Journal of Veterinary Medicine and Animal Health Vol. 5(3), pp. 67-72, March 2013
Available online at http://www.academicjournals.org/JVMAH
DOI: 10.5897/JVMAH12.056

Full Length Research Paper

Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district

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Accepted 31 January, 2013

A cross-sectional study was carried out to determine the prevalence of subclinical mastitis in lactating dairy cows from August 10, 2011 to May 25, 2012 in three purposively selected commercial dairy farms in Holeta district, Ethiopia. The study was carried out through field screening surveys by California mastitis test for each quarter milk sample, followed by bacteriological examination to identify the causative agents of intra-mammary infection. A total of 546 milking cows were examined, out of which 224 (41.02%) were found positive for subclinical mastitis on the basis of California mastitis test. Milk samples collected from 224 positive cows were subjected to microbiological culture for the isolation of pathogenic bacteria. One hundred eighty three (81.7%) of the samples were found positive for bacterial isolation. The major isolate pathogens were *Staphylococcus aureus* (13.8%), *Streptococcus uberis* (12.1%), *Staphylococcus epidermidis* (11.7%), *Escherichia coli* (11.6%), *Streptococcus dysagalactiae* (10.6%), *Pseudomonas aeruginosa* (9.7%), *E. coli* $O_{157:H7}$ (6.9%), *Micrococcus* species (6.5%) and *Streptococcus agalactiae* (6.4%) and others (10.7%). Subclinical mastitis is endemic in Holeta dairy farms and thereby necessary measures are needed to be taken to prevent further losses.

Key words: California mastitis test, bacteriological culture, prevalence, subclinical mastitis.

INTRODUCTION

Despite many years of research, mastitis subclinical remains the most economically damaging and zoonotic potential disease for dairy industry and consumers worldwide irrespective of the species of animal (Ojo et al., 2009). Economic losses caused by mastitis include value of discarded milk, reduction in quality of milk and cost of treatment (Radostits et al., 2007). Bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning or interference with manufacturing process or in rare cases, provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw cow milk include brucellosis, caseous lymphadenitis, leptospirosis, listeriosis, melioidosis, Q-fever, staphylococcal food poisoning, toxoplasmosis and tuberculosis (Mungube et al., 2005; Radostits et al., 2007).

The prevalence of subclinical mastitis in dairy herds is often surprising to producers, moreover, sub-clinically infected udder quarters can develop clinical mastitis and the rate of new infections can be high (Zdunczyk et al., 2003). Previous studies conducted in different countries indicated the distribution and economic importance of the disease. Contreras et al. (1997) from Spain, Moshi et al. (1998) from Tanzania, Ameh and Tari (2000) from Nigeria, Ndegwa et al. (2000) from Kenya and Kozacinski et al. (2002) from Croatia reported different prevalence rates of mastitis in dairy cattle. The disease has been reported by several authors in different parts of Ethiopian country (Mungube et al., 2005; Lakew et al., 2009; Gebreyohannes et al., 2010; Megersa et al., 2010). Several of these studies have shown the occurrence of a range of

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mastitis causing bacteria, indicating *Staphylococcus*, *Escherichia coli* and *Streptococcus* as dominant and pathogenic species. Some authors (Mungube et al., 2005) reported a substantial economic loss in Ethiopian highland crossbred dairy cows due to subclinical mastitis.

Subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), the modified white side test, somatic cell count, pH, and catalase tests. These tests are preferred as the screening tests for subclinical mastitis as they can be used easily, yielding rapid, as well as satisfied results (Joshi and Gokhale, 2006).

In some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects (Mekebib et al., 2009; Megersa et al., 2010). This study aimed: (i) to evaluate the prevalence of subclinical mastitis in apparently healthy dairy cows in Holeta district, (ii) to determine the most frequency of intra-mammary infection, causative agents, and (iii) to evaluate associated risk factors affecting on subclinical mastitis.

MATERIALS AND METHODS

Study area

The study was conducted in dairy farms of Holeta town located 45 km away from Addis Ababa in the south west direction, 9° 3' N and 38° 30' E, at an altitude of 2,400 m above sea level in central highlands. The area is characterized by mild subtropical weather with minimum and maximum temperature ranging from 2 to 9°C and 20 to 27°C, respectively. The area receives annual rainfall of 1060 mm (CSA, 2010).

Study population

A total of 546 dairy cows were examined in three different dairy farms in Holeta town. The dairy cows were distributed according to breed (136 Holstein Friesian breed, 150 Jersey and 260 Holstein × Borena cross breed cows), age (322 cows aged less than 6 years young and 224 cow aged greater than or equal to 6 years old). All dairy cows had no clinical symptoms. They lived nearly under the same conditions of breeding from the habitat, hygiene and feeding systems. All animals were subjected to clinical and physical examinations, with special interest towards the udder and teats. At the time of each examination, the breed of the cow, age of the cow, health status of the mammary glands and the respective farm names were recorded.

Study design, sample size and sampling method

A cross sectional study was conducted. Three dairy farms were purposively selected for their ease of accessibility. Simple random sampling technique was followed to select the study animal, and the desired sample size was calculated according to the formula given by Thrusfield (2007). Milk samples were taken from apparently healthy animals in these dairy farms. A total of 546 dairy cows were examined in three different dairy farms in Holeta district, Ethiopia, and spread out over ten months (during the period from August 10, 2011 to May 25, 20112).

Physical examination of mastitis

Udder attachment, parity number, any physical abnormalities such as swelling of the udder, presence of lesions, anatomical malformations and tick infestation were recorded. The milk was examined for its color, odor, consistency and other abnormalities prior to milking.

California mastitis test (CMT)

The California mastitis test was carried out as described by Hogan et al. (1999) and Quinn et al. (2004). A squirt of milk, about 2 ml from each half was placed in each of 2 shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. Based on the thickness of the gel formed by CMT reagent-milk mixture, test results were scored as 0 (negative/trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive). Positive CMT-cows were defined as having at least one CMT-positive quarter.

Milk sample collection, handling and transportation

Aseptic procedures for collecting quarter milk samples as described by Hogan et al. (1999), Sears et al. (1991) and Quinn et al. (2004) were followed. The time chosen for milk sample collection was before milking. Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton alcohol pads. A separate pledged of cotton was used for each teat. The first streams of milk were discarded and 10 ml of milk was collected into horizontally held vial. After collection, the sample was placed in an icebox and transported to the laboratory for analysis.

Microbiological culture

Each positive CMT milk sample was collected under aseptic conditions in a sterile screw caped bottle numbered to identify the particular quarter and cow. All milk samples were sent directly to the laboratory, with a minimum delay for routine culture techniques. Milk samples were cultured onto 10% sheep blood agar and MacConkey agar plates according to Athar (2006), Coulon et al. (2002) and Quinn et al. (2004). Suspected colonies were identified morphologically, microscopically and biochemically according to National Mastitis Council (NMC) (2004), Iqbal et al. (2004) and Quinn et al. (2004). Cultures with fine bacterial growth were considered as positive and cultures with no visible growth taken as negative, but polluted cultures with disturbed media were considered as contaminated according to Shakoor (2005). Pure isolates of *E. coli* were inoculated into 10 ml of brain-heart infusion (BHI) broth (Oxoid Ltd, Basingstoke, Hampshire, UK), supplemented with yeast extract (Oxoid) followed by incubation at 37°C for 8 h, to further identify serotype of *E. coli* according to Quinn et al. (2004).

Statistical analysis

The data was compiled and analyzed with Statistical Package for Social Sciences (SPSS statistical package version 17). Prevalence estimation of commonly isolated pathogens in Holeta town dairy farms was determined using standard formulae (that is, the number

of positive animals/samples divided by the total number of animals/samples examined). Descriptive statistics such as percentages and frequency distributions was used to describe/present the nature and the characteristics of the data.

RESULTS

California mastitis test (CMT)

Out of 546 lactating cow examined, 224 (41.02%) were diagnosed with subclinical mastitis in the study area, out of which 130 (58%), 58 (26%) and 36 (16.1%) were from A, B and C dairy farms, respectively. Significant difference in mastitis prevalence ($P < 0.05$) was observed among studied farms (Table 1). The prevalence of subclinical mastitis did not vary among age group. However, relatively higher prevalence of subclinical mastitis was recorded in adult (46.42%) followed by young age group (37.3%). There was no significant difference ($P > 0.05$) in infection among age groups (Table 1). Prevalence of subclinical mastitis did not vary along with the lactation stages of animal, but relatively highest prevalence was seen in animals at mid lactation stage (50%), followed by animals at late lactation (47.2%) and a least in early lactation stage (37.5%). The result of statistical analysis revealed no significant difference ($P > 0.05$) among the lactation stages (Table 1).

Mastitis causing pathogens

Out of 224 positive samples for subclinical mastitis, only 183 (81.7%) samples showed growth on 10% sheep blood agar and 28 (12.5%) samples showed no growth, and about 13 (5.8%) were contaminated samples. From 183 culture positive samples, a total of 596 bacteria of seven genera were isolated. The relative prevalence of various bacterial species isolated from subclinical mastitis cases are shown in (Table 2). The most prevalent isolated pathogens were *Staphylococcus aureus* (13.8%), *Streptococcus uberis* (12.1%), *Streptococcus epidermidis* (11.7%), *E. coli* (11.6%), *Enterobacter aerogenes* and *Klebsiella pneumonia* (10.7%), *Streptococcus dysagalactiae* (10.6%) and *Pseudomonas aeruginosa* (9.7%). Other bacterial isolates includes *E. coli* O157:H7 (6.9%), *Micrococcus* species (6.5%) and *S. agalactiae* (6.4%).

DISCUSSION

The present epidemiological study was applied through combination of the CMT with bacteriological cultures. Thus, subclinical mastitis was defined as a state when mammary glands without clinical abnormalities give apparently normal milk but was bacteriologically positive and with positive CMT (Mungube et al., 2005).

Karimuribo et al. (2006) concluded the CMT is still the superior screening diagnostic aid for subclinical mastitis, while bacteriological examination is still the most suitable technique of diagnosis. This study detected the subclinical mastitis in 224 out of 546 milking cows examined, which result in a prevalence of 41.02% subclinical mastitis in dairy farms of Holeta District. This result is in agreement with previous studies by Mekebib et al. (2009), Sori et al. (2005), Workineh et al. (2002) and Girma (2010) who reported prevalence of 34.8, 40.6, 38.6 and 34.4%, respectively. However, the prevalence of subclinical mastitis in this study is relatively higher than previous 23.0% by Biffa et al. (2005) and 9.81% by Lakew et al. (2009) in Southern Ethiopia and Khartoum, respectively. Because mastitis is a complex disease involving interactions of several factors, mainly of management, environment, and factors relating to animal and causative organisms, its prevalence is expected to vary from place to place.

All 224 CMT positive subclinical samples were cultured on bovine blood agar and accordingly, 183 (81.7%) were found culture positive. The failure to isolate the bacteria from the CMT positive milk samples could be partly associated with spontaneous elimination of infection, low concentration of pathogens in the milk, intermittent shedding of pathogen, and intracellular location of pathogens and presence of inhibitory substance in the milk (Radostits, 2007). A total of 596 isolates of seven (7) different microbial species were isolated.

The present study also revealed a close positive relationship between isolation of bacteria from mastitic milk samples and California mastitis test. As almost all milk samples were positive to CMT, specific bacteria were isolated. This means that CMT was a good diagnostic tool in the detection of sub-clinical mastitis; hence it could be most the reliable test to be conducted to investigate sub-clinical mastitis in the dairy farms. On the other hand, the culture method may be used to confirm and aid proper treatment (Tefera, 2001; Barnouin et al., 2005; Bitew et al., 2010; Bekele and Molla, 2001).

Mastitis has a multifactorial nature that predominates with a clear interaction between host, agent and environment (Thusfield, 2007). For this reason, the studied factors here were determined as breed, age and lactation stage (Riekkerink et al., 2008). Considering the breed factor, it was found that the Holstein-Borena breed (50%), all kept in farm A, were found more susceptible than Jersey breed (38.7%), all kept B, and Holstein-Frisian breed cows all kept in C (26.5%) were found least susceptible. Thus, breed difference was found to be statistically significant ($P < 0.05$). The high prevalence of subclinical mastitis in farm A could be associated with breed susceptibility, poor hygienic and managemental conditions. It was observed that subclinical mastitis frequently encountered in the examined dairy cows were more common in middle (50%) and late lactation stage (47.62%) than early lactation stage (36.7%). Hence, regime could be possibly among the major factors contributing to high prevalence at middle stage. During a dry period, due to low bactericidal

Table 1. Association between some of factors with occurrence of subclinical mastitis.

Risk factor		Cow		Prevalence (%)	X^2	P value
		Total	Infected			
Age	Young[a]	322	120	37.3	2.299	0.21
	Adult[b]	224	104	46.42		
Farms/breed	A[c]	260	130	50	10.454	0.03
	B[d]	150	58	38.7		
	C[e]	136	36	26.5		
Lactation stage	Early[f]	360	132	36.7	0.133	0.13
	Mid[g]	144	72	50		
	Late[h]	42	20	47.62		

[a]Young: < 6 years, [b]Old: ≥ 6 years, [c]A: Holeta agricultural research center dairy farm (Holstein × Borena breed), [d]B: Ada'a Berga agricultural research center dairy farm (Jersey breed), [e]C: Holeta cattle genetic improvement center dairy farm (Holstein-Friesian breed), [f]Early: 1 to 120 days of lactation, [g]Mid: 120 to 240 days of lactation, [h]Late: >240 days of lactation.

Table 2. Frequency of mastitis causing pathogen isolated from subclinical mastitis in dairy cows.

Species of bacteria identified	No. of isolates/farms			Total No. of isolate	Percentage
	A[c]	B[d]	C[e]		
E. coli	45	15	9	69	11.6
E. coli O157:H7	31	10	0	41	6.9
S. aureus	28	33	21	82	13.8
M. species	19	12	8	39	6.5
S. epidermidis	46	8	16	70	11.7
S. uberis	40	14	18	72	12.1
S. dysagalactiae	33	19	11	63	10.6
S. agalactiae	22	8	8	38	6.4
P. aeruginosa	23	24	11	58	9.7
Others*	25	27	12	64	10.7
Total	312	170	114	596	100

*Other include *E. aerogenes and K. pneumonia*, [c]A: Holeta agricultural research center dairy farm (Holstein × Borena breed), [d]B: Ada'a Berga agricultural research center dairy farm (Jersey breed), [e]C: Holeta cattle genetic improvement center dairy farm (Holstein-Friesian breed).

and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply. The increased prevalence of mastitis with advancing lactation number agrees with the findings of previous investigators (Harmon, 1994; Radostits et al., 2007; Zerihun, 1996).

The prevalence of mastitis with age seen in this study is similar to reports by Biffa et al. (2005). The high prevalence of subclinical mastitis in aged multiparous animals might be due to increase in teat patency and frequency of previous exposure (Harmon, 1994).

In present study, most major pathogen isolated were *S. aureus* (13.8%), which was not similar with reports by Sori et al. (2005), Sharif et al. (2009) and Mekebib et al. (2009). This variation may be due to season, managemental conditions at the farm, area, difference in sample handling in the laboratory and use of antibiotics. *E. coli* identified in the present study (11.6%) was not similar with reports by Mekebib et al. (2009), Bitew et al. (2010) and Sori et al. (2005) with an isolation rate of 43.13, 20.3 and 26.57%, respectively. This lower report of isolates might be partly associated with effective udder washing and drying, post milking teat dip and keeping cleanness of washing towels. The present study also identified a low prevalence of *Micrococcus* spp. (8.15%) and *Corynebacterium bovis* (1.7%), which was in-line with findings of Workineh et al. (2002), Bitew et al. (2010) and

Sori et al. (2005). *S. agalactiae* was isolated with a proportion of 6.4%. The result of present study was similar with those described by Lakew et al. (2009) and Bitew et al. (2010) who reported 4 and 8.8%, respectively.

The prevalence of streptococcal isolation during this study (29.03%) was lower than that reported for the same species by Okeke et al. (2005) (80.95%) in dairy cows. The lower isolation rate in this study might be associated with the wide spread use of penicillin in the area for treatment of mastitis. It has been recognized that mastitis caused by *Streptococcus* species is susceptible to eradication via use of penicillin. *S. uberis* isolation (12.1%) in this study was higher than that reported by Mekebib et al. (2009) (6.53%), but lower than that of Zerihun (1996) and Iqbal et al. (2004) who reported 27 and 49.98%, respectively.

In this study, the prevalence of subclinical mastitis was accompanied with analysis of different risk factors including farm and breed differences, lactation stages and isolation of major bacterial pathogens in subclinical mastitis cows. Cross-breed was more stuck by subclinical mastitis than Jersey and Holstein-Frisian breeds. Aged cows showed most sensitivity for subclinical mastitis. Mid lactation stage was seen with higher prevalence.

CONCLUSION AND RECOMMENDATIONS

In a spite of a large research efforts aimed to gain prevalence and to develop a new control tools for mastitis, the subclinical occurrence of the mastitis remains a substantial problem for dairy producers. The result of the present study indicated a relatively high prevalence of subclinical mastitis in dairy cattle of the study area. The relatively high prevalence reported in this study clearly indicated lack of strategic control measures against the disease, as well as poor surveillance measures. Lack of maintenance of strict hygiene and good sanitary environment may be contributory factors in the cause of subclinical mastitis. It is therefore important that farmers should ensure strict personal hygiene and that of animals, and general sanitary condition of the farms should be improved and maintained. Furthermore, all dairy producers should know that early detection of intra-mammary infection is important for selecting and implementing proper therapy. Unfortunately, most infections are not detected until they become clinical, and by then, extensive and costly damages could result. Routine milk cultures should be an ongoing part of any mastitis control program. The sampling strategies for any ongoing program require the input of the herd veterinarian, as well as herd management.

ACKNOWLEDGEMENTS

Authors would like to thank Holeta Agricultural Research Center (HARC) and School of Veterinary Medicine of Wollo University for their material and technical supports.

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