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ORIGINAL RESEARCH

Seroprevalence and associated risk factors of bovine brucellosis in Addis Ababa dairy farms

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Abstract A cross-sectional study was carried out on bovine brucellosis in Addis Ababa dairy farms from November 2003 to April 2004. A total of 1,202 blood samples were collected from non-vaccinated, cross-bred dairy cattle. The Rose Bengal plate test (RBPT) was used as a screening test. Those serum samples reacting positively to RBPT were subjected to the complement fixation test (CFT) for confirmation. The RBPT detected 30 of 1,202 (2.5%) of the samples as brucellosis positive. The positive sera when further retested using CFT, 18 out of the 30 RBPT positive sera were confirmed to be positive. The prevalence of brucellosis based on CFT in the study area was 1.5%, and all positive sera were from female cattle. Result of the questionnaire survey revealed that percentage of 4.4% abortion and 9.5% retained fetal membranes. Abortion and retained fetal membranes were associated with *Brucella* antibodies ($P<0.05$). A total of 153 cattle attendants and owners in the farms were interviewed, and 73.5% were found to have no knowledge of brucellosis, only 20.8% wear protective gloves during handling aborted material and 39.6% responded that they consume raw milk. Results of this study showed that prevalence of bovine brucellosis in the study area is low and a test-and-slaughter policy can be used in order to control the diseases in dairy farms of Addis Ababa.

Keywords Addis Ababa · Bovine brucellosis · Dairy farms · Risk factor · Seroprevalence

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Introduction

Specialized intensification of animal production is threatened in a permanent way to the penetration and fast spreading of infectious diseases, for example brucellosis. Farm animals primarily affected include cattle (*Brucella abortus*), sheep (*Brucella ovis* and *Brucella melitensis*), goats (*B. melitensis*), and camels (*B. abortus* and *B. melitensis*) (OIE 2008; De Massis et al. 2005).

The susceptibility of animals to brucellosis depends on their natural resistance, age, level of immunity, and environmental stress. Brucellosis in cattle is characterized primarily by abortion late in pregnancy, frequently followed by fetal membrane retention and endometritis which may be the cause of infertility in subsequent pregnancies (Ahmad et al. 2009; Radostits et al. 2007).

Transmission occurs mainly by ingestion of contaminated feed and water by the organisms which are present in large numbers in aborted fetuses, fetal membranes, and uterine discharges (Munoz et al. 2005; Al-Majali et al. 2009). However, infection through injured/intact skin, the mucosa of the respiratory system, and conjunctiva occurs frequently (Georgios et al. 2005; Kebede et al. 2008). Bulls that are themselves infected and discharge semen containing organisms are mostly unlikely to transmit the disease,

but the chance of spread from the bull is very great if the semen is used for artificial insemination (De Massis et al. 2005; Eshetu et al. 2005).

The seroprevalence of bovine brucellosis in east Africa as summarized by Asfaw et al. (1998) shows that in Djibouti, it was 4%; Somolia, 11.9%; Kenya, 19%; Rwanda, 34.9%; Sudan, 6.5–22.5%; and Uganda, 1.8%. In Ethiopia, investigations have shown the endmicity of bovine brucellosis, for example, Ibrahim et al. (2009) studied the seroprevalence of bovine brucellosis and found that there is 3.1% overall seroprevalence out of 1,595 cattle and the individual animal level seroprevalence indicated that in non-pregnant animals, it was 11.1%; in pregnant, 2.7%; in lactating, 22.3%; and in non-lactating animals, 2.4%, in Jimma zone of the Oromia region. Though these studies were done in different localities of the country and showed its epidemicity, there is still a gap of information for many parts of the country and to see if there is any turn down about its seroprevalence, especially in Addis Ababa where about half of the dairy farms of the country is located. Therefore, this study was carried out to determine the seroprevalence and to know the risk factors of bovine brucellosis in dairy cattle in Addis Ababa.

Materials and methods

Study area

The study was conducted in Addis Ababa dairy farms and the seroprevalence investigation at Shola Regional Veterinary Laboratory which is also located in Addis Ababa. Most of the animal farms in Addis Ababa city are diary type. The farms visited consist of a minimum number of 12 cows and maximum number of 200 cows. Some of these farms are also comprised of bulls important for service, semen collection. Addis Ababa receives mean annual rainfall of 1,300 mm in bimodal distribution, with 11°C and 24°C as minimum and maximum temperatures, respectively (National Metrology Service of Ethiopia 2010).

Study population and study design

The target population was cattle, which consist of breeding females, replacement heifers, and available bulls. A total of 1,202 animals were selected, and the study is dealt with these animals. Of these animals 928, 262, and 12 were dairy cows, heifers, and bulls, respectively. The breeds of these animals were crosses of Holstein type. None of the animals tested were vaccinated against brucellosis.

The study was a cross-sectional type. Animals were selected by a systematic random sampling method based on the sample frame provided by Shola Regional Laboratory. A total of 51 dairy farms were first selected on the basis of random selection which covers the whole Addis Ababa city area. The number of animals included in the study from each farm depends on the number of animals found in the farm, for example, the largest number of animals was selected from the 37th farm of the 51 farms which has 200 dairy cows. The sample size of the study population was calculated on the basis of 5.2% prevalence of bovine brucellosis in Addis Ababa region done by Asfaw et al. (1998) computed with worst acceptable error of 1% and at 95% confidence interval (Thrusfield 2005).

Sampling and data collection

Approximately 10 ml of blood sample was obtained from the jugular vein of each animal using plain vaccutainer tubes and needle. After identification, each animal was labeled on the corresponding vaccutainer tube; the tubes were set tilted overnight at room temperature to allow clotting. Next morning, sera were removed from the clot, unretracted blood being centrifuged, by siphoning into another sterile test tube to which the animals' identity was transferred, and then Rose Bengal test was conducted. Finally, serum samples were kept at −20°C at Shola Regional Veterinary Laboratory until tested using complement fixation test (CFT).

Questionnaires have three types of contents: the first type is to collect information about each sampled animal, the second is to collect farm data, and the third is to collect data about hygienic procedures from the animal attendants. These questionnaires that were set into one which contain 42 questions were forwarded to each farm (for 9 farms only one questionnaire, for 11 farms each two questionnaires, for 13 farms each three questionnaires, 7 farms each four questionnaires, and 11 farms each five questionnaires) intended to know the farm size, use of maternity pen, breeding method, disposal of materials, replacement strategies, fate of aborted cows, and history of vaccination. The questions were two in kind (open and close ended). The open-ended ones are designed to reflect the data that were recorded during each sample collection: the sex, breed, sources, history of abortion, and retained fetal membrane. Furthermore, knowledge on brucellosis, on whether they were using protective gloves during handling of aborted material or not, and on consumption of raw milk were included in the questionnaire survey.

Serological analyses

The Rose Bengal plate test (RBPT) was used as a screening test of the serum samples for the presence of *Brucella* agglutinins at Shola Regional Veterinary Laboratory.

Positive sera were then retested for confirmation by the CFT at the National Veterinary Institute, Debre Zeit.

Data analysis

Data from the laboratory results and questionnaires were stored in personal computer, using Microsoft Excel spreadsheet program. Descriptive statistical analysis of various risk factors and dependant variables was done using Intercooled STATA 7.0 (Stata Corporation 2008). The chi-square test or Fisher's exact test was also used.

Results

Descriptive results

A total of 153 cattle attendants and owners in the farms were interviewed, and 72.5% (111/153) of cattle attendants and owners have no knowledge of brucellosis and only 20.8% (33/153) wear protective gloves during handling of aborted cows and retained fetal membranes. Furthermore, 39.6% (63/153) responded positively on the consumption of raw milk.

Results of the questionnaire survey on some of the epidemiological risk factors such as presence of maternity pens, type of mating used, farm replacement, fate of aborted cows, disposal of aborted materials, and history of vaccination are indicated in Table 1. It was observed that 41.2% of dairy farms culled aborting cows, and there was no history of vaccination in all dairy farms.

Table 1 Result of questionnaire survey: indicating the management systems and the percentage with each other

Farm management activities	Percent of farms
Presence of maternity pens	6/51 (11.8%)
Type of mating	
Use of natural mating	1/51 (2%)
Use of AI	40/51 (78%)
Use of AI and natural mating	10/51 (20%)
Farm replacement	6/51 (11.8%)
House	
Market	11/51 (21.6%)
Both	34/51 (66.7%)
Fate of aborted cow	
Culled	21/51 (41.2%)
Not culled	30/51 (58.8%)
Disposal of aborted material	51/51 (100%)
History of vaccination	–

Seventy-eight percent of the farms use artificial insemination when their cows show estrus and 58.8% of the farms do not cull their cows due abortion

Serological and statistical analyses

Out of the 1,202 serum samples that were tested by the RBPT for screening of brucellosis, 30 (2.5%) were positive. Out of 30 RBPT positive sera, 18 were positive for CFT and all of them were female cattle.

During collection of blood samples, history of abortion and retained fetal membrane were recorded among sampled cows. There were 4.4% and 9.5% history of abortion and retained fetal membranes, respectively. It was found that brucellosis was highly associated with abortion and retained fetal membranes as shown above in Table 2.

Discussion

The present study revealed an overall prevalence rate of 1.5% of bovine brucellosis in Addis Ababa dairy farms. The low seroprevalence was in line with Haileselassie et al. (2010) who found out an individual level seroprevalence rate of 1.2% in western Tigray Region and Kassahun et al. (2010) who reported a prevalence of 1.6% in Sidama Zone of the southern Ethiopia. However, other researches reported higher seroprevalence rate than the present study. For example, Mussie (2005) has indicated a 4.6% in Bahirdar milkshed; Ibrahim et al. (2009), 15.0% in Jimma zone of Oromia region; Hunduma and Regassa (2009), 11.2% in east Shewa zone of the Oromia region; and Gebretsadik et al. (2007), 42.3% in the extensive cattle production system of Tigray Region of Ethiopia.

This study shows that the current seroprevalence of brucellosis is statistically different ($P<0.05$) from the study of Asfaw et al. carried out in Addis Ababa in 1998 which was 5.2%. This difference might be due to the creation of awareness to dairy farm owners in which positive reactors could have been removed. This was supported by the questionnaire survey which indicated that 51 dairy farms 41% (21/51) follow culling strategy of repeatedly aborting cows for any reason. On top of this, the Shola Regional Veterinary Laboratory provides a veterinary assistance to the health problem of dairy cows in Addis Ababa. Whenever there is abortion, the laboratory conducts screening test by RBPT, and positive reactors are recommended to be culled. Moreover, health education is given to dairy farm owners in Addis Ababa by the animal health sector concerning the transmission, control, and prevention of brucellosis. These all can contribute to the reduction of the prevalence of the disease in the study farms.

Latent infections occur in some animals whose serological tests gave negative results. In addition, serological

Table 2 Association of brucellosis with abortion and retained fetal membranes

Result of Test	History of abortion		Total	Chi-square (χ^2)
	Aborted Cows	Non-aborted Cow		
Association with abortion				
CFT+	5	13	18	23.5
CFT−	36	874	910	
Total	41	887	928	
Association with retained fetal membranes				
CFT+	10	8	18	45.6
CFT−	79	813	910	
Total	89	839	928	

For association of abortion, χ^2 cal=23.5, *df*=1, $P<0.05$; χ^2 tab=3.84. For association with retained fetal membranes, χ^2 cal=45.6, *df*=1, $P<0.05$; χ^2 tab=3.84

diagnosis is considered to be unreliable when applied during the period of 2 to 3 weeks before and after abortion or calving suggesting that false-negative results could occur (Haileselassie et al. 2010; Muma et al. 2006).

The RBPT is generally considered to be a sensitive test (OIE 2008). The false-positive results in the RBPT are due to cross-reactions with other bacteria since none of them have been vaccinated against brucellosis. The complement fixation test is recognized as the most reliable diagnostic test now in routine use for individual animals (OIE 2008; De Massis et al. 2005).

The positive reactor animals in this study were all females, but this does not mean that male animals were not tested. It had been reported that males are usually more resistant than female cattle (Bayemi et al. 2009; Muma et al. 2007; Gebretsadik et al. 2007). This idea has been supported by different investigators in this country (Mussie 2005; Tolosa et al. 2008; Asfaw et al. 1998). Different factors are probably involved in the variation in sex susceptibility, including physiological and behavioral differences between males and females. Because of the preferential growth of *Brucella abortus* in gravid uterus, it can enter the uterus as it disseminated from the principal sites of carrier states (udder, supramammary lymph node). In latently infected cows, depending on the number of pregnancy events and presence of infection, this will give the organism sufficient contact with the lymphoid system to stimulate a significant immune response.

Using the questionnaire survey, 4.55% abortion and 9.5% retention of fetal membranes were recorded. The prevalence rate of abortion in the study area was in agreement with that of Ibrahim (2003) in which he reported a prevalence rate of 3.2% in Kombolcha. However, a higher prevalence rate of 6.7% in north Gondar zone was reported by Tadesse (2003) and 7.4% in Tigray by Abreha (2003). This difference in prevalence rate might be due to the variation in cattle husbandry and management system. Moreover, the control strategy followed by Addis Ababa dairy farm owners that is culling of aborted cow independent of the cause of abortion could have contributed to the difference. The prevalence rate retained fetal membranes in the study area was found to be 9.5%. This was in agreement with the report of Abreha (2003) who indicated 9.3% prevalence rate.

The questionnaire survey of 153 cattle attendants and owners in the farms indicates that 72.5% have no knowledge of brucellosis, only 20.8% wear protective gloves and 39.6% consume raw milk. Presence of high association between brucellosis and abortion as well as retained fetal membranes (Table 2) is indicative of risk to cattle attendants and professionals working in the area without precautions and protective clothes. Most cases of brucellosis in human are occupational and occur in the farm attendants, veterinarians, and butchers (Radostits et al. 2007). Since 39.6% of cattle attendants have habit of milk consumption without boiling or pasteurization, the risk from the disease could be high. The possibility of infection occurring by drinking milk necessitates the pasteurization or boiling of milk (Nielsen et al. 2005; OIE 2008).

Conclusion

The problem of bovine brucellosis in Addis Ababa, the capital of the Ethiopia, is still a predicament no matter how it seems that there is a better perception of disease control and farm management systems than decades before. The allied risk factors for the occurrence of brucellosis in the study area are overlooked and brucellosis remains, year after year, a setback of these farms. Therefore, the seroprevalence of brucellosis may exist for long if this consideration is not taken in every health apprehensive activity of the farms. We hope that this research will address the problem of brucellosis to all the farms in Ethiopia, in general, and in Addis Ababa, in particular, so that they will take measures to make the problem subside and increase the productivity of the dairy farms. Since results of this study showed that prevalence of bovine

brucellosis in the study area is low, a test-and-slaughter policy can be used in order to control the diseases.

References

Abreha, T., 2003. Brucellosis in cattle and small ruminants in selected sites of Tigray Region, North Ethiopia, (Unpublished DVM Thesis, Addis Ababa University).

Ahmad, M. A., Adbelsalam, Q. T., Mustafa, M. A. and Mohammed, M. A., 2009. Seroprevalence and risk factors for bovine brucellosis. Jordan Journal of Veterinary Science, 10, 61–65

Al-Majali, A.M., Talafha, A.Q., Ababneh, M.M., Ababneh, M.M., 2009. Seroprevalence and risk factors for bovine brucellosis in Jordan. Journal of Veterinary Science, 10, 61–65.

Asfaw, Y., Molla, B., Zessin, K., and Tegegn, A., 1998. A cross sectional study on bovine brucellosis and test performance in intra and peri-urban dairy production system in and around Addis Ababa. Bulletin of Animal Health and Production in Africa, 46, 217-224.

Bayemi, P. H., Webb, E. C., Nsongka, M. V., Unger, H. and Njakoi, H., 2009. Prevalence of Brucella abortus antibodies in serum of Holstein cattle in Cameroon. Tropical Animal Health and Production, 41, 141–144.

De Massis, F., Giovannini, A., Di Emidio, B., Ronchi, G.F., Tittarelli M., Di Ventura, M., Nannini, D. and Caporale, V., 2005. Use of the complement fixation and brucellin skin tests to identify cattle vaccinated with Brucella abortus strain RB51. Veterinaria Italiana, 41, 291–299.

Eshetu, Y., Kassahun, J., Abebe, P., Beyene, M., Zewdie, B. and Bekele, A., 2005. Seroprevalence Study of Brucellosis on Dairy Cattle in Addis Ababa, Ethiopia. Bulletin of Animal Health and Production in Africa, 53, 211-214.

Gebretsadik, B., Kelay, B. and Yilkal, A., 2007. Seroepidemiological Investigation of Bovine Brucellosis in the Extensive Cattle Production System of Tigray Region of Ethiopia. International Journal of Applied Research in Veterinary Medicie, 5, 65-71.

Georgios, P., Nikolaos, A., Mile, B. and Epaeinondas, T., 2005. Brucellosis. The New England Journal of Medicine, 352, 2325-2336.

Haileselassie, M., Shewit, K. and Moses. 2010. Serological survey of bovine brucellosis in Barka and Arado breeds (Bos indicus) of western Tigray, Ethiopia. Journal Preventive Veterinary Medicine, 94, 28-35.

Hunduma, D. and Regassa, C., 2009. Seroprevalence Study of Bovine Brucellosis in Pastoral and Agro-Pastoral Areas of East Showa Zone, Oromia Regional State, Ethiopia. American-Eurasian Journal of Agricultural and Environmental Science, 6, 508-512.

Ibrahim, N., Kelay, B., Fikre, L. and Merga, B., 2009. Sero-prevalence of bovine brucellosis and its risk factors in Jimma zone of Oromia Region, South-western Ethiopia. Tropical Animal Health and Production, 42, 35–40.

Ibrahim, O., 2003. Study on major reproductive health problems of small holder dairy farms in and around Kombolcha, (Unpublished DVM Thesis, Addis Ababa University).

Kassahun, A., Yilkal, A., Esayas, G. and Gelagay, A., 2010. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. African Journal of Agricultural Research, 5, 257-263.

Kebede, T., Ejeta, G. and Ameni, G., 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue de Medecine Veterinaire, 159, 3-9.

Muma, J.B., Samui, K.L., Siamudaala, V.M., Oloya, J., Matope, G., Omer, M.K., Munyeme, M., Mubita, C. and Skjerve, E., 2006. Prevalence of antibodies to Brucella spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia. Tropical Animal Health and Production, 38, 195-206.

Muma, J.B., Samui, K. L., Oloya, J., Munyeme, M. and Skjerve, E., 2007. Risk factors for brucellosis in indigenous cattle reared in livestock-wildlife interface areas of Zambia. Journal Preventive Veterinary Medicine, 80, 306-317.

Munoz, P., Marin, C., Monreal, D., Gonzales, D., Garin-Bastuji, B., Diaz, R., Mainar-Jaime, R., Moriyon, I. and Blasco, J., 2005. Efficacy of several serological tests and antigens for the diagnosis of bovine brucellosis in the presence of false positive serological results due to Yersinia enterocolitica O: 9. Clinical and Diagnostic. Laboratory Immunology, 12, 141–151.

Mussie, H., 2005. Seroprevalence study of brucellosis in cattle and human in Bahirdar milkshed, (Unpublished Masters Thesis, Addis Ababa University).

National Metrology Service, 2010. National Metrology Service of Ethiopia, weather forecasting and information work process owner for 2010. Gondar printing enterprise, Gondar, Ethiopia.

Nielsen, K., Smith, P., Yu W., Nicoletti, P., Elzer, P., Robles, C., Bermudez, R., Renteria, T., Moreno, F., Ruiz, A., Massengill, C., Muenks, Q., Jurgersen, G., Tollersrud, T., Samartino, L., Conde, S., Forbes, L., Perez, B., Rojas, X. and Minos, A., 2005. Towards a single screening test for brucellosis. Review of Scientific Techniques of Office for International Epizootics. 24, 1027–1038.

OIE, 2008. Bovine brucellosis. In: Manual of Diagnostic Tests and Vaccines for terrestrial animals (mammals, birds and bees). 6th. ed. Office International des Epizootics, OIE, Paris, France, 2: 624-659.

Radostits, M., Gay, C., Hinchcliff, W. and Constable, D., 2007. Veterinary Medicine, A text book of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. Grafos, S.A. Arte Sobre Papel, Spain.

Stata Corporation. Intercooled Stata 7.0 for Windows 98/95 NT, 2008. College Station, Texas, USA: Stata Corporation.

Tadesse, Y., 2003. A survey of bovine brucellosis in selected areas of North Gondar Zone. (Unpublished DVM Thesis, Addis Ababa University).

Thrusfield, M., 2005. Veterinary Epidemiology; 2nd ed. Blackwell Science Ltd. Cambridge, USA.

Tolosa, T., Regassa, F. and Belihu, K., 2008. Seroprevalence Study of Bovine Brucellosis in Extensive Management System in Selected Sites of Jimma Zone, Western Ethiopia. Bulletin of Animal Health and Production in Africa, 56, 25-37.