Analysis of Milk Somatic Cell Counts (SCC) of Dairy Heifers in Early Lactation from Cherry Dairy Farm

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Background

Practical methods are desired to monitor mastitis in early lactation. Because of the direct relationship between inflammatory cells and intramammary infection, milk somatic cell counts (SCC) have been the most widely used measurement to monitor udder health. It is generally recognized that milk SCC are elevated at and immediately after calving in both uninfected and infected quarters. Milk SCC decrease with time after parturition, with a more rapid decline observed in uninfected quarters (Brooks et al., 1982; Barkema, 1998). Numerous studies have attempted to determine how long SCC remain elevated in early lactation, with results ranging from 5 to 35 days (Reichmuth, 1975; Sheldrake et al., 1983). The majority of these studies examined multiparous cows, although Dohoo (1993) found generally similar patterns in heifers. Many Dairy Herd Improvement programs begin monitoring SCC early postpartum as indicators of mastitis. Thus, the variation of SCC seen following parturition needs to be considered for proper interpretation of SCC used in surveillance programs to evaluate early lactation intramammary infections in heifers. The purpose of this study was to evaluate milk cultural results and somatic cell counts (SCC) of dairy heifers in early lactation.

Materials and Methods

Thirty-four dairy heifers from Cherry Dairy Farm at the Center for Environmental Farming Systems were observed in early lactation during September-November 2005. Quarter milk samples were collected up to 4 times during days 0-2, 3-5, 6-13 and 21 days after calving. Samples were classified into 4 defined intervals of days into milking (DIM) time periods: DIM 0-2, DIM 3-5, DIM 6-7 and DIM 21-24. Milk samples were analyzed for SCC using a DeLaval Cell Counter (DCC, DeLaval Inc., Kansas City, MO) and milk bacteriological cultures were performed on aseptically collected milk samples using standard methods to determine the presence of intramammary infection. Both SCC and bacteriological determination were performed using the same milk samples.

Quarter infection status was defined as a binary outcome, with each quarter classified as positive or negative for intramammary infection. Quarter infection status was defined based upon serial samples because previous work (Timms and Schultz, 1987; Schepers et al., 1997) reported that defining infection status on consecutive samples (as compared to the basis of a single sample) improved accuracy of diagnosis of intramammary infections (IMI). A quarter was considered infected when the same pathogen was isolated at any level in repeated samples from the same quarter, or when >1000 colony-forming units/ml of a bacterial species was cultured in any one sample. If a sample was contaminated, results from the remaining samples were used to define infection status. Samples without both SCC measurement and bacteriological examination were excluded from the dataset.

Analysis

Five of the heifers were treated for clinical mastitis and were removed from the study. Data from 6 heifers were excluded from the final dataset due to lack of milk samples or because collection times were not consistent with the study. Samples reported as “flow error” indicated that the milk was unable to flow adequately for a SCC measurement. Samples for DCC analysis reported as “flow error” were entered as 5,000,000 cells/ml, because this finding was considered to likely represent a quarter with a significantly elevated SCC.

Data were transformed using the natural logarithmic of SCC (lnSCC) to approximate a normal distribution. Features of SCC distributions were calculated according to quarter infection status for each of the four time periods (DIM 0-2, 3-5, 6-7, 21-24). Prevalence of infection by specific pathogen was calculated along with the number of infected quarters per heifer. Percentage of milk samples with SCC values below three thresholds were also calculated according to infection status at each of the 4 DIM time periods. SCC thresholds used were 200,000, 500,000 and 750,000 cells/ml.

Associations between SCC and IMI over the first 24 days post-calving were tested for statistical significance using a mixed model ANOVA (SAS, Inc., Cary, NC). Quarter within heifer was the unit of analysis. Factors considered in the model included quarter infection status, DIM and the interaction between infection status and DIM. Statistical significance was assessed at $P < 0.05$. In this preliminary model we did not account for the possible heifer effect on SCC; in other words, quarters were treated as independent observations. A hierarchical model, taking into consideration the importance of the heifer in explaining the variation in quarter SCC, may be considered in future modeling.

To evaluate the validity of SCC for monitoring infection in early lactation, sensitivity and specificity were calculated at selected thresholds. Sensitivity was defined as the proportion of infected quarters with SCC above the selected threshold, and specificity was the proportion of non-infected quarters with SCC below selected thresholds. An optimal SCC threshold was determined as a function of test sensitivity/specificity. Threshold values selected were 200,000, 500,000, 750,000 and 1,000,000 cells/ml.

Results and Discussion

Data consisted of a total of 291 quarter milk samples from 87 quarters of 23 dairy heifers. Bacteriologically negative quarters accounted for 55.2% (n=48) of the quarters. The most frequently isolated bacteria (both pure and mixed) were *Staphylococcus aureus* (40.5% of positive samples), environmental streptococci (29.3% of positive samples) and coagulase-negative staphylococci (28.4%). At the heifer level, 26.1, 13, 34.8, 8.7, and 17.4% of heifers had 0, 1, 2, 3 and 4 quarters infected, respectively.

The overall geometric mean SCC of all samples collected was 330,000 cells/ml. The geometric mean SCC of infected and non-infected quarters were 639,000 and 186,000 cells/ml, respectively. Features of SCC distributions according to DIM and infection status are shown in Table 1.

Table 1. SCC (x1000 cells/ml) by quarter infection status over the first 24 days post-calving from 87 quarters of 23 dairy heifers

	DIM 0-2		DIM 3-5		DIM 6-7		DIM 21-24	
	No growth	Infected	No growth	Infected	No growth	Infected	No growth	Infected
Mean SCC[1]	671	1,939	159	812	92	347	47	106
Minimum SCC	40	67	21	57	18	22	19	9
Maximum SCC	5,000	5,431	4,873	4,906	2,779	5,020	165	941
25% percentile	370	1,216	71	227	40	184	30	39
75% percentile	1,548	4,336	344	2,969	167	809	63	365

[1]Geometric mean SCC

Results from the statistical model indicate that infection status was a significant (P< 0.01) source of variation in lnSCC. DIM also explained a significant (P< 0.01) effect of the variation in lnSCC. More importantly, the interaction of infection status by DIM was statistically significant (P< 0.049) indicating that infection status and time have an effect on lnSCC. We speculate that the effect of the heifer in the model would not modify the results significantly, and this has been shown in at least one other study. Djabri et al (2002) reported that taking the cow into account had a very limited impact on interpreting quarter SCC, revealing that the variation was mainly due to processes involving the quarter.

Mean lnSCC was significantly higher from milk of infected quarters compared to culture negative quarters in all time periods (Table 2). In both infected and non-infected quarters, there was a linear decrease in lnSCC over the first 24 days post-calving. Mean lnSCC decreased from 6.51 (671,000 cells/ml) at 0-2 DIM to 3.86 (47,000 cells/ml) at 21-24 DIM in non-infected quarters. In infected quarters, mean lnSCC was 7.58 (1,958,000 cells/ml) at 0-2 DIM and 4.66 (105,000 cells/ml) at 21-24 DIM. By 7 days into milking, SCC of non-infected quarters decreased by 30.4% compared to 22.8% observed in infected quarters. This is in accordance with previous studies reporting a more rapid decrease in SCC of non-infected quarters vs. infected quarters. The difference between infected and non-infected quarters was most profound at DIM 3-5.

Table 2. Descriptive statistics for lnSCC (x1000 cells/ml) by infection status over the first 24 days post-calving of milk samples from 87 quarters of 23 dairy heifers

	Stage of Lactation											
	DIM 0-2			DIM 3-5			DIM 6-7			DIM 21-24		
	N	X	SD	N	X	SD	N	X	SD	N	X	SD
Noninfected	48	6.51	1.28	48	5.06	1.25	48	4.53	1.13	14	3.86	0.60
Infected	39	7.58	1.13	39	6.70	1.44	39	5.85	1.24	16	4.66	1.34

The percentage of non-infected quarters and infected quarters with SCC below selected thresholds are displayed in Figure 1. Consistent with the ANOVA model analysis, the results show that the percentage of quarters with elevated SCC generally decline over time in

both infected and non-infected quarters. The percentage of culture-negative quarters with SCC less than 200,000 cells/ml increased dramatically from 18.8% at DIM 0-2 to 81.2% at DIM 6-7. Although the number of infected quarters with significantly elevated SCC (>1000 cells/ml) decreased over time, the percentage of infected quarters less than 200,000 cells/ml increased. This was not expected since most studies suggest that quarters with SCC less than 200,000 are not likely to be infected. Because the effect of minor vs. major pathogens on SCC was not studied, perhaps those quarters infected with minor pathogens accounted for the low SCC.

Figure 1. Prevalence of quarter milk samples with SCC (x1000 cells/ml) < 200, 201-500, 501 to 1000 and > 1000 for infected (solid bar) and non-infected (lined bar) by days in milking

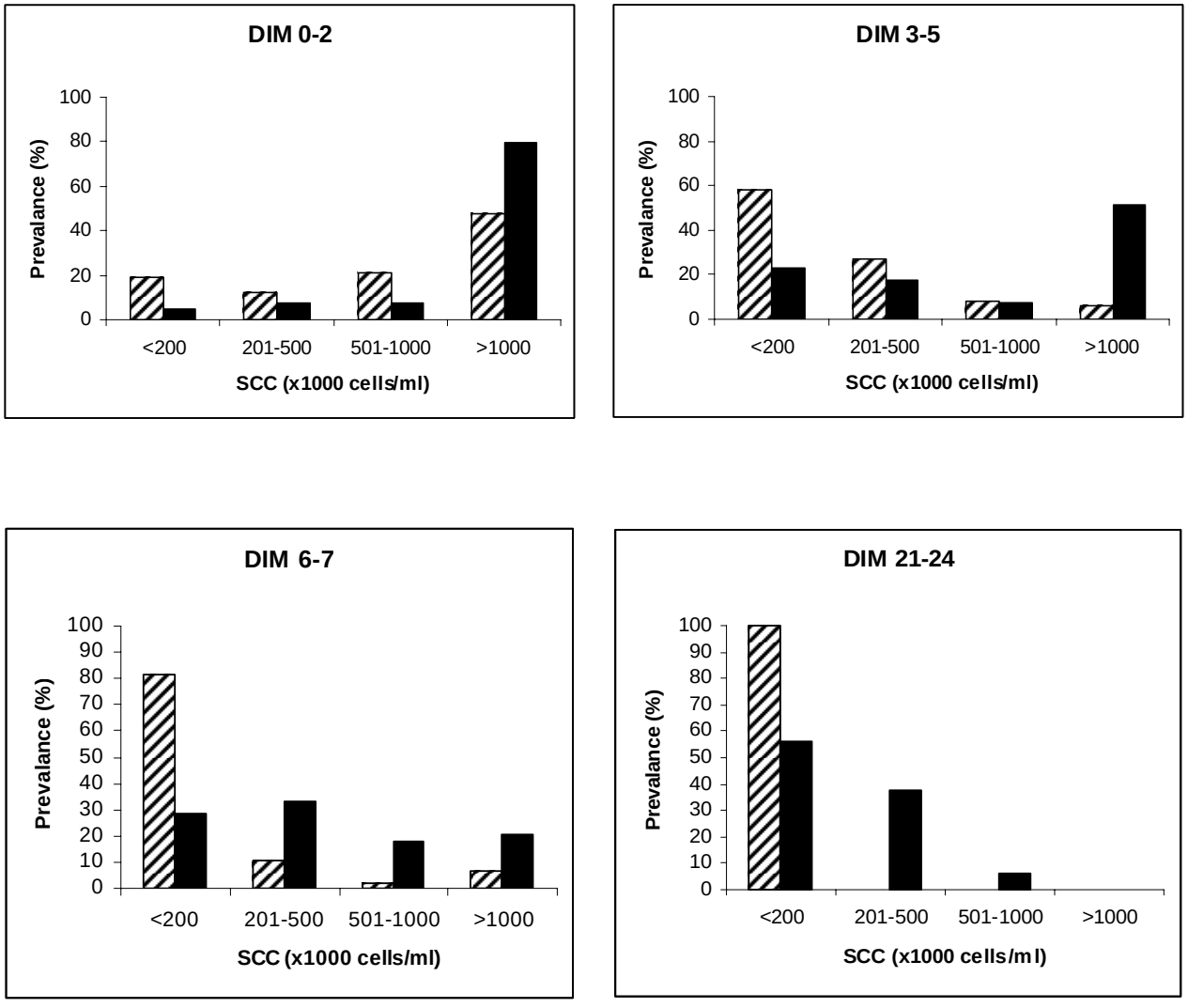

Table 3 shows that over time, the sensitivity of SCC for identifying infected quarters decreased and the specificity increased. Also, as SCC threshold increases, the sensitivity decreases and specificity increases. When sensitivity and specificity are considered equally important, the most direct approach in selecting optimal thresholds to define presence of IMI is to

select thresholds resulting in the lowest total number of diagnostic errors, defined as false positive diagnoses plus false negative diagnoses (Smith, 2006). The results indicate that the maximum sensitivity and specificity was seen at a threshold of 200,000 cells/ml within 7 days post-calving. At this time and threshold, the sensitivity and specificity were 71.8% and 83.3%. However, because selected thresholds depend on the economic consequences of false negative results compared to false positive results, the above value may not as relevant in practice.

Table 3. Sensitivity, specificity, and 95% confidence interval of SCC threshold values (x1000 cells/ml) for identifying infected quarters in the first 7 days of lactation

	DIM											
	0-2				3-5				6-7			
	>200	>500	>750	>1000	>200	>500	>750	>1000	>200	>500	>750	>1000
Sensitivity (95% CI)	94.9 (82.6-99.2)	87.2 (72.6-99.2)	82.1 (66.5-92.4)	79.5 (63.5-90.7)	76.9 (60.7-88.8)	56.4 (39.6-72.2)	53.8 (37.2-69.9)	51.3 (34.8-67.6)	71.8 (55.1-85.0)	38.5 (23.4-55.4)	28.2 (15.0-44.9)	20.5 (20.5-93.7)
Specificity (95% CI)	18.8 (9.0-32.6)	33.3 (20.4-48.4)	50.0 (35.2-64.8)	54.2 (39.2-68.6)	58.3 (43.2-72.4)	85.4 (72.2-93.9)	91.7 (80-97.6)	93.7 (82.8-98.6)	83.3 (69.8-92.5)	91.7 (80-97.6)	91.7 (80-97.6)	93.7 (82.8-98.6)

Results from DIM 21-24 may not be representative of the population since sample size was limited (infected n=16, non-infected n=14), and the definition of an infected quarter becomes complicated at this time, leading to potential misclassification of infection status. New infections may have emerged since the first week post calving and transient infections may have cleared. To test if the criteria used in the study to define an IMI may have skewed the results, regardless of the small sample size, SCC from DIM 21-24 were evaluated according to the same test day culture results, thus being independent of the previous culture samples. This revealed 22 quarter samples with no growth on culture and 8 samples showing some form of growth. All 22 negative samples had a SCC below 200,000 cells/ml. Six of the eight positive samples had SCC between 200-500,000 cells/ml, verses 6 of 16 when IMI was defined upon prior culture results.

Conclusion

Although the data used in the study examined one herd with a limited number of heifers, the results are in accordance with other studies which report a linear decrease in SCC following freshening. These results may be used to guide interpretation of milk SCC in early lactation in dairy heifers. However, the data also could be interpreted as presenting evidence that an exact SCC threshold which always permits separation of infected from non-infected cows does not exist. It is our general opinion that post-calving quarter milk SCC are useful surveillance tools for monitoring heifer mastitis, although the values require appropriate interpretation due the normal physiological variation seen after calving.

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