RELATIONSHIPS BETWEEN NORMAL LEVELS OF SOMATIC CELLS AND THE DURATION OF MASTITIS INFECTIONS

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INTRODUCTION

As an alternative to direct selection for mastitis resistance, many countries record and genetically evaluate somatic cell concentrations (**SCC**). This approach is effective because SCC increases in the udder of infected cattle as neutrophils are recruited to fight the infection. The critical role of these cells has caused some concern that selection of bulls with low estimated breeding values (**EBV**) for SCC could jeopardize the ability of cattle to resist or eliminate infections (Kehrli and Shuster 1994). However, Nash *et al.* (2001) examined this relationship and reported that daughters of sires with low EBV for SCC tended to be relatively resistant to infections. Nevertheless, Detilleux and Leroy (2000) suggested that evaluations for SCC could be improved by considering the fact that data for SCC actually represent a mixture of records for two distinct traits, the "natural" SCC (**NSCC**) of healthy cows and the generally increased SCC of infected cows. Differences observed in sire EBV for SCC could be largely based on differences in the proportions of daughters expressing the infected SCC trait rather than mean differences among sires in either trait. Individually, these two traits could have different relationships with mastitis. Low NSCC in the uninfected state could be associated with increased incidence of mastitis, but this relationship would not be detected by conventional analyses if disproportionately high numbers of cattle with low NSCC are infected and thus express more often the infected SCC trait. In fact, evidence suggests that this is not the case, as a recent study (Rupp and Boichard 2000) showed that cattle with phenotypically low NSCC tend to have decreased risk of mastitis. One hypothesis to explain this interesting result is that the NSCC may be a more important factor in the elimination of mastitis after the entry of bacteria into the udder than in the initial resistance to infection. Physiological factors other than NSCC may play a greater role in preventing initial infection. In a mastitis challenge study, Schukken *et al.* (1994) found that the pre-challenge mean SCC of cattle that quickly eliminated the introduced bacteria was greater than the pre-challenge SCC of cows that contracted mastitis. However, their study was small and this difference no level of significance was reported for this observed difference. The objective of this study was to to determine if a relationship exists between NSCC before infection and duration of mastitis after infection, based on a large set of SCC data from the milk recording program in Italy.

MATERIALS AND METHODS

Data. Data were test-day (**TD**) records of somatic cell scores (**SCS**) from nine provinces in Italy (Samoré et al. 2001). This information was edited to include only TD from the first the first three lactations and <600 days in milk, resulting in 12,584,941 records from 740,159 cows. No data were available for mastitis, so mastitis was defined based on SCS. A threshold

of 5 (=400,000 cells/ml) was taken as an indicator of mastitis, according to the approach of Li *et al.* (2001). Records of SCS $\geq$ 5 were assumed to indicate infection and SCS < 5 were assumed to indicate freedom of infection. These data were used to form two files. File 1 contained data for NSCC. This file contained all first-parity TD-SCS records for cows prior to their first TD with an SCS $\geq$ 5 (including many cows that never reached an SCS $\geq$ 5). Only records from the first 305 days of lactation were saved. These data were 3,007,742 TD records from 500,302 cows. File 2 included observations for "duration" of mastitis, which was defined as the number of TD between an initial TD-SCS $\geq$ 5 and the first successive TD-SCS < 5. When cows completed a given lactation without dropping below the threshold of SCS = 5, the record was considered censored at the last TD. These data initially included 801,809 records from 528,608 cows but a further step of editing saved a single record per cow. About 20% of the records were censored.

Analyses. The effects of NSCC (or NSCS) on duration of mastitis were evaluated with Weibull survival analyses using the Survival Kit software of Ducrocq and Sölkner (1998). Four definitions of NSCC were used, in separate analyses:

1) Mean of phenotypic NSCS: obtained by calculating for each cow the simple mean of TD-SCS based on the data in File 1. When merged with File 2, the resulting data included 337,221 records for duration of "mastitis" (i.e. number of days with SCS>5).
2) Mean of adjusted phenotypic NSCS: same as above, but TD-SCS were adjusted for effects of herd-TD, age, and days in milk (N = 337,221).
3) Sire EBV for NSCS, obtained by a genetic evaluation of all 3,007,742 TD records in File 1. A TD animal model with repeated records was used. Effects in the model included fixed herd-TD, age (4 classes) and days in milk (14 classes). Heritability for NSCS was assumed to be 0.05 and repeatability was assumed to be 0.15. When the file of sire EBV was merged with File 1, the resulting data contained 370,610 records.
4) Independent sire EBV for NSCS, obtained by a genetic evaluation of TD records in File 1, but using only those cows that were not in File 2 (to eliminate the possibility of residual covariances affecting the results). This genetic evaluation was based on 1,127,954 records. The same model as described in (3) was used. The file for the subsequent survival analysis contained 362,978 records.

In addition, the effect of standard sire EBV for SCS (from the Italian national SCS evaluation) on duration of mastitis was examined. The official EBV are published on a standardized 0 to 12 scale for which sires that transmit low SCS take higher values. For this analysis, scale was inverted to match the other definitions of NSCS. This file contained 333,535 records.

In addition to the previously described NSCS variables, the survival analysis included effects of herd-year-season (HYS) of infection (based on first TD >5 SCS), age (4 classes in each lactation), and stage of lactation at infection (12 stages). The default length of seasons was six months, but 6-mo HYS were split into 2-mo intervals if these new HYS contained $\geq$5 cows. The Weibull distribution was assumed to describe the baseline "risk" of "cure" (i.e. having SCS drop below 5). The effects of HYS were assumed to be distributed randomly according to a gamma distribution and were iterated out of the model. All other effects were fixed. Effects of NSCS were evaluated in two ways, first as a covariate and second as class effects. Because the ranges in variables differed, the classes were not defined in the same ways for all measures

of NSCS. For phenotypic mean, Class 1 corresponded to mean SCS <0.0 and the remaining classes represented increasing NSCS in half-point increments. For adjusted mean, Class 1 included mean values less than 2.0 SCS below the grand mean and other classes increased by 0.5. For sire EBV for NSCS, Class 1 included sires with EBV less than –0.50 and other classes increased by increments of 0.25. For official SCS, the standard EBV were used except that Class 1 included bulls with EBV 0 or 1 and class 11 included bulls with EBV 11 or 12. Likelihood ratios were used to test the significance of the NSCS effects.

RESULTS AND DISCUSSION

Table 1 has regression coefficients of the different measures of NSCS for the duration of mastitis. Regressions were significant ($P < 0.0001$) for all measures of NSCS. Coefficients were all negative, indicating that decreased NSCS was associated with shorter duration of mastitis (at least with shorter duration of SCS>5). The regression for phenotypic mean was very similar to that for adjusted phenotypic mean. Coefficients of regression of duration on phenotypic measures of NSCS were much larger than were regressions on sire EBV for NSCS. This result is not surprising considering that NSCS was assumed to have a low heritability and sire EBV for NSCS contributes $\leq$1/4 of the variance in a cow's genetic component for NSCS. The coefficient based on sire EBV from on all records in File 1 was greater than that based exclusively independent data from half sibs of infected cattle. Regression of official EBV for SCS on duration was also highly significant, but this was expected because sires with high EBV for SCS are likely to have many daughters whose SCS are high for long periods of time.

Table 1. Regression coefficients and risk ratios of different classes for effects of natural somatic cell concentration(NSCS) on duration of mastitis.

	Definition of NSCS				
	Phenotypic Mean	Adjusted phenotype	Sire EBV	Independent sire EBV	Official sire EBV for SCS
Regression	-0.139	-0.138	-0.0219	-.0169	-0.0571
NSCS Class					
1	1.29	1.18	1.13	1.04	1.23
2	1.18	1.17	1.06	1.08	1.13
3	1.18	1.16	1.03	1.02	1.16
4	1.12	1.13	1.00	1.00	1.09
5	1.06	1.06	0.94	0.95	1.02
6	1.00	1.00	0.92	0.90	1.00
7	0.92	0.94	0.88	0.92	0.91
8	0.85	0.86	0.84	…	0.86
9	0.77	0.79	…	…	0.80
10	0.68	0.72	…	…	0.75
11	0.68	0.62	…	…	0.75

Estimates of risk ratios associated with class definitions of NSCS are also presented in Table 1. For all definitions of NSCS, "risk" of dropping below the threshold of 5 (i.e. of fast elimination

of the infection) decreased as NSCS increased. These decreases in risk were continual for all definitions except for independent sire EBV. A very interesting result was that the "risk" continued to increase with decreased NSCS, even for the extremely low classes of NSCS. For example, for phenotypic mean NSCS, risk for class 1 (NSCC < 12,500 cell/ml) was 1.29 versus 1.18 for class 2 (NSCC < 25,000 cell/ml). Concerns have been expressed that cows with extremely low NSCC could lack the capacity to respond to infection, the cows in this study obviously had some capacity to increase their SCC (only cows with at least one TD-SCS $\geq$ 5 were included in this study.

Obviously, results of this study must be interpreted with some caution because definitions of mastitis status were made based on SCS, and SCS and presence of bacteria in the udder are not correlated one-to-one (Weller *et al.* 1992). An ideal study would have defined mastitis status based on periodic (weekly) milk culture and clinical incidence data, but such a study would be financially infeasible if done on a scale comparable to this study. Because infection data were not available, in theory, it is possible that cows with low NSCC initially responded to infections and increased SCS above 5, but then dropped and remained below the threshold without eliminating the infection. Another possibility is that cows with high NSCC responded to infections by increasing SCC and then maintained SCC at a high level even after the invading bacteria are eliminated. However, we are not aware of any biological evidence that demonstrate that either of these two phenomena occur.

CONCLUSIONS

Based on the assumption that infection status of cows can be reasonably accurately defined by SCC, low levels of circulating somatic cells in udders free of mastitis are associated with the increased ability of the immune system of cows to cure eliminate infections. If so, this result suggests that selection for low SCS should not impair immune function of cattle. Perhaps the ability to recruit neutrophils to the udder only at times of infection and the killing ability of these cells are more important traits for mastitis resistance than is their concentration during times of health.

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