Genetic Variability of Alternative Somatic Cell Count Traits and their Relationship with Clinical and Subclinical Mastitis

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Keywords: clinical mastitis, subclinical mastitis, somatic cell count, genetic parameters

Introduction

Mastitis, both clinical and subclinical, is a common and costly disease in dairy cattle, associated with reduced milk yield, discarded milk, reduction in milk price due to high somatic cell counts (SCC), veterinary and treatment costs, increased labor, and increased culling rate (Nielsen, 2009). It is generally accepted that undesirable genetic relationships exist between production and mastitis (e.g., Emanuelson *et al.,* 1988). The heritability of clinical mastitis has been shown to be low, 0.07 to 0.10 in different populations (Heringstad *et al.,* 2005; Zwald *et al.,* 2006; Pérez-Cabal *et al.,* 2009; Hinrichs *et al.,* 2011); therefore, lactation average log (SCC) has often been used as an indicator of clinical mastitis (e.g. Heringstad *et al.,* 2000).

Recently, other SCC-derived traits have been proposed as alternatives for lactation average, to improve breeding for udder health (de Haas *et al.,* 2008); SCC traits were defined on the basis of lactation stage, occurrence of excessive SCC, and SCC traits on the basis of patterns in peaks of SCC. In a previous study (Urioste *et al.,* 2010), we focused on the genetic variability of novel traits that could be derived from information present in the test-day (TD) SCC records, using a small research data with weekly observations, and explored the feasibility of applying our findings into monthly records of SCC. The traits of interest, according to that study, are further studied in this study in a large field data set.

Material & Methods

Data on clinical mastitis and TD-data with SCC records were extracted from the Swedish milk recording scheme, and were edited to include records from the first 3 lactations of Swedish Holstein cows having their first calving between 2002 and 2009. Only test-day records between 5 and 366 DIM were included. The clinical mastitis data were merged with the TD data. The diagnosis date was assigned to a given lactation, if it was between a preceding calving date (-10 days before calving) and the following calving date -10 days. Defined minimum and maximum ages for first, second, and third calving were 19 to 38, 31 to 52, and 42 to 66 mo, respectively. Cows belonging to a herd-year class with fewer than 5 observations, and from sires with fewer than 40, 30 or 20 daughters in the data (lactations 1, 2 or 3, respectively) were excluded from the analyses. Pedigree files were constructed, using pedigree information of the cows back to third generation. After editing, data contained 178 613, 116 079, and 64 474 lactation records from 778, 702 and 521 sires in the first 3 lactations, respectively.

Traits

For simplicity and coherence with earlier studies, clinical mastitis (**CM**) was defined as presence of a veterinary-treated clinical case from 10 d before calving to 10 d prior to the following calving; it was scored as present (1) in a given lactation if at least one

case of veterinary treatment was recorded; otherwise it was scored as absent (0). Subclinical mastitis (**SCM**) was defined as the number of periods (TD± 15d.) from DIM>45 with a SCC>150,000 cell/mL and without a treatment for clinical mastitis in that period. Test-day SCC (**SCC150D**) were averaged over the early lactation period (5-150 d) and used as a kind of reference trait, to maintain coherence with previous work (De Haas *et al.*, 2008; Urioste *et al.*, 2010) and assuming a high genetic correlation with TD SCC in the second part of lactation.

Various alternative SCC-traits were used in this study, as potential indicator traits for clinical and subclinical mastitis, capturing different aspects of mastitis. Definition details can be found elsewhere (Urioste *et al.*, 2010); a brief definition of the used traits follows:

a) Binary traits were defined as the presence of at least one TD between 41,000 and 80,000 (**TD41-80**), or at least one TD >500,000 (**TD>500**).
b) An infection peak was defined as a period of increased SCC (>150,000) between two low (≤ 150,000) TD observations. The number of peaks (**NPeak**) was considered as a trait.
c) We defined average days diseased per peak (**ADSick**), as total number of days diseased divided by total number of peaks, trying to distinguish between short and long durations, the latter often associated with contagious pathogens.
d) Standard deviation of log of SCC-TD during lactation (**SCCSD**), as proposed by Green et al. (2004) was also used in our study.

Statistical Analysis

The following linear animal model was used:

$$y_{ijkl} = hy_i + year_j + month_k + age_l + a_m + + e_{ijklm}$$

where y_{ijkl} denotes the response trait; hy_i is the random effect of *i*th herd by year of calving ~ND(0, Iσ^2_{hy}); $year_j$ is the fixed effect of *j*th year of calving; $month_k$ is the fixed effect of the month of calving; age_k is the fixed effect of *k*th age at calving, organized in 6 classes within lactation; a_m is the random effect of *m*th animal ~ND(0, Aσ^2_a) and e_{ijklm} is the random residual effect ~ND(0, Iσ^2_e).

A threshold liability approach (e.g. Gianola and Foulley, 1983) was used for traits expressed as a discrete (0/1) response. Continuous variables (**SCCSD, ADSick, SCC150D**) were log-transformed to improve parameter estimation.

Herd-year, additive genetic and residual (co)variances were drawn from the posterior distributions using a Bayesian approach and Gibbs sampling, as implemented for threshold and/or linear trait analyses in the program Thrgibbs1f90 (Misztal *et al.*, 2002). Based on visual inspection of trace plots in earlier runs (a binary and a continuous variable were tested with chains of 50,000, 100,000 and 250,000, thinning intervals of 25 and 50 samples, 25 or 50% of burn-in, and found to converge to the same values), a chain of 125,000 iterations was run for each trait, including a burn-in of 25,000 iterations, keeping every 25th sample for inference of posterior features (4,000 effective samples). Estimates of genetic correlations between traits and between lactations for the same trait were obtained from bivariate analyses; heritabilities (h^2) were averaged over the bivariate runs.

Results

Genetic parameters for mastitis traits (Table 1)

Heritability estimates for CM (0.07-0.08) were well in accordance with what is known from literature when using a threshold approach (e.g. Heringstad *et al.*, 2005; Zwald *et al.*, 2006; Pérez-Cabal *et al.*, 2009) and our previous study in a research herd (Urioste *et al.*, 2010). This low value indicates that the use of traits genetically correlated with CM would be beneficial for selection purposes.

Heritability for SCM (0.12 to 0.17), as defined in this paper, has not been reported before, and was twice the genetic variability found for CM. One explanation could be that

SCM is a continuous trait. Genetic variability seems to decrease in the 3rd parity. De Haas *et al.* (2008) found very low heritabilities (0.02-0.03) for SCM, defined as a 0/1 trait and analyzed with a linear model.

Posterior genetic correlations between CM and CSM were 0.744, 0.716 and 0.618 in the first 3 parities, respectively. This decline in genetic correlation with parity number agrees with the trend observed by Windig *et al.* (2010), who obtained estimates of 0.578, 0.554 and 0.259 in parities 1, 2 and 3, respectively, although the definition of SCM was different. Two conclusions can be drawn: that CM and SCM are distinct traits, and that selection against one of them will bring genetic improvements in the other.

Genetic correlations of CM and SCM with the alternative SCC-traits were positive and very high (0.67 to 0.82 for CM, and 0.94 to 0.99 for SCM. This was expected, because SCM is directly derived from TD, and CM is not. While SCCSD and TD>500 show similar correlations with CM as SCC150D, they are probably capturing more of the biological background: they are phenotypically more associated to CM (Urioste *et al.*, 2010) because they describe the effect of clinical infection on SCC. The impact of infection on maximum SCC or a TD with > 500,000 is likely to be greater than that on mean SCC because the mean is influenced by all SCC readings during lactation (Green *et al.*, 2004). Additionally, TD>500 could be associated with environmental pathogens, both for CM and SCM (De Haas *et al.*, 2008).

There seems to be a trend for lower correlations with CM with increasing parity, whereas correlations with SCM were more stable. As a general picture, our results are closely related to those obtained by Windig *et al.* (2010) in Dutch dairy herds, the only comparable study. Genetic correlation estimates between mastitis and somatic cell scores obtained by Carlén *et al.* (2004), also working with Swedish Holsteins, ranged between 0.66 and 0.77.

In Urioste *et al.* (2010), TD41-80 was a trait positively associated with clusters of healthy cows when measurements were monthly recorded (recall that a TD41-80 = 0 identifies a cow that never has got a TD with SCC between 41,000 and 80,000 SCC). A recent review (Schukken *et al.*, 2003) reported that uninfected quarters have a mean SCC of approximately 70,000 cells, with some variation around this mean, which calls for a closer look to a trait reflecting such features. The genetic nature of such a trait has not been described before, except for its genetic variability in our study mentioned above. Here, it was the only alternative trait that did not show very strong genetic correlations with CM and SCM; correlations were weak to moderate for CM (-0.22 to -0.50), and moderate to strong (-0.48 to -0.85) with SCM.

Genetic parameters for the alternative SCC-traits (Table 2)

Estimates for third parity are not shown but were similar to those from 2nd parity. Heritabilities were well in accordance with those estimated by us (Urioste *et al.*, 2010) using a monthly dataset of SCC records, with the exception of ADSick, where we had a lower estimate (0.05 vs. 0.14 in this study). The more "classic" SCC150D varied between 0.13 and 0.16. For the same trait, de Haas *et al.* (2008) reported values of 0.08 to 0.10. The estimates of Koeck *et al.* (2011), presented in a preliminary report on alternative traits, varied between 0.01 and 0.07.

There were two levels of heritability for the alternative traits: 0.12-0.17 for SCCSD, TD>500 and ADSick, and 0.06-0.10 for TD41-80 and NPeak (and ADSick in 3rd parity). The genetic variability in the first group is then at the same level as SCC150D, which was also anticipated from our previous study. Our findings are consistent with those of de Haas *et al.* (2008), who found heritabilities of traits describing the dynamics of SCC to be between 0.03 and 0.11, and 0.01 to 0.05 for patterns of peaks.

Genetic correlations among traits in each parity were very high (0.93- 0.99 in first parity, 0.92-0.98 in second parity, and 0.78-0.99 in third parity), and similar to the results of Windig *et al.* (2010) and Koeck *et al.* (2011), whose estimates often were above 0.95. The only exception was TD41-80,

which showed moderate to strong negative correlations with the rest of the traits. The high positive correlations suggest that any of the new traits with heritability similar to SCC150D could be used in its place, but they do not add very much information. This is probably a partial effect of autocorrelation; all traits are built from the same information. The only trait which seems to add new useful information is TD41-80, because it identifies the "healthy" and not the "sick" cow, and therefore could be useful for use in a selection index.

Genetic correlations across parities

Genetic correlations of the same trait across parities were positive and very high (0.83 to 0.99), suggesting that they could be considered as the same trait. The only exception is the genetic correlation for TD41-80 in parities 1 and 3, which was moderately positive (0.69). Carlén *et al.* (2004) obtained estimates of clinical mastitis across lactation above 0.7 and somatic cell scores above 0.8, whereas in Windig *et al.* (2010), correlations among alternative SCC traits in different lactations ranged between 0.54 and 0.99. These results suggest i) that the use of simpler repeatability model could be used for traits with several parities, and ii) that selection decisions can be taken already in the first parity.

Conclusions

This research, performed with a large field dataset, has shown that clinical and subclinical mastitis are distinct albeit correlated traits, and that alternative SCC traits show genetic variability and are closely associated to both CM and SCM, confirming their potential use as biologically valuable indicator traits. While most traits are positively associated to CM and SCM, TD41-80 is more related to healthier cows, thus showing negative genetic correlations with mastitis and SCC-traits. Estimated genetic parameters could be useful for testing alternative indices, as suggested by Windig *et al.* (2010), and should be the next step in research and development of genetic evaluations for more robust dairy cows. The results have particular value for less developed recording conditions, because not all countries have records on mastitis cases, but standard BLUP methodology based on SCC-traits can be used for selection and culling purposes.

Acknowledgments

This work was carried out as part of the RobustMilk Project, which is financially supported by the European Commission under the Seventh Research Framework Programme, Grant Agreement KBBE-211708. The content of this paper is the sole responsibility of the authors, and it does not necessarily represent the views of the Commission or its services. The authors are grateful to Swedish Dairy Association and farmers for providing the data. First author greatly acknowledges Dr. H. Naya, Institut Pasteur Montevideo, for facilitating the numerous and tedious runs done in Uruguay.

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Table 1. Posterior heritability estimates for clinical (CM) and subclinical (SCM) mastitis and posterior genetic corrlelation estimates with alternative SCC-traits.

	Parity 1		Parity 2		Parity 3	
	CM	SCM	CM	SCM	CM	SCM
Heritabilities†	0.076	0.166	0.082	0.160	0.066	0.125
SCC-trait‡						
SCC150D	0.817	0.952	0.814	0.962	0.762	0.943
SCCSD	0.816	0.972	0.787	0.969	0.743	0.941
TD>500	0.804	0.942	0.868	0.937	0.783	0.918
TD41-80	-0.225	-0.484	-0.497	-0.808	-0.261	-0.851
NPeak	0.792	0.963	0.740	0.936	0.667	0.838
ADSick	0.812	0.988	0.762	0.990	0.665	0.976

† Posterior means and standard deviations are average of 7 bivariate analyses.

‡ CM: clinical mastitis; SCM: subclinical mastitis; SCC150D: average somatic cell counts in early lactation (5-150d); SCCSD: standard deviation of SCC; TD4180: at least one TD between 41,000 and 80,000 cell/mL; TD>500: at least one TD > 500,000 cell/mL; NPeaks: number of peaks; ADSick: average days diseased.

Table 2. Posterior estimates of heritability and genetic correlations among SCC-traits (1st parity below, 2nd parity above diagonal).

		SCC150D	SCCSD	TD>500	TD4180	NPeak	ADSick
SCC-trait‡	$h^{2\dagger}$	*0.157*	*0.163*	*0.169*	*0.086*	*0.082*	*0.147*
SCC150D	*0.135*	x	.982	0.965	-0.811	0.932	0.972
SCCSD	*0.144*	0.969	x	0.994	-0.793	0.923	0.972
TD>500	*0.119*	0.939	0.943	x	-0.800	0.886	0.972
TD41-80	*0.052*	-0.374	-0.456	-0.489	x	-0.651	-0.791
NPeak	*0.099*	0.959	0.959	0.927	-0.430	x	0.952
ADSick	*0.136*	0.970	0.986	0.971	-0.503	0.975	x

† Posterior means and standard deviations are average of 7 bivariate analyses.

‡ Trait acronyms as in Table 1.