mixed thoroughly (a stick being used only once).
- The plate was rocked by hand for about 4 minutes.
- Results were read in a good light source.
- When micro agglutination was suspected, a magnifying glass was used.

Interpretations

Reaction were identified as o, +, ++, +++ according to Nielsen et al. [19].

1=No agglutination

+= Barely perceptible (using magnifying glass)

++=Fine agglutination

+++=Course clumping, definite cleaning

Those samples identified with no agglutination (0) were recorded as negative while those with+, ++,+++ were recorded as positive.

Complement fixation test

All the reagents required for CFT were evaluated by titration sheep red blood cell suspension at 2% was prepared before being used in the test proper. The preparation of regents and CFT procedure was according to the protocols of Bunds institute Furgesundheitlieher verbuschenrchute and veterinary Medizin (Bguv) service laboratory (Berlin, Germany) and Nielsen et al. [19].

Test proper

- 25 ul of VBD was added in all wells.
- Serial dilution of test sera starting from 1: pre dilution pipette 25 ul per well, the first column was left for negative and positive control.
- 25 ul of 1:40 working dilution of brucella antigen was added into all wells.
- 50 ul of complement (1:40) working dilution of was added in wells.
- The plated were covered with another plate, agitated with a shaker and incubated for 30 minutes at 37°C.
- 50 ul 1 of (2% SRBC+amboceptor) mixture was added into all the wells.
- Plates were sealed with other empty plates and placed on a shaker (Working) housed and incubator 37°C) for 3 minutes.
- Plates were taken out from an incubator and results were read after being left on the table for 10 minutes.

Interpretation

Sera with at least 50% fixation titration of the complement at dilution of 1:10 were taken as positive. A. hemolytic reaction of 50% or less at a dilution of 1:5 was considered as the minimum sero-positive threshold [20].

Data analysis

The data was entered to MS. Excel (2003) and analysis using SPSS software 111.0 (2002) and then the association of brucellosis with abortion and retained fetal membrane were analyzed. The apparent prevalence rate was calculated by dividing the number of RBPT/CFT positive animals y the total number of animals tested [16].

Results

A total of 240 serum samples were tested the RBPT for screening of brucellosis, 9 (3.75%) were positive. Out of 9 RBPT positive sera, 5 (2.08%) were positive for CFT and all of the positive sera were from female cattle and most of the positive sera were old aged cows (Tables 1 and 2).

Number of sera tested	RBPT positive	CFT positive	RBPT+/CFT+
240	9(3.7%)	5(2.08%)	5(2.08%)

Table 1: Sero-prevalence of bovine brucellosis in the study area.

Risk factors	Number of animal tested	RBPT+	RBPT+ /CRT+
Sex			
Male	26	-	-
Female	214	9	5
Age (years)			
0.5-5	37	-	-
03-Jun	89	5	1
>6	114	8	4

Table 2: prevalence of bovine brucellosis according to age and sex.

Most of the positive sera were old age cows. During collection of blood samples history of abortion and retained fetal membrane were recorded. A total of 161 dairy cows were examined 96 dairy cows were from two semi-intensive dairy farms and small holders. 65 dairy cows were from extensively managed cattles of the study area. The abortion prevalence rate in semi intensively managed cattle was found to be 2.08%, which was less as compared to extensive managed system. The variation was statistically significant ($P<0.05$). A relatively higher retained fetal membrane prevalence rates were found in extensively managed cattle (13.8%) then semi-intensively managed cows (4.1%). The difference in prevalence is statistically significant ($p<0.05$) (Table 3).

The association of brucellosis with abortion and retained fetal membrane was tested using chi-square. It was found that brucellosis was highly associated with abortion and retained fetal membranes (Tables 4 and 5).

Management system	Number of observed cows	Aborted cows	Cows with retained fetal membrane
Semi-intensive	96	2(2.08%)	4(4.1%)
Extensive	65	7(10.7%)	9(13.8%)
Total	161	9(5.5%)	13(8.07%0

Table 3: Abortion and fetal membrane retention prevalence rates.

Result of test	History of abortion		Total
	Aborted cows	Non aborted cows	
CFT+	3	2	
CFT-	6	150	156
Total	9	152	161

Table 4: Association of brucellosis with abortion.

Result of test	History of fetal membrane retention		Total
	Cows with retained placenta	Cows with no Retained placenta	
CFT+	4	1	5
CFT-	9	147	156
Total	13	148	161

Table 5: Association of bovine brucellosis with retention of fetal membrane.

Discussion

The present study revealed that an overall prevalence rate of bovine brucellosis in the study area was 2.08%. The low prevalence was in agreement with the expected prevalence 1.8% which was reported by Fekadu [16] in Eastern Amhara region. The result was also in agreement with assigned (1987), who reported prevalence of 2.1% around shoa.

However, in previous studies carried out I different parts of the country, bovine brucellosis was reported with high prevalence as follows: Bayleyegn [4] 7.82% in Arsi; Endrias [22] 11.6% in sidama region; Yilkal [4] 8.11% in and around Addis Ababa. The result of the preset study was found by far low from almost all previous studies. This could be partly due to the fact that most of the previous studies were undertaken in intensive dairy farms and ranches where there is close contact between animals, which facilitate the spread, and transmission of the disease as compared to present study, which was carried out where there is minimum intensification of cattle.

The existence of the disease in the study area possesses risk to the human population, and the people in the area cohabit with animals and also there is habit of drinking raw milk. The absence of positive reactors in the two dairy farms included in the study could be due to the small number of animal they possess; these farm owners do not purchase animals from open market.

No positive reactor animals were detected among the smallholder dairy cows. This could be due to less chance of contact between infected and non-infected animals owned by different small holders and also due to small herd size. Infection of brucellosis is facilitated with large herd sizes [4].

The positive reactor animals in this study were all females, 4 females of greater than 6 years of age, and 1 female was 3-6 years old. It had been reported that males are usually more resistant than females and young cattle are less susceptible to *B. abortus* than older sexually mature cattle [7,11].

Latent infections can occur in some anima, which are serologically negative. In addition, serological diagnosis is considered to be unreliable when applied during the period of 2-3 weeks before and after abortion or calving [7]. Suggesting that false negative could have been occurred and this might contribute to the low prevalence of bovine brucellosis

The 4 females false positive results in the RBPT may be due to cross-reactions with other bacteria since none of them have been vaccinated against brucellosis. The FT is recognized as the most reliable diagnostic test now in the routine use for individual animals [19]. It rarely exhibits on-specific reactions and do not wane as the disease becomes chronic [7].

Using the questionnaire survey the overall abortion and retained placenta were 5.5% and 8.07% respectively. The prevalence rate of abortion in the study area was in agreement with that of Ebrahim [23] in which he reported prevalence rates of 3.2% in Kombolcha, place here the current study were undertaken. However, a higher prevalence was reported by Adane 111.8% in jersey cows at Wolaita.

A relatively high abortion prevalence rate 10.7% was recorded in extensively managed cattle. This might be due to poor hygienic conditions like exposure to aborted fetus, placentas, and vaginal discharges. When the above-motioned are found on common grazing land, transmission is favored [7,8].

A relatively high retained fetal membrane prevalence rate in extensive managed cattle might be attributed to various cause, extensive research on cow indicate the involvement of several causal factors, such an abortion, still birth, multiple birth, dystocia, age of the dam [24].

Limitation of the Study

This study showed only specified area of the study site. Wider area and larger sample size were not taken during the study where the power representativeness becomes less. Molecular study is encouraged in addition to serological test to increase the precision and strain circulating in the area for better brucellosis control and prevention system.

Conclusion

A cross-sectional investigation of bovine brucellosis in an around shows a low prevalence of infection. In and around d Kombolcha there was no existing brucellosis vaccination program for cattle. From the test result, females of old age animals can considered as risk factors. The over al prevalence rates of 2.08% (based on RBPT+/CFT+ is observed in the present study can be considered as low. But brucellosis positive animals will be a potential hazard to the population and consumers unless they are controlled and removed. Based on the test result brucellosis is highly associated with abortion and retained fetal membrane. Hence, it is recommended to test and slaughter of positive reactors since the prevalence is low, serological screening of animals for brucellosis before they are purchased for dairy purpose is advisable, and continues surveillance of bovine brucellosis in and around Kombolcha is necessary to follow the detail status of brucellosis.

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