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Milk yield and associated economic losses in quarters with subclinical mastitis due to *Staphylococcus aureus* in Ethiopian crossbred dairy cows

Gebreyohannes Y. Tesfaye · Fekadu Gudeta Regassa · B. Kelay

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Abstract The objective of the study was to estimate the prevalence and losses associated with subclinical mastitis (SCM) caused by *Staphylococcus aureus* in Ethiopian crossbred dairy cows. A split-udder trial was carried out to determine milk yield losses in udder quarter with *S. aureus*-caused SCM. Each quarter of the study cows was examined using the California Mastitis Test (CMT) and quarter milk production was measured over a period of 8 days. Milk yield losses for CMT positive quarters were estimated by comparing production of quarters with CMT score 0. Mean milk yield for uninfected healthy quarters was 1.66 kg per milking (95% CI, 1.66–1.55 kg per milking), and the rate of milk reduction for quarters with CMT scores of 1+, 2+, and 3+ was 25%, 33%, and 48%, respectively. Economic losses at different farm-size levels were calculated by multiplying the prevalence of CMT scores with milk yield losses associated with respective CMT scores. In Debre Ziet dairy herds, a quarter with SCM due to *S. aureus* lost an average of 34.5% of its potential milk production while the total milk yield loss per cow was estimated at 6.8%. Losses were highest in large-scale (13%) farms and lowest (3.7%) in small-scale. Based on the prevalence, the overall financial loss for each cow per lactation was 984.64 Eth Birr (US$78.65) and losses in large farms (1,882.40 Eth Birr or US $150.35) were over 3.5 times the loss in small-size farms. These figures possibly underestimate the potential benefits of mastitis control program as they do not include other direct and indirect costs.

Keywords Dairy cows · Milk loss · *S. aureus* · Subclinical mastitis

Introduction

Dairy production is a biologically efficient system that converts large quantities of roughage which is the most abundant feed in the tropics to milk (Reugg 2001). In Ethiopia, where there is access to a market, dairying is preferred to meat production since it makes more efficient use of feed resources and provides a regular income to the producer. Despite many years of research, mastitis remains the most economically damaging disease that severely reduces milk yield, profit margins, and quality of milk and milk products in all dairy-producing countries of the world (Owens et al. 1997). Mungube et al. (2005) reported a substantial economic loss in Ethiopian highland crossbred dairy cows due to subclinical mastitis (SCM). However, most dairy farmers in the

G. Y. Tesfaye
MOA, Agricultural and Rural Development,
Bahir Dar, Amhara Regional State, Ethiopia

F. G. Regassa (✉) · B. Kelay
Faculty of Veterinary Medicine, Addis Ababa University,
Debre Zeit P.O. Box 34, Ethiopia
e-mail: regassaf@yahoo.com

country normally do not recognize subclinical mastitis, which incidentally occurs at a much higher frequency than clinical mastitis, while quite few ignore the disease (Mungube et al. 2004). *Staphylococcus aureus* belongs to the contagious mastitis pathogens and is one of the most prevalent mastitis-causing pathogens in Ethiopia (Workineh et al. 2002; Kerro and Tareke 2003; Getahun 2006). Also, it is the most frequently isolated mastitis pathogen in some European countries (Osteras et al. 2006). Besides, among other bacterial mastitis pathogens, *S. aureus* stands out for its resistance to antibiotic treatment and its tendency to recur chronically (Janson 2006). In Ethiopia, there is no study related to a pathogen-specific SCM, particularly that of *S. aureus* and its effect on milk production. The objective of this study was to estimate the prevalence of SCM due to *S. aureus* in crossbred cows in Debre Ziet dairy farms and losses associated with milk production.

Materials and methods

Description of the study area

The study was carried out at Debre Zeit, from October 2007 to May 2008. The town is located at 9°N and 40°E 47 km south east of Addis Ababa. It has a human population of about 95,000. The altitude is about 1,850 m asl. It is an important small town where most governmental institutions and national research centers and the nationally biggest Ada'a-Liben Dairy Cooperative are located. The area experiences a bimodal rainfall pattern with heavy rains (84%) extending from June to September and short rains from March to May with an average annual rainfall of 800 mm. The mean annual minimum and maximum temperatures are 12.3°C and 27.7°C, respectively, with an overall average 18.7°C, and the mean relative humidity is 61.3% (NMSA 2003; CSA 2001).

Study type and sampling method

A cross-sectional study was carried out to estimate the prevalence of SCM caused by *S. aureus* and associated economic loss in Debre Zeit dairy farms while the split-udder trial was used to determine milk production losses. The study population was all milking dairy cows belonging to the members of Ada'a-Liben dairy cooperative. The sample size was determined according to Thrusfeild (2005). A sampling frame containing the list of current members of Ada'a-Liben Dairy Cooperative was acquired at the beginning of the study. Dairy farms/herds were selected from this list using a stratified random sampling procedure based on herd size according to the previous work on the urban and peri-urban production systems (Lemma et al. 2001). A total of 217 cows were selected from 55 dairy farms of which 44 were small (<5 heads of cattle), nine medium (six to 50 heads), and two large (≥50 heads).

Milk sample collection, screening, and bacteriological culture

A total of 850 quarters were screened for *S. aureus* SCM using the California Mastitis Test (CMT) and bacteriological culture to determine the number of affected quarters. Milk samples were collected from each quarter which was diagnosed positive for SCM by following strict aseptic procedures as recommended by NMC (1990). Only quarters with CMT score trace, 1+, 2+, and 3+ were considered positive for SCM. The individual milk samples were also cultured and analyzed for growth in accordance with standard bacteriological procedures (Quinn et al. 1999). A loopful of milk was streaked on 5% sheep blood agar, and plates were incubated at 37°C for 24 h before being examined for growth. The colonies were provisionally identified on the basis of Gram stain reaction, morphology, and hemolytic pattern. The suspected colonies were further subcultured on blood agar plates and nutrient slants for biochemical characterization of the isolates. The cultures were subject to catalase test, and those positive organisms were considered as staphylococci. Then, the catalase positive cultures were subcultured on Mannitol Salt Agar (Difco) and incubated at 37°C and examined after 24–48 h. The presence of growth and change of pH in the media (red to yellow color) regarded as presumptive identification of *S. aureus* or coagulase-positive staphylococci. These organisms were further subcultured into Brain Heart Infusion Broth and incubated at 37°C for 24 h. Then 0.1 ml of broth culture and 0.3 ml of sterile rabbit plasma were put into a narrow sterile tube along with a control tube containing a mixture of 0.1 ml of sterile Brain Heart

Infusion Broth and 0.3 ml of rabbit plasma. The tubes were incubated at 37°C and examined for clot formation after 4 and 24 h for identification coagulase-positive organisms. To differentiate pathogenic staphylococci with coagulase-positive from other *Staphylococci*, the suspected colonies were inoculated on Purple Agar Base (Difco) plate with 1% of maltose and incubated at 37°C for 24 h. Rapid fermentation of maltose by *S. aureus*-caused yellow discoloration of the medium.

Estimation of milk yield loss

A split-udder trial was carried out to collect data on quarter milk loss specifically due to *S. aureus* under tropical conditions. Based on bacterial culture results, only 12 dairy cows with pure *S. aureus* isolates were obtained and selected from the two large farms. All cows were almost under similar management and housing conditions. Also, the cows were with varying lactation stages and parities to reduce the effect of variations within cows to the minimum. Another inclusion criterion in this split-udder study was that a cow had to have at least one healthy quarter (Mungube et al. 2005). Thus, 20 quarters had CMT score of “0” while 8, 10, and 7 had 1+, 2+, and 3+, respectively, and were also culture-positive for *S. aureus*. Quarters with CMT trace and 1+ were combined, as the difference in the milk yield between the two CMT scores was considered negligible. Three quarters were blind. Each cow was hand-milked over an 8-day period, and milk from each quarter was put separately in a bucket. Although cows were milked twice daily, only the late-afternoon milking was used for this study.

The milk production loss due to *S. aureus*-caused SCM in each farm-size level was estimated according to Mungube et al. (2005) after those quarters with SCM of other causes were excluded. To estimate the overall quarter milk loss, the number of different CMT scores of the positive quarters was multiplied by the corresponding milk production using the formula: $ML_{\mathrm{y}} = \left(CMT0_{\mathrm{y}} \times ML_{CMT0}\right) + \left(CMT1_{\mathrm{y}} \times ML_{CMT1}\right) + \left(CMT2_{\mathrm{y}} \times ML_{CMT2}\right) + \left(CMT3_{\mathrm{y}} \times ML_{\mathrm{CMT3}}\right) / CMT0_{\mathrm{y}} + CMT1_{\mathrm{y}} + CMT2_{\mathrm{y}} + CMT3_{\mathrm{y}}$ where ML_{y}, milk yield loss per quarter in the respective farm level; $CMT0_{\mathrm{y}}$, 1_{y}, 2_{y}, 3_{y}, number of quarters with respective CMT scores in the farms; $ML_{\mathrm{CMT0,1,2,3}}$, production losses determined in the split-udder trial.

Economic losses at different farm-size levels were calculated based on the data obtained from the cross-sectional study and the data reported by Mekonnen et al. (1985) who reported that a healthy crossbred dairy cow in Ethiopia yielded a mean of 8.8 kg of milk per day and a total mean of 2,896 kg over a 328-day lactation period. The losses are expressed as losses per cow per lactation. In this study, economic loss estimates were only of financial losses derived from milk lost in the infected quarters. Although the health status of an individual quarter could not be taken as a constant throughout an entire lactation, it was assumed that the overall health status of the study population was more or less constant, i.e., that for every case cured there was a new case in another quarter. This assumption seemed reasonable as the study population included cows with various parities and lactation stages. Milk prices used in the calculations were obtained from the office of the Ada’a-Liben Dairy Cooperative which processes raw milk. All small- and medium-scale farmers supply majority of their milk, at the rate of 5 Eth Birr/kg, to this cooperative of which they are members themselves. However, a few farmers retail a small portion of their milk for higher prices (6–7 Eth Birr/kg) while the government-owned research stations sell milk at relatively lower price (~4 Eth Birr/kg) for their own community.

Data analysis

All the data were entered and stored in Microsoft Excel. Data from the split-udder investigation were analyzed using SPSS 11.5 version (SPSS 2002). Univariate analysis of variance was used to compare different means. Independent variables were CMT scores and udder quarter. Proportions of SCM at the cow and quarter level were compared using the chi-square tests.

Results

Prevalence

The overall prevalence of SCM at the cow level and quarter level was 69.1% and 42.6%, respectively, and there was no significant difference ($P>0.05$) between different farm sizes. Of the overall prevalence, the

Table 1 The overall prevalence of subclinical mastitis due to all causes and that caused by *S. aureus* at farm level

Level of estimation	Cow			Quarter		
	n^a	Number positive	Prevalence (%)	n^b	Number positive	Prevalence (%)
Overall	217	(150) 36[c]	(69.1) 16.7	850	(362) 61	(42.6) 7.2
Farm size						
Small	129	(85) 10	(65.9) 7.8[d]	508	(220) 18	(43.3) 3.5[d]
Medium	55	(44) 11	(80.0) 20.0[e]	211	(98) 18	(46.5) 8.5[de]
Large	33	(21) 15	(63.6) 45.5[e]	131	(44) 25	(33.6) 19.1[f]

[a] *n* sample size at each level

[b] *n* total quarters examined

[c] Numbers in parentheses are values for subclinical mastitis due to all causes including *S. aureus*, and figures within the same columns are significantly different ($P>0.05$)

[d,e,f] Values within the same columns with different superscripts are significantly different ($P<0.05$)

contribution of *S. aureus* to SCM was 16.6% and 7.2% at the cow and quarter level, respectively. Both at cow ($P<0.05$) and quarter ($P<0.01$) levels, there was a significant difference in the prevalence of *S. aureus*-caused SCM between farm-size levels (Table 1). The average CMT score of *S. aureus*-caused SCM for both small- and medium-size herd was 2.1 and that for large herd was 1.8.

Milk production loss

In the split-udder trial, the average milk production per healthy quarter (*CMT*=0 and *S. aureus* culture negative) was 1.6 kg (95% CI, 1.66–1.55 kg) per milking. The difference in the average milk yield between uninfected quarters (*CMT*=0), and infected ones with CMT scores of 1+, 2+, and 3+ were 0.40 (25%), 0.54 (34%), and 0.78 kg (48%), respectively. There was a significant ($P<0.001$) difference between the amounts of quarter milk production for the different CMT scores (Fig. 1). Also, the mean milk production for healthy quarters was significantly higher compared with quarters with different CMT scores while quarters with CMT score 3+ had significantly lower yield than those with CMT 1+ and 2+. In this study population, an overall milk production loss per quarter due to *S. aureus*-caused SCM was estimated at 4.4% of possible production, and loss in quarters with SCM was 34.4%. The CMT scores from the prevalence study and the associated milk yield losses based on the split-udder investigation are shown in Table 2.

Economic losses per cow per lactation estimated for this study population for different milk prices are shown in Fig. 2. The estimated total loss caused by SCM due to *S. aureus* was 984.64 Eth Birr (US $78.65) per cow per lactation, and losses in large farms (1,882.40 Eth Birr or US$150.35) were over 3.5 times the loss in small-size farms.

Discussion

In this study, the prevalence of SCM for all causative pathogens was somewhat similar to that reported for the region in the previous studies (Workineh et al. 2002; Kerro and Tareke 2003; Mungube et al. 2004).

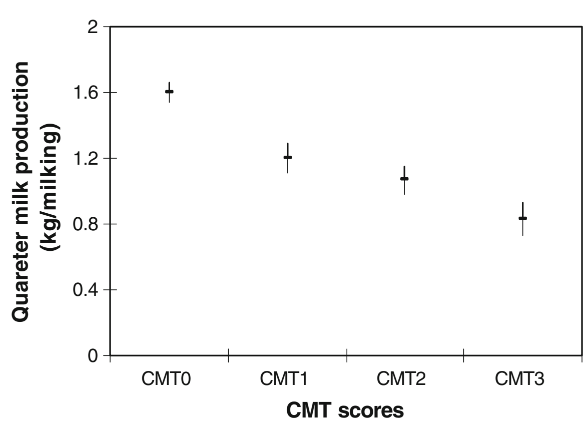

Fig. 1 Least-square means±SEM for quarter milk production of 12 crossbred dairy cows per different CMT scores

Table 2 Quarter CMT scores and associated milk production losses due to *S. aureus* caused at farm level

Level of estimation	CMT 0	CMT 1	CMT 2	CMT 3	Total loss (%)	Loss in quarters with CMT (%)
Loss (%)	0	25	33	48		
All cows	442	12	37	12	6.8	34.5
Farm size						
Small	264	2	12	4	3.7	35.6
Medium	101	3	10	5	8.8	35.9
Large	77	7	15	3	13.0	32.7

Also, the present results showed that the share of *S. aureus* from the overall prevalence of SCM due to all pathogens was over one fourth. This is in agreement with previous studies in the region (Workineh et al. 2002; Kerro and Tareke 2003; Getahun 2006). Prevalence of SCM caused by *S. aureus* at the farm level was higher in large-scale farms than in small-scale farms. The reason for this could be poor milking management and indiscriminate use of intra-mammary antibiotics in farms with large herds. The primary reservoirs of *S. aureus* are infected quarters and exposure of uninfected quarters is limited to the milking procedure (Fox and Gay 1993). Also, its resistance to antibiotic treatment and tendency to recur chronically makes it the most frequently isolated pathogen (Janson 2006). During this study, it was observed that, invariably, hand-milking was practiced with little or no regard for transmission of pathogens from cow to cow. This is more damaging to large farms where a single milker has been usually assigned to more than ten cows per milking.

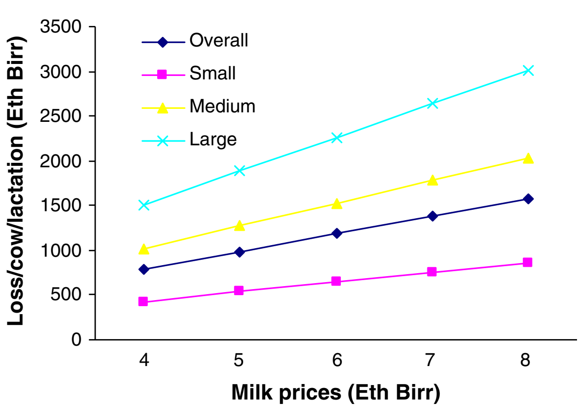

Fig. 2 Economic losses in Ethiopian Birr due to *S. aureus*-caused subclinical mastitis at different milk price level

The milk production of the cows used for split-udder trial was higher than 0.82 kg per quarter per milking reported by Mungube et al. (2005) in one of the government research farms. In the present study, the average quarter milk yield of 1.6 kg per milking in private farms would amount to 6.4 kg per cow per milking. This could be due to difference in management, environment, and pathogens involved. Under Debre Zeit conditions, the split-udder investigation revealed that, overall, a quarter with *S. aureus* SCM lost on average 34.5% of the potential milk production. Again, this result was twice that reported for the region by Mungube et al. (2005) and for Costa Rica (DeGraaf and Dwinger 1996). The explanation for this is that the previous studies on SCM determined milk loss irrespective of the causative pathogens and did not account for the role of specific pathogenic agent of SCM. These results also showed that milk production losses increased substantially as CMT scores increased. This is in agreement with previous studies reported for the Ethiopian highlands (Mungube et al. 2005) and European (Miller et al. 2004) and American dairy cows (Dobbins 1977; DeGraves and Fetrow 1993; DeGraaf and Dwinger 1996). In the cows participating in this split-udder trial, there was a more pronounced difference in milk yield between CMT scores 0, 1+, 2+, and 3+ than that reported by previous studies (DeGraaf and Dwinger 1996; Mungube et al. 2005) leading to higher quarter milk reduction rates per CMT score. This could be due to difference in the causative pathogens and/or the bias that arose from scoring the CMT results since it is a semi-quantitative and subjective measurement.

Except for direct financial losses, the costs of other factors contributing to economic losses were not considered due to unavailability of reliable data. However, Schepers and Dijkhuizen (1991) reported that 70% of mastitis losses are attributable to reduced milk yield associated with SCM. The overall financial losses of 984.64 Eth Birr per cow per lactation in this study were higher than that reported by Mungube et al. (2005) for Ethiopian highland dairy cows. The reason for this is, as mentioned above, besides the increased milk price at the present day, the quarter milk reduction rates of CMT scores for SCM caused by non-specific pathogens (all mastitis agents) reported by these authors were lower compared with the present results for pathogen-specific SCM. Also, studies revealed that bacterial mastitis is a problem of high-producing cows (Grohn et al. 2004), and different mastitis pathogens have clearly shown difference in their pathogenesis, epidemiology, and clinical presentations (Sears and Wilson 2003). For example, recently, isolation of *S. aureus* from the milk of cows with clinical mastitis (Grohn et al. 2004) and clinically healthy cows (Reksen et al. 2007) has been related to more severe reduction in milk yield than that of other bacterial mastitis pathogens. The overall losses caused by SCM due to *S .auerus* in this study indicate a remarkable financial scope for measures to improve udder health in crossbred dairy cows. Also, the figures may well underestimate the potential benefits of mastitis control program as they do not include other direct and indirect causes like costs of treatment, culling, and replacement of a diseased cow. Although the prevalence of *S. aureus*-caused SCM was higher in large-scale as opposed to both small- and medium-scale farms, the severity of the condition was less. The reason for this could be that awareness of the higher-grade mastitis was higher in large-scale farms because nowadays they have started keeping milk yield records.

These results indicate that, in addition to reducing the prevalence, it is possible to reduce the total loss by simply reducing the severity of SCM in the farm. The presence of variation in milk yield loss among the causative pathogens for a given CMT score may also be possible. Thus, the use of somatic cell count for screening SCM could avoid the subjective bias related to CMT and improve the estimation of the extent of tissue damage and milk yield losses caused by different mastitis pathogens.

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