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Associations Between Somatic Cell Count and Intramammary Infection in Early Lactation of Dairy Cows

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Abstract: Association between values for the Somatic Cell Count (SCC) and intramammary infection (IMI) were studied in 80 dairy cows from dairy herd in Iran during the first 5-15 days post calving. Samples were cultured for bacterial presence and were tested for SCC. Intramammary infection was defined as the presence of one or two bacterial species in milk samples taken within 5-15 day postcalving. Prevalence of IMI was large; 65% of milk samples were infected. Approximately 30% of the cows classified as infected with Coagulase-negative *Staphylococci* (CNS) and *S. aureus* had the pathogen identified on 5-15 days post calving. *Streptococcus* agalactia accounted for 10% of the IMI. Remaining IMI were by other pathogeneses among which *Escherichia coli*, *Proteus* sp., *Klebsiella* sp. Arcanobacter pyogenes. All milk samples from dairy cows in early lactation had SCC ranged from 5.24×10^5 cells mL^{-1} in the first parity to 5.5×10^5 in the third parity with a mean value of 5.45×10^5. No significant differences were observed ($p>0.05$) in SCC values between parities. Also, no significant differences were found ($p>0.05$) between SCC values and infections. Thus, these testing strategies may not be ideal for making decisions about individual animals, such as identifying individual cow with *S. aureus* for segregated milking.

Key words: Intramammary infection, somatic cell counts, early lactation

INTRODUCTION

Mastitis is the most costly disease of dairy cattle due to economic loss from reduced milk production, treatment costs, increased labor, milk withheld following treatment, death and premature culling (Vliegher *et al*., 2001). Identifying and eliminating intramammary infection in early lactation may have significant economic benefits. The type of bacteria most frequently isolated in milk samples of cows, with mastitis in previous studies, such as *S. aureus*. Therefore, the frequency of IMI present at the time of calving, as well as knowledge of the specific pathogens implicated, would provide a way to monitor the effectiveness of existing udder health programs and assess the need for new control strategies.

Bacteriological culture is the standard method for identifying IMI. However, logistic and financial considerations involved in sampling all quarters at the time of calving have precluded widespread adoption of this strategy in dairy industry. If an effective means to identify fresh cows at a high risk for IMI were available and validated it might increase the adoption of milk culture. The SCC has been widely implemented as a screening test to identify IMI in lactating cows. A threshold of 200,000 cells mL^{-1} has been shown to have a high sensitivity and specificity for identifying infections (Dohoo and Leslie, 1991; Moroni *et al*., 2006). Therefore, the potential exists to use SCC in early postpartum period as a screening method to select quarters for bacteriological culture (Sargeant *et al*., 2001).

The goals of the present study were, to assess the prevalence of IMI in dairy herds during early lactation, to identify the pathogens causing IMI and to study relationship among udder infection and SCC.

MATERIALS AND METHODS

Animals: Animals were selected from a Holstein dairy farm included 680 milking cows located in Tabriz in East Azerbaijan province of Iran. In herd, cows were housed in free stall barns. Cows were in 1st to 8 lactation and were milked three times daily by machine milking. Cows were fed ad libitum by a total mixed ratio that had been formulated to meet the nutritional

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requirements of a 650 kg cow, yielding 20-45 kg of milk/day with about 3.5% milk fat and 3.4% protein. All cows were subjected to post-milking teat disinfection, those were dried off approximately 2 months before expected calving and all quarters of cows were infused with a dry cow antibiotic preparation following the last milking of lactation.

Milk sampling: Foremilk samples were obtained (August 2007 up to February 2008) from quarters of 80 cows. Any cows had not evidence of clinical mastitis at time of sampling. Teat ends were cleaned with ethyl alcohol 70% before sampling. First streams of foremilk were discarded and then 10 mL of milk was collected aseptically from each teat into sterile vials. The milk samples were stored at 4°C in a refrigerator until analysis. Milk samples intended for the SCC determination were preserved by potassium dichromate (0.2% w v^{-1}) (Seifu *et al*., 2007).

Determination of SCC: Composite milk samples were collected aseptically on 5-15 day post calving and were analyzed with the Fossomatic 5000 (Foss Electric, Hillerod, Denmark) (Vliegher *et al*., 2004).

Bacteriological procedures: Bacteriological culturing of milk samples was performed according to standards of the National Mastitis Council (NMC, 1999). Ten micro liters each milk sample was spread on blood agar plate (5% defibrinated sheep blood). Plates were incubated aerobically at 37°C and examined after 24 h. Colonies were provisionally identified on the basis of Gram stain, morphology and hemolysis patterns were recorded. Gram-negative isolates were identified by using colony morphology, gram-staining characteristics, oxidase and biochemical reaction on MacConkeys agar.

Outcome evaluation: A milk sample positive for IMI was defined as one with one or two pathogens isolated at either 5-15 days post calving. Bacteriological causes of IMI were categorized as major pathogens [*E. coli*, *Kelebsilla* sp. *Staphylococcus aureus*, *Streptcoccus agalactiae*) or minor pathogens (coagulase-negative *Staphylococci*, *Cornyobacterium bovis*] (Harmon, 1994). If both major and minor pathogens were isolated from one or both quarter milk samples, the cow quarter was classified as infected with major pathogen.

Statistical analysis: The data for SCC was transformed to logarithmic value (log 10) prior to statistical analysis. Univariate Analysis of Variance was used to analyse effect of infection and parity on SCC. Differences were considered to be statistically significant at ($p<0.05$). Statically analyses were performed with SPSS.11.5 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Milk samples were collected from August 2007 up to February 2008. Among the 80 milk samples that were evaluated in this study 65% showed the presence of IMI. Within these positive samples coagulase-negative *Staphylococci* (CNS) was present in 22.5% of the cases. Individual species of CNS were not identified. Streptococcus dysglactiae was not identified in any sample. Approximately, 30% of the cows classified as infected with coagulase-negative *Staphylococci* and *S. aureus* on 5-15 days post calving. *Streptococcus* agalactia accounted for 10% of the IMI. Remaining IMI were by other pathogeneses among which *Escherichia coli*, *Proteus* sp., *Klebsiella* sp. *Arcanobacter pyogenes* (Table 1). All milk samples from dairy cows in early lactation had SCC ranged from 5.24×10^{5} cells mL^{-1} in the first parity to 5.5×10^{5} in the third parity with a mean value of 5.45×10^{5}. All milk samples had SCC greater than the threshold value of 500,000 cells mL^{-1} for subclinical mastitis. Therfore, 100% of cows had subclinical mastitis. Effect of parity on number of SCC by one –way ANOVA were not significant ($p>0.05$) (Table 2). Effect reciprocal parity and infection on SCC were not significant ($p>0.05$) (Table 3).

Table 1: Distribution of pathogens present among the infected samples (n = 80)

Species	Frequency	(%)
No growth	28	35.0
Escherichia coli	6	7.5
Staphylococcus aureus	6	7.5
Streptococcus aglactiae	8	10.0
Proteus sp.	4	5.0
Coagulase- negative Staphylococci	18	22.5
Klebsiella sp.	4	5.0
Arcanobacter pyogenes	6	7.5

Table 2: Effect of parity on SCC

	Mean square	
Parameter	df	SCC
Between parity	2	661^{ns}
F	77	426^{ns}

Ns = Not significant ($p>0.05$)

Table 3: Effect of reciprocal parity and udder infection on SCC

Parameters	df	MS	F	p-value
Parity	2	0.446	1.947	0.150^{ns}
Infection	2	0.530	1.090	0.342^{ns}
Parity × Infection	4	0.473	0.970	0.428^{ns}
Error	71	0.486		

ns = Not significant ($p>0.05$)

DISCUSSION

There is an increasing focus on milk quality in the dairy industry. Producing high quality milk will require effective udder health programs at the herd level. Management practices at the time of dry-off and during the dry period are essential to this effort and the fresh cow period is an ideal time to evaluate these efforts. In the present study, intramammary infection.

Stage of lactation had not clear effect on IMI. Probability for IMI tended ($p = 0.05$) to increase with increasing parity number up to >5. But in our study no correlation between Infection and parity were observed.

The most prevalent group of mastitis pathogens associated with IMI in dairy cows at parturition are the Coagulase-negative *Staphylococci* (22.5% of positive samples). Previous study reported the prevalence of IMI due to CNS in heifers' most common pathogens (Oliver and Mitchell, 1983). Bassel *et al.* (2000) reported an increasing prevalence of *S. aureus* IMI at calving with 3% of composite samples from first-calf heifers were positive composite with 8% in cows in third parity or higher. In our stuy the prevalence of *S. aureus* (7.5%) was higher than in comparision to previously reported at the time of calving of 5.3% (Sargeant *et al.*, 2001). The prevalence streptococcal was higher than in comparision to estimates at the time of calving of 3.2 (Todhunter *et al.*, 1995) and 6.3% (Smith *et al.*, 1985), but the prevalence of coliform bacteria (7.5%) was lower than previously reported (Smith *et al.*, 1985). In the SCC analyse, the sensitivity and specificity of selecting quarters for bacteriological culturing were only moderate for all IMI and for infection with minor pathogen (Sargent *et al.*, 2001). However, our result that were not considerably for distinguishing infections with minor or major pathogens. Although, the literature reports that subclinical infection with *S. aureus* is associated with elevated SCC (Wilson *et al.*, 1997), numbers were insufficient in the present study to provide meaningful estimates of sensitivity and specificity of using SCC thresholds to sample cows specifically for pathogen. The lack of perfect sensitivity in this screening tests means that some individual quarters that are infected will not be identified (Sargeant *et al.*, 2001). Thus, these testing strategies may not be ideal for making decisions about individual animals, such as identifying individual cow with *S. aureus* for segregated milking. Further research with large sample sizes is also needed to determine the sensitivity and specificity of these tests for identifying specific pathogens. In summary, SCC in early lactation con not be used to select milk samples for bacteriological culture to verify infection with major or minor pathogens. Based on our results, Milk samples taken 5-15 days post calving should be cultured to determine whether an IMI is present and to identify the specific pathogen type. Information on IMI status of the herd at calving will aid producers in monitoring existing udder health progras and assessing the impact of new control practices.

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