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Sero-prevalence Study of Bovine Brucellosis and Reproductive Problems in Small-scale Dairy Farms of North Shewa, Ethiopia

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Abstract

Brucellosis is one of the highly infectious major bacterial diseases of animals and humans. A cross sectional study was carried out between January and March 2012 in order to investigate the sero-prevalence of bovine brucellosis, reproductive problems of dairy cows, and the associated risk factors in North Shewa, Ethiopia. The study population comprised of lactating crossbreed cows managed under small scale dairy farms. Sera samples collected aseptically from 384 cows were screened by Rose Bengal plate test (RBPT). The test result indicated that 3 (0.78%) of cows were found to be positive. These sera were further confirmed by complement fixation test (CFT) and all of them were found to be negative. The questionnaire survey was also administered to 96 households in order to determine the associated risk factors among different age groups, and parity. This study revealed that abortion and retained fetal membrane were found to be the major reproductive problems. The result of chi square analysis showed that there was no significant effect between abortion and retained fetal membranes (RFM), between the age groups, and between parity (P >0.05).The prevalence of abortion and retained fetal membranes were found to be 25 (6.6%) and 31 (8.1%), respectively. The questionnaire results indicated that insufficient animal feeding, poor housing, inadequate veterinary infrastructure and improper management practices were the main causes of low productivity. More effective breeding techniques and reproductive health management should be taken into account in order to improve the fertility of the herd in the study area. Awareness creation and training should be provided to the farming community about the reproductive health problems, improved sanitation, and good hygienic practices. It is emphasized that detailed investigation should be undertaken to elucidate the causes of abortion and retained fetal membranes in the study area. Since brucellosis has public health implications, precautions are needed to prevent the transmission of infection to humans.

Key words: Abortion, Brucellosis, Complement Fixation Test, Dairy Cows, Rose Bengal Plate Test, Retained Fetal Membranes, Sero-Prevalence, Zoonosis

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Introduction

Ethiopia is an agricultural based country, where livestock plays a pivotal role in the nation's economy. The livestock population of Ethiopia is believed to be 56.71 million cattle, 29.33 million sheep, 29.11 million goats, 7.43 million donkeys, 2.03 million horses,1.16 million camels, 0.4 million mules, and 56.87 million poultry (CSA 2015). Even though the country has such a huge livestock population, animal diseases were still the major constraints to their production and productivity, and this has the strongest impact on socio-economic and public health aspects of the country. There are many infectious diseases of multiple etiologies, which affect cattle, and other animals both in developing and developed countries (Pal, 2007). Among these, brucellosis is a widely prevalent infectious disease, which affects social and economic developments especially in the developing nations of the world. Brucellosis has continuously been a re-emerging bacterial zoonotic disease, causing serious impact on human health (Pal, 2013). It remains one of the most important anthropozoonoses resulting into over 50,000 human cases annually throughout the world (Hadush and Pal, 2013). The disease causes significant economic losses to the dairy farms, because of abortion, infertility, and also results in extensive chronic morbidity in humans (Al Dahouk *et al*., 2013).

Brucellosis is caused mainly by *Brucella abortus*, *B. melitensis, B. suis, B. canis, B. ceti, B. ovis, B. neotomae, B. microti, B. inopinata*, and *B. pinnipedialis* (Pal, 2007; Hadush and Pal, 2013). *Brucella* is a Gram-negative, non-capsulated, non-spore forming, non-motile, small cocco-bacillus and intracellular organism (Pal, 2007). The disease is widespread in animals as well as in humans in African continent (Hadush and Pal, 2013; Hadush *et al.,* 2013; Mekonnen *et al*., 2015). Brucellosis affects many species of animals such as cattle, buffaloes, goats, pigs, sheep, camels, equines, deer, and dogs (Pal, 2007). Sexually matured and pregnant cattle are more susceptible to *Brucella* infections. The transmission of the disease in humans is by direct or indirect contact with infected animals; and also by ingestion of unpasteurized milk and dairy products (Pal, 2007). The risk factors for human infection include the handling of infected animals, ingestion of contaminated animal products such as unpasteurized milk and milk products, meat, and handling of cultures of *Brucella* spp. in laboratories (Pal, 2007; Smirnova *et al*., 2013). A plethora of tests such as RBPT, standard tube agglutination test, Coomb's test, mercaptoethanol test CFT, and ELISA are employed to demonstrate circulating *Brucella* antibodies (Pal, 2007; Alemu *et al*., 2014). However, CFT is considered as a gold standard and a prescribed test for international trade (Alemu *et al*., 2014). It is an important occupational zoonosis of butcher, livestock handler, dairy farmer, veterinarian, abattoir worker, and laboratory personnel. It is pertinent to mention that human brucellosis often coincides with livestock infection (Pal *et al*., 2013). Human brucellosis is usually prevented by controlling the infection in animals (Pal, 2007). Several studies on the sero-prevalence of brucellosis have been conducted in local and crossbreed animals in different agro-ecological and production systems in

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Ethiopia, and the results indicated that bovine brucellosis is an endemic and widespread disease with different prevalence rates (Asmare *et al*., 2010; Jergefa *et al.*, 2009, Kassahun *et al*., 2010; Alemu *et al.* 2014). The prevalence of 0.52% and 3.23% was reported in Bishoftu and Asella, respectively (Minda *et al.*, 2016). Alemu and co-investigators (2014) described 2% sero-prevalence in cattle of Eastern, Showa. In Southern part of the country, Yilma (2016) recorded the sero-prevalence of 1.04%.

The prevalence of the major reproductive health problems in the dairy cows was reported by Tesfaye and Shamble (2013). The highest prevalence of abortion (12.2%) was observed by Duguma and others (2014). In contrast, Dawit and Ahmed (2013) reported a relatively lower prevalence (6.3%). The reported prevalence of retained fetal membrane also varies depending on the management systems of the farming community. However, 16.8% prevalence of retained fetal membrane was the highest as recorded by Gebre-Mariam (1996) in the Northern part of Ethiopia. These reproductive health problems can cause considerable economic loss of the farms, and are also the major animal health constraints to production and productivity of the livestock sector. This study was focused on bovine brucellosis, abortion and retained fetal membranes in North Shewa Zone of Oromiya Regional, National State, as this area has a huge potential of milk markets for the growing dairy industry in and around Addis Ababa city. However, a great concern should be made relating to health, risk of the consumer, due to the presence of zoonotic pathogens, and antimicrobial drug residues in milk (FAO, 2003; Pal, 2007; Pal *et al*., 2016). The approaches used to control brucellosis are immunization, test and slaughter policy of confirmed animals. Pasteurization of dairy products and wearing protective clothing are the important safety measures where the disease is endemic (Pal, 2007). Hence, the epidemiological investigation of the disease is found to be great significance. Therefore, the objective of this study was to determine the prevalence of bovine brucellosis, abortion and retained fetal membranes, and the associated risk factors in this area.

Materials and Methods

Study Area

The study area is located at an altitude of 9^{o} 48N and38^{o} 44E North West of Addis Ababa between Mukaturi and Goha Tsion town of North Shewa, Oromiya National Regional State. It covers 1,174,500 ha of land from which 40% are crop land, 25% is grazing land, 13% is forest and bush area, 7% is construction area and 15% is unproductive land. The cattle population is about 1,113,200. 42% of the area is highland that is suitable for crop cultivation and livestock husbandry and the herd structure is characterized by a higher number of cows. The area has two annual rainy seasons: from February to May (short rainy season), and from June to October (long rainy season). This study area has a high potential of mixed crop and livestock production. As the area is conducive for dairy production, farmers rear dairy cattle for milk production. The farmers of this area are the main source of milk supplier to the city of

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Addis Ababa. The Holsteins breed and their crosses are found alongside native zebu cattle. The farming systems were managed under extensive, intensive and semi-intensive farming conditions.

Study Animals and Study Design

A sero-epidemiological study was carried out in six districts of North Shewa, between January and March 2012. The animals were selected using simple random sampling method. The peasant associations (PAs, were selected based on the willingness of the owners. The sample size was calculated on the basis of 50% expected prevalence of bovine brucellosis in the study area with the expected precision at 5% and at 95% confidence interval. The sample size was calculated according to method described by Thrusfield (2005). The study subjects included lactating crossbred dairy cows, which were found in different lactating stages and reared under intensive, semi-intensive, and extensive management systems. A standard questionnaire format was used to collect information relevant to the epidemiological investigation, such as age, herd size, and reproductive disorders (abortion, retained fetal membrane). Data related to lactation stages, origin of the animal, breeding and management practices of the farms were also gathered. A questionnaire was prepared and administered to 96 households. The questionnaire was pre-tested in the field and adjusted as required. Purposive sampling was used to select the key informants. A total of 384 lactating crossbred dairy cows found in 96 households were sampled randomly. Blood samples were collected aseptically from the jugular vein of each cow using plain vacutainer tubes. The samples were left at room temperature overnight to allow clotting for serum separation. Sera were collected into cryogenic vials and stored at –20°C until test was performed.

A cross sectional epidemiological study was conducted to determine prevalence of bovine brucellosis, reproductive problems such as abortion, retained fetal membrane, and the role of risk factors such as age, herd size, and under different management systems. The management systems were grouped into semi intensive, intensive and extensive systems, which accounted 267 (69.5%), 26 (6.8%) and 91 (23.7%), respectively. Herd size was categorized into less than 7 animals (small size), 8–15 animals (medium size), and above 16 animals (large size). The age stratification was made in 2.5- 4 years and above 4 years.

Rose Bengal Plate Test (RBPT)

Serum of 30µl was mixed with 30µl of antigen on a white tile or enamel plate to produce a zone approximately equal to 2 cm in diameter. The mixture was rocked gently for four minutes at ambient temperature and then observed for agglutination. Any visible reaction was graded positive and otherwise negative. The test was conducted in Asella Regional Veterinary Laboratory (ARVL), according to the procedure of OIE (2004). An animal was classified as positive if both the RBT and the CFT were

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positive. RBPT is a simple screening test, and its sensitivity and specificity values were 97.6% and 77.6%, respectively (Blasco *et al*., 1994).

Complement Fixation Test (CFT)

The test was conducted at National Animal Health Diagnostics and Investigation Center (NAHDIC), Ethiopia, according to the procedure of (OIE, 2004). Screening of serum was done using Rose Bengal plate test (RBPT). Sera positive by RBPT were confirmed further by complement fixation test (CFT). The sensitivity and specificity of CFT was considered 88.1% and 100%, respectively (Blasco *et al*., 1994).

Data Analysis

Data generated from the questionnaire survey and laboratory investigations were recorded and coded using a Microsoft Excel spreadsheet and analyzed using SPSS version 22. The sero-prevalence was calculated as the number of sero-positive samples divided by the total number of samples tested. The total prevalence was calculated by dividing the number of RBPT and CFT positive animals by the total number of animals tested. The degree of association between or among each risk factor was assessed using the Chi-square ($\chi 2$) test. For all analyses, a p-value of less than 0.05 was taken as statistically significant.

Results and Discussion

Out of the 384 animals sera samples screened using RBPT, only 3 (0.78%) were found positive. In this study, all sera were found to be negative based on the CFT confirmatory test**.** The overall prevalence of the current findings was in agreement with Bashitu and co-workers (2015), which was 0.2% in Ambo and 0.7% in Debre Brehan towns in the west and east of Addis Ababa. The significance of RBPT as a rapid, simple and low cost test for the detection of circulating *Brucella* antibodies at field level has been documented by several researchers (Blasco *et al*., 1994; Hadush *et al*., 2013; Alemu *et al*., 2014; Lemu *et al*., 2014; Yilma, 2016).

The current study identified abortion and RFM as the major reproductive health problems. The results of the questionnaire survey were indicated that the total prevalence of abortion and retained fetal membrane (RFM) found to be 25 (6.6%) and 31 (8.1%), respectively (Table 1). The total prevalence of abortion and RFM in the study area was in conformity with the observations of Mekonnen and others (2015) who recorded abortion (6.4%) and RFM (11.4%) in cross breed dairy cattle in and around Mekele. A closely similar finding from different parts of Ethiopia was recorded by Dinka (2013) and Esheti and co-investigators (2014). To the contrary, the higher prevalence of abortion 12.5% was also reported by (Benti *et al*., 2014). The lower abortion and RFM prevalence were reported by (Edilu *et al*., 2016). The higher abortion and RFM prevalence rate was reported by Khan and co-investigators (2014) in North Eastern India, which was 11.25% and 14.35%, respectively. Infectious abortion is an important reproductive

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disease of animals, which may occur in sporadic as well as in epidemic form, and is caused by a diverse types of agents (Pal, 2006). The disease is global in distribution, and is of great concern to the livestock raisers (Pal, 2006). Some of the infectious agents, such as *Brucella abortus, Campylobacter jejuni, Chlamydophila psittaci, Coxiella burnetii, Leptospira, Listeria monocytogenes, Toxoplasma gondii, Yersinia enterocolitica*, etc. responsible for abortions in animals, have public health significance (Pal,2006; Pal, 2007). Brucellosis is one of the leading causes of abortion in dairy animals, and is of great economic losses in the dairy farms (Pal, 2015). The late term abortions have been estimated to cost between USD 500 to 900 per animal (Pal, 2015).The incidence of abortion more than 2 to 5% should be viewed seriously, and efforts should be made to determine the causes (Mainar-Jaime *et al*., 2005). Infertility is the main problem that affects production in both local and crossbred cows and heifers in Ethiopia All cases of abortion should be investigated to ensure that there is no infectious cause that could be transmitted to other animals. The cause should be assumed infectious, and therefore, hygienic precautions should be taken immediately until a definitive diagnosis is available.

The causes of RFM are complex. The main factors involved are insufficient expulsive efforts by the myometrium, failure of the placenta to separate from the endometrium, that could be caused by inflammatory changes, placental immaturity, hormone imbalances, a neutropenia, a lack of polymorph migration to the site of attachment and possibly immune deficiencies, mechanical obstruction, including partial closure of the cervix and also vitamin E, selenium, cobalt and copper deficiencies. Retained foetal membranes cause considerable economic loss of the farm, especially when incidence exceeds the average of 5-10% RFM (Joosten *et al*., 1987). In this study, more than 60% of cows were found in the 1st to 3rd parity group (Table 2). Our findings on the effect of parity on reproductive problems were indicated mostly on the 1st parity. This observation goes parallel with Khan and others (2016) under smallholder's dairy cattle production system in India.

Table 1: The Association Age with Abortion and Retained Fetal Membrane in Bovine

Age group	Number of examined	Number of cows with reproductive problems in %	Chi square (P-Value)
2.5 to 5 years	319	62 (19.4)	0.58(0.44)
5 to 8 years	65	16 (24.6)	
Total	384	78	

The results of chi square analysis showed that there was not significant effect on the prevalence of the both abortion and RFM, between the age groups, and also between parity (P >0.05) (Table 2 and Table 3).

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Table 2: The Association of Parity with Abortion in Cows

Parity	Normally Calved	Aborted	Prevalence	X^2 (P- Value)
1st to 3rd parity	222	14	6.3%	0.34(0.5)
4th to 8th parity	137	11	8.2%	
Total	359	21		

Table 3: The Association of Parity With Retained Fetal Membrane in Dairy Cattle

Parity	Normally expelled	Retained placenta	Prevalence	X^2 (P- Value)
Ist to 3rd	220	16	6.37%	1.09(0.29)
4th to 8th	133	15	11.27%	
Total	353	31	8.07%	

Our observations revealed that both abortion and RFM were found to be the highest in the age group of 2.5 to 5 years **(**Table 1). Low prevalence was found in the age group of > 5 years. However, there was no significant difference between abortions RFM. This finding was in agreement with Tesfaye and Shamble (2013), however, the problems were seen to rise with age. The results of the questionnaire survey showed that 253 (65.9%) of them were born on the farm of the households, while the rest 131 (34.8 %) cows were purchased from the surrounding markets. Further, it was observed that mating by artificial insemination (AI) was the most commonly practiced methods of animal breeding in the area, which accounted 240 (62.5%).Whereas the bulls were used for natural mating, and practicing of either methods, accounted 95 (24.7%) and 49 (12.8%), respectively. Most farmers 24 (100%) reported that their sick animals were not isolated, and the aborted fetal membranes were dumped without using protective cloths /hand gloves. Nearly all participants mentioned that milk, and milk products were consumed without boiling. The general sanitary and hygienic conditions of the farms were very poor (poor ventilation, floor without concrete, small and narrow housing (personal observation). These may play a significant role on the effect of the poor breeding performance, and reproductive problems of the animals. The results of our study indicated that unsatisfactory feeding, poor housing, and inadequate health management practices were the main causes of low productivity of dairy cows in the study area. Lack of awareness, low access to effective and timely breeding, and unsatisfactory animal health services were also observed, which further contributed to lower production and productivity of the dairy animals.

Conclusion

Among the bacterial zoonoses affecting dairy cattle in developing nations of the world, brucellosis is considered one of the most prevalent infectious diseases. Brucellosis remains one of the important public

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health concerns in many countries including Ethiopia. In our study, 0.78% and 0% sero-prevalence of bovine brucellosis were obtained as measured by RBPT and CFT, respectively. The screening of blood sera by RBPT indicates that brucellosis is endemic in cattle in the study area. Our study revealed that the reproductive problems such as abortion and retained fetal membrane were found to be the major problems in the study area. We recommend that the sera positive to RBPT should be confirmed by ELISA, and if possible, modern molecular techniques such as rt-PCR (reverse transcriptase- polymerase chain reaction) should be conducted in order to identify animals, which are not detected by conventional serological methods. The emphasis is given to investigate the causes of abortions and retained fetal membranes among the dairy animals in study area. As abortion is typically the first clinical sign in pregnant animal due to brucellosis, stringent measures must be taken in the handling and disposal of aborted fetus and retained fetal membranes to prevent the spread of disease to humans and other animals.

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