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SEROPREVALENCE AND ASSOCIATED RISK FACTORS OF *BRUCELLOSIS* IN DAIRY CATTLE IN SELECTED TOWNS OF WEST SHEWA, ETHIOPIA

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Abstract

Bovine brucellosis is a contagious disease of cattle causing reproductive failure, loss of milk production and zoonosis worldwide. A cross-sectional epidemiological study was conducted on 816 dairy cattle (449 were cows) from 60 dairy farms to determine the seroprevalence and associated risk factors of *bovine brucellosis* in dairy cattle in selected towns of West Shewa, Ethiopia. Sera were collected, screened by Rose Bengale Plate Test and positive sera were further tested by Complement Fixation Test for confirmation of Brucella seropositivity. Data regarding risk factors were obtained from records and questionnaire. The association of *brucellosis* with risk factors was analyzed by Chi-square/Fischer's exact test. The result showed 0.49% (4/816), 0.9% (4/449) and 3.3% (2/60) seroprevalence in cattle, cows alone and at herd level, respectively. Among the risk factors herd size (X2=4.24), history of abortion (OR=8.94) and retained fetal membrane (OR=8.39) showed significant association ($p<0.05$). The results of questionnaire survey showed 90% of the respondents do not know about *brucellosis* and use no maternity pen for their cows and 86.7% of them use no safety measure during assisting delivery and disposing afterbirth materials. The prevalence of *bovine brucellosis* in the present study was low; however, the risk attributed by a single carrier cow to the public health is high in the presence of lack of awareness and poor hygienic practice. Therefore, at this prevalence level to test and cull seropositive cows at farm level and creating public awareness is suggested.

Key words: *Brucellosis*, Dairy cattle, Seroprevalence, Risk factor, West Shewa

SÉROPRÉVALENCE DE LA BRUCELLOSE ET FACTEURS DE RISQUE ASSOCIÉS CHEZ LES BOVINS LAITIERS, DANS CERTAINES VILLES DE L'OUEST DE SHEWA EN ÉTHIOPIE

Résumé

La brucellose bovine est une maladie contagieuse qui est à l'origine de l'infertilité, de la perte de production de lait et de zoonoses, partout dans le monde. Une étude épidémiologique transversale a été réalisée sur 816 bovins laitiers (449 vaches) issus de 60 fermes laitières, dans le but de déterminer la séroprévalence de la brucellose bovine et les facteurs de risque associés chez les bovins laitiers de certaines villes de l'Ouest de Shewa en Éthiopie. Des sérums ont été prélevés, étudiés au moyen du test au rose bengale, et les sérums qui se sont révélés positifs ont fait l'objet d'un examen plus approfondi utilisant le test de fixation de complément pour confirmer la séropositivité à la Brucella. Les données relatives aux facteurs de risque ont été obtenues à partir des dossiers et d'un questionnaire administré. L'association de la brucellose aux facteurs de risque a été analysée en utilisant le test de Chi-carré / méthode exacte de Fischer. Le résultat a montré une séroprévalence de 0,49% (4/816), 0,9% (4/449) et 3,3% (2/60) respectivement chez les bovins, les vaches seules et au niveau du troupeau. Parmi les facteurs de risque, la taille du troupeau (X2 = 4,24), les antécédents d'avortement (OR = 8,94) et la rétention des membranes fœtales (OR = 8,39) ont montré une association significative ($p <0,05$). Les résultats de l'enquête par questionnaire ont révélé que 90% des répondants ne savaient rien de la brucellose et n'avaient pas d'enclos de vêlage pour leurs vaches et que 86,7% d'entre eux n'utilisaient aucune mesure de sécurité pendant la mise-bas et l'élimination du placenta après la mise-bas. Le taux de prévalence de la brucellose bovine dans la présente étude était faible; cependant, le risque que constitue une seule vache

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porteuse pour la santé publique est élevé compte tenu du manque de sensibilisation et de l'application de mauvaises pratiques d'hygiène. Par conséquent, avec ce taux de prévalence, il est proposé de procéder au diagnostic et à l'élimination de vaches séropositives au niveau de la ferme et à la sensibilisation du public.

Mots-clés : *brucellose*, *bovins laitiers*, *séroprévalence*, facteur de risque, Ouest de Shewa

Introduction

Bovine brucellosis is a major zoonotic disease widely distributed in both animals and humans especially in the developing world. It is a disease of cattle usually caused by Brucella abortus, on occasionally by Brucella melitensis and rarely by Brucella suis (Radostitis *et al.,* 2007). The disease impacts both animal and human health, as well as economic consequences, especially in countries where livestock production play the greatest economic role (Roth *et al.,* 2003).

Bovine brucellosis is a contagious infectious disease of cattle characterized primarily by abortion in late stage of pregnancy, frequently followed by retention of fetal membrane and endometritis, which may cause infertility in subsequent pregnancies (Al-Majali *et al.,* 2009). The common signs in male are orchitis and epididyimitis with frequent infertility (Radostitis *et al.,* 2007). Ingestion of feed and water contaminated by brucella organisms from aborted fetus and fetal materials is the common means of transmission to susceptible animals (Al-Majali *et al.,* 2009). However, it has been reported that infection through injured/intact skin, the mucosa of the respiratory system, and conjunctiva is not uncommon (Kebede *et al.,* 2008). Semen from infected bulls, if inseminated by artificial means, it can definitely transmit the infection to the cows (De Massis *et al.,* 2005).

The disease in man (undulant fever) is transmitted through consumption of raw milk and its products or through contact with afterbirth products from infected animals (Khorasgani *et al.,* 2008). The diseases is acute febrile in man which may progress to a more chronic form and can also produce serious musculo-skeletal, cardiovascular and nervous complications (OIE, 2009).

Currently the prevalence of *bovine brucellosis* is highest in dairy cattle in Latine America and developing countries like in Africa, though the rate of infection varies greatly among the countries. However, most European countries are free of *bovine brucellosis* (Seifert, 1996). In Ethiopia, the dairy production has been growing to meet an ever increasing demand for milk and milk products. Cross breeding indigenous cattle with high yielding exotic cattle is used to bridge the gap between supply and demand for dairy products (Deselegn and Gangwar, 2011). Owners of dairy cattle and institutions promoting the dairy industry require current reliable information on important diseases such as brucellosis. The unlikelihood of treatment and economic loss through abortion, reduction of milk yield and loss of fertility could impede the developing dairy sector and risk to public health shows that the disease is highly important. Studies on *bovine brucellosis* in varies parts of the country have showed the prevalence of the disease at varies rates. However, no similar work concerning *bovine brucellosis* has been conducted in districts of west Shewa zone so far. Moreover, according to the data from district veterinary clinics and artificial insemination technicians, there have been several repeated complaints for poor reproductive performance by dairy cattle owners. Therefore, the objective of this study was to determine the seroprevalence and associated risk factors of *bovine brucellosis* in dairy cattle in selected towns of west Shewa Zone, Ethiopia.

Materials and methods

Description of the study area

The study was conducted in five selected towns namely Ambo, Holeta, Addis Alem, Ginchi and Guder of West Shewa Zone, Ethiopia from October 2013 to January 2014. Ambo is the administrative center for the Zone, which is located at 114 km west of Addis Ababa.

The other four towns are the administrative towns for their respective district (woreda). According to the zonal basic data, the zone is dominated by high land environment followed by mid altitude and small share of low land. All the study towns are dominated by high land and mid altitude, which is good climatic condition for the adaptation of exotic breeds of dairy cattle. As a result the establishment of dairy farms and small holders targeting milk production is increasing in the area.

Target population and study Animals

The target populations were crossbred dairy cattle in urban and peri-urban areas kept for commercial purpose in the selected towns. List of dairy farm owner was obtained from the respective districts livestock agency from which volunteer dairy farm owners holding at least 3 cross breed cattle in their herd were included in the study. Cattle kept under small (holding less than 30) and medium (holding 30 and above) dairy farm, managed in either intensive or extensive system and selling raw milk for the community were included in this study. The study animals were cross breed dairy cattle with the age of 6 months or above, which belonging to volunteer dairy farmer/ farm owners.

Sampling method and study design

Sixty volunteer dairy owners were selected and a total of 816 dairy cattle owned by these owners were sampled to screen *bovine brucellosis* using cross-sectional study. Screening of sera was performed using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) was used for confirmation of RBPT positives sera. In addition survey was conducted using a semi-structured questionnaire, and 60 dairy owners were interviewed to assess the different risk factors such as herd composition, herd size, breeding system, animal management, knowledge about brucellosis, hygienic disposal of aborted material and presence of maternity pen. Before the actual interview and blood sample collection verbal consent was obtained from each farmer. Data regarding history of reproductive failure (poor breeding performance rate, abortion, retained fetal membrane) in the herd were also compiled.

Ethics statement

The seroprevalence study involves interview with dairy cattle owners and blood sampling from dairy cattle. The study protocol was assessed and approved by the Ambo University Animal Research Ethics Review Committee (ARERC) on December 10, 2012, with its reference number RCCSD/ AREC/002/2012. The dairy farmers were informed about the purpose and the methods of the study and informed consent was obtained from all individual participants included in the study.

Blood sample collection and serological analysis

Approximately 10ml of blood sample was collected from the jugular vein of each cattle using sterile needle and plain vacutainer tube and labeled. The collected sera were allowed to clot overnight in a slant position at room temperature. After 24 hrs, sera were separated from the clot into cryovials by decanting; unretracted blood was centrifuged to separate serum. Finally the sera samples were stored at -20^{0}C until tested. All sera samples were screened by RBPT, using an antigen suspension consisting of B. abortus according to the procedures described by the OIE (2012) at Ambo University veterinary laboratory. Thirty micro liters of serum was mixed with an equal volume of antigen suspension on a plate and agitated. After 4 minutes of shaking, visible agglutination was considered as a positive result. The test was repeated when test results were unclear. Sera positive for the RBPT were further tested using CFT complying with the standard protocol OIE (2012) at National Veterinary Institute, Debre Zeit, Ethiopia. A standard B. abortus antigen for CFT was employed to detect the presence of antibodies against Brucella in the sera. The control sera and complement are both obtained from the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Germany.

Data management and analysis

Data obtained both from serological tests and questionnaire survey were entered into Microsoft excel spreadsheet and analyzed using the SPSS 20.0 statistical package (SPSS Inc., Chicago, Illinois, USA). Animals tested positives to both RBPT and CFT were defined as seropositive and herds having at least one seropositive cattle were considered positive. Animal level seroprevalence was computed by dividing the number of positive animals by total number of animals tested and for herd level seroprevalence the number of positive herd was divided to the total number of herds tested. Data from questionnaire was summarizes by simple descriptive way. The associations of serological status of *bovine brucellosis* with risk factors such as age, sex, herd size, parity, breeding system, management system and study towns as well as history of reproductive disorders was assessed using Chi-square test/ Fischer's exact test. For statistical inference 0.05 was considered as level of significance.

Result

Sixty small and medium level dairy farms from five towns namely Ambo (N=20), Holeta (N=15), Ejere (N=9), Guder (N=8) and Ginchi (N=8) were investigated. Out of the total 816 sera samples tested, the overall individual animal level seroprevalence was 0.49% (4/816). All the seopositive cattle were cows 0.9 % (4/449) and the herd level seroprevalence was 3.3% (2/60). Individual animal level seroprevalence was 1.08% (3/277) at Ambo, 1% (1/285) at Ejere and 0% in the rest of towns. Herd level seroprevalence of 11.1% (1/9), 5% (1/20), and 0% was recorded at Ejere, Ambo and the rest the towns, respectively. However, the variation among the towns was not statistically significant (Table 1).

Among the risk factors only herd size showed marginally significant association ($p<0.05$), with cattle in herds holding >30 had higher prevalence 1.01% (4/390) to those holding less than 30 cattle 0% (0/426). The other factors namely age, sex and management system did not significantly influence the occurrence of brucellosis infection. However, the seroprevalence was observed to be numerically higher in intensively managed animals 0.75% (3/401); in cattle older than 6 years of age 1.37% (3/219) and only in female animals 0.53 % (4/756) as compared to their counterpart (Table 2).

Seroprevalence of *bovine brucellosis* in cattle alone was significantly associated with history of abortion and retained fetal membrane with higher risk of acquiring infection in cows with history of abortion ($OR=8.94, p<0.05$) and retained fetal membrane ($OR= 8.39, p<0.05$). The remaining factors namely parity, breeding system and history of poor breeding performance did not show significant association ($p>0.05$) with brucellosis seroprevalence. However, a numerically higher seroprevalence as compared to their counterpart was recorded; 1.47% (3/204) in dairy cows having more than 2 calves, 1.31% (4/306) in cows served either by bull or AI and 1.30% (2/154) in cows with poor breeding performance rate (Table 3).

The result of questionnaire survey indicated 90% (54/60) of the respondents did not have any information regarding brucellosis and 86.67% (52/60) did not use protective precaution while assisting animals with difficult parturitions and discarding aborted fetus and fetal membrane. Moreover, only 10% (6/60) of the respondents had maternity pen for their dairy herd (Table 4).

Discussion

The low overall individual animal level seroprevalence (0.49%) of brucellosis in the present study was comparable with 0.4% report of Asmare et al. (2007) from urban dairy farms of Northern Ethiopia and Sebeta and 0.5% Tolosa et al. (2010) and Degefu et al. (2011) from cattle managed under extensive system. Relatively higher prevalence were reported in dairy cattle by other researchers; for instance seroprevalence of 1.5%, 1.7%, 2.5%, 3.9% and 10% were reported by Tesfaye et al. (2011); Tschopp et al. (2013); Asmare et al. (2007); Abebe et al. (2009); Eshetu et al. (2005),

Table 1: Individual and herd level prevalence of *bovine brucellosis* in the study towns

Towns	No. of animals tested	No. of CFT positive cattle (%)	No. herd tested	No. of CFT positive herd (%)
Ambo	277	3 (1.08)	20	1(5.00)
Guder	116	0	8	0
Holeta	285	0	15	0
Ejere	100	1 (1.00)	9	1(11.11)
Ginchi	38	0	8	0
Total	**816**	**4 (0.49)**	**60**	**2(3.33)**

X^2=4.69, p>0.05 (individual level) X2=3.01, p>0.05 (Herd level)

Table 2: Association of seroprevalence of *bovine brucellosis* with risk factors

Factor	Category	Total No. examined	CFT positive	Chi- square	P-Value
Management system	Intensive	401	3 (0.75)	4.16	0.12
	Semi-intensive	358	1 (0.28)		
	Extensive	57	0		
Age	<2years	272	0	5.04	0.08
	2-6 years	324	1 (0.31%)		
	>6 years	219	3 (1.37%)		
Sex	Male	60	0	0.32	0.736
	Female	756	4 (0.53%)		
Herd size	<30 animals	426	0	4.24	0.048
	> 30 animals	390*	4 (1.03%)		

*<: less than, >: greater than, *: P<0.05*

Table 3: Association of *bovine brucellosis* seroprevalence with the risk factors in dairy cows

Risk factor	Category	Total No. examined	Brucellosis positive	Odds Ratio	95% CI	P-Value
Parity	<2	245	1 (0.41%)			
	>2	204	3 (1.47%)	3.64	0.37-35.28	0.26
Breeding method	Only AI	143	0 (0.70%)			
	Mixed	306	4 (1.00%)	0.92	0.894-1.018	0.761
History of abortion	Absent	432	3 (0.69%)	-	-	-
	Present	17	1 (5.88%)	8.94	0.88-90.72	0.026
History of retained fetal membrane	Absent	431	3(0.70%)	-	-	-
	Present	18	1(5.56%)	8.39	0.83- 84.93	0.032
History of poor breeding performance	Absent	295	2(0.68%)	-	-	-
	Present	154	2 (1.30%)	0.52	0.07- 3.72	0.506

<: less than, >: greater than, CI: confidence interval, AI: Artificial insemination

Table 4: Result of questionnaire survey concerning knowledge and practice of brucellosis

Knowledge and practice	Category	Percent of farms/owners
Knowledge about brucellosis	Yes	6/60 =10%
	No	54/60 =90%
Precaution during assisting delivery and handling aborted fetus and fetal membrane	Yes	8/60 =13.3%
	No	52/60 =86.7%
Presence of maternity pens	Yes	6/60 =10%
	No	54/60 =90%

respectively. Even higher prevalence of 39% was recorded by Meyer (1980) while Asmare *et al.,* (2011) from Nazareth, Gondar and Mekele; Belihu (2002) from Salale and Addis Ababa and Alem and Solomon (2002) from central Ethiopia were unable to find a single positive reactor in intensive dairy farms. The herd level seroprevalence of 3.3% in the present study was also lower compared to 15%, 42.31% and 45.9% the report by Ibrahim et al. (2010); Berhe et al. (2007) and Kebede et al. (2008), respectively. The lower individual animal and herd level seroprevalence in the current study might be due to culling of seropositive dairy cattle owned by private and government farms based on recommendation given by regional veterinary laboratory following screening *bovine brucellosis* in 2010 (personal communication). Moreover this could also be indicative of less level of intensification of the dairy farming in the present study areas as compared to central high lands of Ethiopia. However, the variation in seroprevalence reported by different authors might be due to variation in breed of study animals, level of management intensification, herd size, breeding system, and serological tests employed (Hailesilasie et al. 2010; Tesfaye et al. 2011).

The numerically higher seroprevalence in intensive dairy farm than the semi-intensive and extensive in the present study is in agreement with the reports of Bekele et al. (2000) and Hailesilasie *et al.,* (2010). In line with this it has been well documented that the level of brucellosis infection tends to be higher in intensive farms and the risk of infection increase with intensification when aborting cows are present in the herd and hygienic practices is low (FAO/WHO, 1986). This could be due to higher chance of contact between healthy and infected animals in these systems and inattention of some famers to segregate infected animals and proper disposal of infectious materials.

It is well known that sexually mature animals are more susceptible to Brucella abortus infection (Radostitis *et al.,* 2007). Accordingly a fairly higher seroprevalence in cattle of older than 6 years and seronegativity in animals below 3 years old was observed in the present study. The same trend has been reported by Hailesilasie et al. (2007), Abebe et al. (2008) and Degefu et al. (2011).

Although there is disproportional sampling of male (n=60) over female (n=256) in the present study, the absence of male reactors is in agreement with the findings of Asmare *et al.,* (2007), which might be due to the limited serological response of male animals to Brucella infection because of low antibody titer of testes of infected males (Crawford, 1990).

In the current study the proportion of seropositive animals was significantly higher in herds holding greater than 30 animals (1.03%) than those holding less than 30 animals (0%), which is in agreement with the report of similar authors (Hailesilasie *et al.,* 2007; Asmare *et al.,* 2007). This could be due to the cumulative effect of intensive management system and stocking density, which facilitate rate of contact in dairy herds.

It is well-known fact that in susceptible non-vaccinated pregnant cattle, abortion after the 5th month of pregnancy is cardinal feature

of bovine brucellosis, and retention of fetal membrane and metritis are common sequels to abortion (Walker, 1990; Schelling, 2003). Different researchers have reported significant association between history of abortion and retained fatal membrane with brucellosis (Al-Majali, *et al.,* 2009; Deselegn and Gangwar, 2011; Berhe, *et al.,* 2007). In line with this the present study revealed significant association ($P<0.05$) of *brucellosis* seropositivity with history of abortion and retained fetal membrane, though, the number of cows with history of abortion (n=17) and retained fetal membrane (n=18) were small. This could be due to the reason that some farmers could hide of cows with history of abortion and retained fetal membrane for fear of risking acceptance of their dairy product as their lively hood is based on the income they generate from milk and milk products. Moreover, this study considered two years information, since cows usually abort once perhaps at the first pregnancy (OIE, 2009), cows might have aborted in their earlier calving and their number might be small. The cause of abortion and retained fetal membrane in seronegative cow with history of abortion and retained fetal membrane might be other factors such as malnutrition, deficiency diseases and other infections. According to Asmare et al. (2013) abortion, retained feral membrane and poor breeding performance were more associated with Neospora than Brucella in breeding dairy herds.

Brucellosis in humans is an occupational hazard to animal attendants, slaughter house workers and veterinarians (Radostitis *et al.,* 2007; OIE, 2009). In line with this, the lack of information regarding brucellosis in 90%, absence of maternity pen in 90% and poor hygienic practice in 86.67% of the respondents in the current questionnaire surveys showed the increased zoonotic risk of brucellosis for the dairy personnel in the study area.

In conclusion the present study detected brucellosis in two out of the five studied towns and large herd, presence of history of abortion and retained fetal membrane were the detected risk factors. Although the seroprevalence is low, the lack of awareness about the disease and poor hygienic practices in the preset study alarms the need for due attention to reduce public health risk. Hence, eliminating carrier dairy cows is suggested at this prevalence level together with raising public awareness regarding brucellosis. Moreover, beyond the epidemiological survey, detection and typing the prevailing strains of Brucella is recommended to aid its control at national level.

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