Impact of Early Lactation Somatic Cell Count in Heifers on Somatic Cell Counts Over the First Lactation

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ABSTRACT

The objective of this study was to estimate the impact of somatic cell count in early lactation (SCC_{el}) from Belgian dairy heifers on test-day somatic cell count (SCC) in first lactation. Geometric mean SCC_{el} [5 to 14 d in milk (DIM)] of the 14,766 available samples was 104,000 cells/mL, and decreased from 178,000 at 5 DIM to 74,000 cells/mL at 14 DIM. Proportion of SCC_{el} >200,000 cells/mL was 27.5. Heifers calving in the period April–June had highest SCC_{el}.

In total, 117,496 monthly SCC were measured. A multilevel regression analysis revealed that an increase of the natural log-transformed SCC_{el} (LnSCC_{el}) by one unit on average resulted in an increase of test-day natural log-transformed SCC (LnSCC) by 0.22 unit. The impact of LnSCC_{el} on LnSCC depended on when LnSCC_{el} was measured; an elevated LnSCC_{el} at 14 DIM was more consequential than an equally elevated LnSCC_{el} at 5 DIM. The probability of having a test-day SCC >200,000 cells/mL during the first lactation, also increased with an increasing LnSCC_{el}. The negative effect of an elevated LnSCC_{el} was still present, although to a lesser extent, in heifers with a second test-day SCC ≤50,000 cells/mL.

This study indicates that udder health problems in heifers in early lactation have a high prevalence and stresses that heifers should have a low SCC_{el}, because an elevated SCC_{el} will negatively influence test-day SCC during the whole first lactation.

(Key words: dairy heifer, early lactation, somatic cell count, udder health)

Abbreviation key: LnSCC = natural log-transformed SCC, LnSCC_{el} = natural log-transformed SCC_{el}, MY = milk yield at test-day (kg), OR = odds ratio, SCC_{el} = SCC in early lactation (between 5 and 14 DIM).

INTRODUCTION

A mastitis prevention program (Neave et al., 1969) has value in reducing the prevalence and incidence of subclinical mastitis. This type of program has primarily focused on lactating and dry cows, overlooking young, primigravid, and recently calved heifers. This should change, as many studies have reported a high prevalence of IMI in heifers around calving. Nearly all studies indicate that CNS are responsible for the majority of the IMI in nonlactating and freshly calved heifers. *Staphylococcus aureus* and environmental pathogens also play an important role (Pankey et al., 1991; Oliver et al., 1992; Roberson et al., 1994; Myllys, 1995; Oliver et al., 2003). Reported prevalence differs between studies, but one study reported that as many as 45% of all sampled quarters were infected with CNS (Oliver et al., 2003), whereas prevalence of quarters infected with *Staph. aureus* and environmental pathogens ranged from 0.6 to 4.7% (Oliver et al., 1992; Myllys, 1995) and from 4.6 to 8% (Myllys, 1995; Oliver et al., 2003), respectively.

Intramammary infection at calving results in an increased SCC, particularly if it is caused by a major pathogen (Barkema et al., 1999). The probability of clinical mastitis increases with an increasing SCC in early lactation (SCC_{el}) (Rupp and Boichard, 2000). An elevated SCC_{el} could result in a permanently elevated SCC and an increased risk of subclinical mastitis during the subsequent first lactation. A higher mean SCC in the first lactation is associated with a higher risk of clinical mastitis in the second lactation (Rupp et al., 2000), stressing the importance of having low SCC from the start until the end of the first lactation.

Coffey et al. (1986), using DHI test-day records from heifers from 30 herds, concluded that the initial rank of SCC classes in early lactation (<100,000, 100,000 to 400,000, and >400,000 cells/mL) was maintained throughout the remainder of the first and subsequent lactations. Probably because of a relatively small sample size, initial test-day SCC were categorized in 3 classes, and day of measurement of the first test-day SCC was not part of the evaluation. Because SCC rap-

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idly decreases in the first couple of weeks after calving (Dohoo, 1993; Laevens et al., 1997; Barkema et al., 1999), this may have resulted in a biased classification of heifers (Dohoo, 1993; Barkema et al., 1999). Additionally, Coffey et al. (1986) studied the effect of first test-day SCC class on the lactational-average SCC, ignoring variation in SCC during lactation. Using the information of all test-day measurements instead of a lactational average makes studying a changing effect of SCCel on SCC throughout lactation possible.

Since the publication of this study (Coffey et al., 1986), statistical software and computer power have progressed considerably, making it possible to account for clustering of heifers within herds (McDermott and Schukken, 1994). Ignoring clustering for continuous outcome variables can result in unbiased estimates of the regression coefficients, but standard errors and associated *P* values might be strongly affected. Ignoring clustering for discrete data will, in addition, lead to biased estimates, especially with limited samples sizes (Dohoo et al., 2003). Multilevel modeling takes into account clustering of animals within an environment and can be used to identify the level where the greatest variation resides, as interventions at that level would seem to have the greatest chance of success (McDermott and Schukken, 1994; Dohoo et al., 2001).

The objective of the present study was to estimate the impact of SCCel on test-day SCC in the ongoing first lactation using multilevel regression analysis.

MATERIALS AND METHODS

Initial Data Set and Data Handling

Four-weekly milk recordings from 2000 and 2001 of all lactating cows and heifers were used from the herds enrolled in the Belgian DHI program (Flemish Cattle Breeding Association, Oosterzele, Belgium). No data were recorded before 5 DIM. The database included: SCC, milk yield on test-day (**MY**) (kg of milk), breed, DIM, and date of measurement. The latter was categorized into 4 "calving seasons": January–March, April–June, July–September, and October–December.

Somatic cell count was measured in composite milk samples collected from 2 successive milkings and was analyzed using the Fossomatic 5000 (Foss Electric, Hillerød, Denmark).

All dairy heifers in which the first test-day SCC was measured between 5 and 14 DIM (SCC_{el}) in the year 2000, were selected (*n* = 14,766). Monthly-measured test-day SCC of the 14,766 heifers during the first lactation (until 365 DIM) were extracted from the database using Microsoft Access (Microsoft Corporation, Mountain View, CA).

Table 1. Structure of the data: Test-records in early lactation and during the course of the first lactation.

Dataset ¹	Level	n	Average number per unit at next-higher level	Range
Early	Herd	3287	...	...
	Heifer	14,766	4.5 ²	1 to 58 ⁴
	Test	14,766	1 ³	1 to 1 ⁵
Full	Herd	3264	...	...
	Heifer	14,234	4.4 ²	1 to 56 ⁴
	Test	117,496	8.2 ³	1 to 2 ⁶
Sub	Herd	2827	...	...
	Heifer	7807	2.8 ²	1 to 22 ⁴
	Test	65,458	8.4 ³	1 to 2 ⁶

¹Early = Test-day records in early lactation (between 5 and 14 DIM) from 14,766 dairy heifers; Full = test-day records between 15 and 365 DIM from 14,234 heifers (full dataset); Sub = test-day records between 15 and 365 DIM from 7807 heifers with a second test-day SCC ≤50,000 cells/mL milk (data subset).

²Average number of heifers per herd.

³Average number of tests per heifer.

⁴Range of heifers per herd.

⁵Range of tests per heifer.

⁶Range of tests per heifer within separate 30-d DIM intervals.

In total, 117,496 additional test-day SCC (full data set) from the first lactation (measured after 14 DIM) were available from 14,234 (96.4%) of the 14,766 heifers. These animals belonged to 3264 herds (average 4.4 heifers per herd) (Table 1).

A subset of data was created by selecting heifers with a second test-day SCC (measured after 14 and before 75 DIM) of ≤50,000 cells/mL. This resulted in data from 7807 heifers (55% of the initial 14,234 heifers) in 2827 dairy herds, with 65,458 measurements in total (on average 8.4 per heifer, not including SCC_{el}) (Table 1).

Statistical Analysis

To approximate the normal distribution, a natural logarithmic transformation of SCC (**LnSCC**) and SCC_{el} (**LnSCC_{el}**) was performed. For presentation purposes, LnSCC and LnSCC_{el} estimates were converted to SCC and SCC_{el} after analysis.

Firstly, LnSCC_{el} was analyzed by multilevel linear regression with herd random effects and fixed effects of DIM (10 levels, 5 to 14 DIM), calving season, and breed (4 levels both, as listed in Table 2), all entered as categorical variables.

Secondly, the effect of LnSCC_{el} on LnSCC was studied. For analysis of the repeated measures of LnSCC of each heifer, both the full data and the data subset were split into 12 subsets referred to hereafter as "30-day DIM intervals" (15 to 45, 46 to 75, 76 to 105, ...

DIM), reflecting the approximately 4-weekly milk recordings. No analysis was performed for the first 2 30-d DIM intervals from the subset because selection of animals was based on the measurements within those 2 intervals. To account for multiple testing of the same hypotheses in the 30-d DIM intervals, all *P* values were multiplied by the number of intervals analyzed (Bonferroni correction; Dohoo et al., 2003). This approach to repeated measures data does not lead to bias in the estimates, but implies some loss of power and renounces any information about the correlation structure over time for each individual. The approach was favored because none of the above issues was considered serious for the study's primary purpose of describing the effect of LnSCC_{cel} on LnSCC over time, and because potential misspecification of complex repeated measures models was considered a greater concern for the study's validity. Multilevel linear and logistic regression models, one model per 30-d DIM interval, were used to analyze LnSCC and the binary outcome variable SCC₂₀₀ (0: SCC ≤ 200,000 cells/mL milk; 1: >200,000 cells/mL), respectively. These models were analyzed by restricted maximum likelihood and penalized quasi-likelihood estimation algorithms, respectively, as implemented in the MLwiN software (Rasbash et al., 2000). The analyses used a second-order algorithm for the full data set and a first-order algorithm for the subset, and constrained in both analyses the variation at the lowest level to be binomial because no substantial extrabinomial variation was detected in the data. The models included random effects for herds and heifers, the latter to account for a small number of heifers with 2 test recordings within the same interval [except for the first (15 to 45 DIM) and twelfth (345 to 365 DIM) intervals]. The full model included regression terms for LnSCC_{cel} (predictor of main interest), DIM (between 5 and 14, the day of assessment of LnSCC_{cel}) and MY, and the categorical variables, test-season and breed (4 levels both; Table 2). Moreover, the model included interaction terms between LnSCC_{cel} and the predictors DIM and breed, as well as a quadratic term for LnSCC_{cel} and its interaction with DIM. The continuous predictors were centered by subtracting their overall mean, providing better interpretations and better numerical stability of estimates (Dohoo et al., 2001). The interactions between DIM and LnSCC_{cel} were introduced to allow for variable effects of LnSCC_{cel} across the different days of testing. Approximate linearity of the DIM effects was checked by fitting DIM as a categorical variable and examining the estimates. After the assumptions of the full model had been evaluated using the residuals, the interaction terms were tested by likelihood-ratio tests and were removed when nonsignificant. To facilitate comparison of models for different 30-d DIM intervals,

a main effect was kept in all models as soon as it was significant in one interval. The significance level for all analyses was set at $P \leq 0.05$. The distribution of variance at the hierarchical levels (test, heifer, and herd) was assessed for the null-models (models without fixed effects) with LnSCC as outcome variable. Probabilities of Black Holstein-Friesian heifers having a SCC >200,000 cells/mL per 30-d DIM interval were calculated based on the final logistic regression models using the full data set. For this calculation, the calving date was set at September 15, and seasonal changes in SCC and MY were accounted for. The probability per 30-d DIM interval was calculated as $e^{\beta}/(1+e^{\beta})$, with β being the predicted value on logistic scale, corresponding to an average heifer and herd (subject-specific interpretation; Dohoo et al., 2003). The sum of all probabilities over the 30-d DIM intervals was interpreted as the expected number of cases of subclinical mastitis between 15 and 365 DIM (more precisely, the number of times the test-day SCC would exceed 200,000 cells/mL) per Black Holstein-Friesian heifer with a calving date of September 15.

RESULTS

Descriptive Analysis of SCC_{cel}

Geometric mean SCC_{cel} of the 14,766 samples was 104,000 cells/mL (Table 2). The interquartile range was 183,000 cells/mL. Approximately 70% of the samples were recorded in July to December, reflecting the Belgian calving pattern (Table 2). A difference in LnSCC_{cel} between calving seasons ($P < 0.001$) was noted, with April–June having higher average LnSCC_{cel} compared with July–September and October–December (Table 2). Of the heifers, 55% were Black Holstein-Friesian, and 28% were Red Holstein-Friesian. There was a difference in LnSCC_{cel} between breeds ($P < 0.001$) (Table 2). Somatic cell counts in early lactation progressively decreased from 178,000 cells/mL at 5 DIM to 74,000 cells/mL at 14 DIM (Figure 1) ($P < 0.001$). The proportion of SCC_{cel} exceeding 50,000, 100,000, 150,000, 200,000, 500,000, and 1,000,000 cells/mL was 68.8, 46.4, 34.3, 27.5, 12.3, and 6.5%, respectively. Proportion of SCC_{cel} above these thresholds decreased with increasing DIM (Figure 2).

Descriptive Analysis of SCC During First Lactation

Monthly test-data SCC from the first lactation, measured after 14 DIM, were available from 14,234 (96%) of the aforementioned 14,766 heifers. Geometric mean SCC_{cel} from these heifers was 112,000 cells/mL.

On average, 8.2 test-day measurements per heifer were available between 14 and 365 DIM (Table 1). The

Table 2. Descriptive statistics of SCC in early lactation and throughout the course of the first lactation ($\times 1000$ cells/mL).

Dataset ¹	n	GM ²	SD	Min	Max
Early					
Calving season					
January–March	3114	121	816	5	16,022
April–June	1343	131	920	5	14,605
July–September	4424	105	691	1	25,088
October–December	5885	90	689	1	8740
Breed					
Belgian White-Blue and unknown breed	823	120	697	5	8475
MRY, ³ Red, Red and White, and Jersey	1682	95	885	1	14,605
Red Holstein-Friesian	4176	100	753	1	25,088
Black Holstein-Friesian	8085	106	707	1	16,022
Total	14,766	104	742	1	25,088
Full					
Test-season					
January–March	28,871	64	477	5	18,398
April–June	37,039	79	455	5	12,965
July–September	22,504	90	399	5	10,829
October–December	29,082	70	480	5	17,677
Breed					
Belgian White-Blue and unknown breed	11,175	91	644	5	18,398
MRY, Red, Red and White, and Jersey	11,682	83	475	5	10,509
Red Holstein-Friesian	32,890	75	413	5	12,965
Black Holstein-Friesian	61,749	71	433	5	17,677
Total	117,496	75	457	5	18,398
Sub					
Test-season					
January–March	16,258	41	311	5	10,199
April–June	20,808	54	318	5	12,965
July–September	12,422	64	270	5	7115
October–December	15,970	44	398	5	10,199
Breed					
Belgian White-Blue and unknown breed	5595	56	301	5	6768
MRY, Red, Red and White, and Jersey	6230	52	348	5	10,199
Red Holstein-Friesian	18,156	49	291	5	12,965
Black Holstein-Friesian	35,477	48	301	5	10,199
Total	65,458	49	303	5	12,965

¹Early = SCC in early lactation (between 5 and 14 DIM) from 14,766 dairy heifers; Full = SCC between 15 and 365 DIM from 14,234 heifers (full dataset); Sub = SCC between 15 and 365 DIM from 7807 heifers with a second test-day SCC $\leq 50,000$ cells/mL milk (data subset).

²Geometric mean.

³Meuse-Rhine-Yssel.

average interval between 2 test-day measurements was 34 d, with a minimum of 21 d. Heifers maximally had 2 test-day measurements within the same 30-d DIM interval. Geometric mean SCC was 75,000 cell/mL (Table 2). The interquartile range was 109,000 cells/mL. Black Holstein-Friesian heifers had the lowest SCC, and the Belgian White-Blue dual-purpose heifers and heifers of unknown breed had the highest SCC (Table 2). Geometric mean SCC was 55,000 in the 15 to 45 and 46 to 75 DIM intervals after which it progressively increased from 60,000 cells/mL in the 76 to 105 DIM interval to 120,000 cells/mL at the end of lactation (345 to 365 DIM).

Of the heifers, 55% had a second test-day SCC $\leq 50,000$ cells/mL milk. This selection resulted in a subset of heifers with lower geometric mean SCC for the

particular heifers compared with all heifers (Table 2). The interquartile range was 58,000 cells/mL. Between 15 and 45 DIM, geometric mean SCC was 25,000 and progressively increased from 31,000 cells/mL at 46 to 75 DIM to 91,000 cells/mL at the end of the first lactation.

Effect of SCCel on Test-Day SCC

Somatic cell counts stratified by 5 SCCel-classes are presented in Figure 3. Heifers having a SCCel $\leq 50,000$ cells/mL, for instance, had on average a SCC 25,000 cells/mL lower during the subsequent lactation compared with heifers starting their lactation with SCCel between 51,000 and 200,000 cells/mL.

Estimates of LnSCC_{el} corrected for other variables of importance (DIM, MY, breed, and season) are pre-

Figure 1. Geometric mean SCC/DIM in early lactation ($\times 1000$ cells/mL; bars represent the inter-quartile ranges) from 14,766 heifers in the year 2000.

sented in Table 3. On average, if LnSCC_{el} of a heifer was one unit higher than for another heifer, the LnSCC was 0.36, 0.31, and 0.26 units higher in the first, second, and third 30-d DIM intervals, respectively. This interpretation assumes LnSCC_{el} of the 2 heifers to be measured at the same theoretical DIM of 9.5 (mean value between 5 and 14). The size of the effect decreased (i.e., had a smaller impact on LnSCC) in the later 30-d DIM intervals but remained significant over the whole lactation. Furthermore, the significant interaction between LnSCC_{el} and DIM showed that the impact of LnSCC_{el}

depended on when it was measured in early lactation. For example, for a recording at DIM 12, the effect in the first 30-d DIM interval would be $0.364 + [0.013 \times (12 - 9.5)] = 0.40$. Figure 4 gives model-based estimates of LnSCC_{el} effects (comparing heifers differing by one unit) at 7, 9.5, and 12 DIM throughout the lactation. The impact of an elevated SCC_{el} measured at 12 DIM is seen to be higher than that of an equally elevated SCC_{el} at an earlier DIM.

The logistic regression models also showed the probability of high test-day SCC values ($>200,000$ cells/mL, interpretable as a case of subclinical mastitis) to increase with increasing LnSCC_{el} (Table 4). For instance, when comparing heifers with a one unit difference in LnSCC_{el} , the heifer with the higher LnSCC_{el} was 1.86, 1.71, and 1.60 times more likely [as measured by the odds ratio (OR)] to have an SCC $>200,000$ cells/mL in the first, second, and third 30-d DIM intervals, respectively. In all 30-d DIM intervals, the OR was significantly above 1. In the same manner as above, this interpretation corresponded to both heifers being measured at the theoretical mean DIM of 9.5, and the significant interaction between LnSCC_{el} and DIM would alter the actual OR for recordings at other DIM (Table 4).

A direct estimate of the impact of LnSCC_{el} on the risk of subclinical mastitis (SCC $>200,000$ cells/mL) throughout the lactation was computed as the expected number of cases of subclinical mastitis, assuming one recording per 30-d DIM interval for an average Black Holstein-Friesian heifer calving on September 15 and housed in an average herd. The expected number of cases ranged from 0.8 at a low LnSCC_{el} (3.3, corresponding to 27,000 cells/mL, 10th percentile) to 2.1 at a high LnSCC_{el} (6.5, corresponding to 665,000 cells/mL, 90th percentile) for a theoretical DIM of 9.5.

Heifers with a second test-day SCC $\leq 50,000$ cells/mL (data subset, Figure 5) had lower test-day SCC values over time during first lactation when compared with heifers in the full data set (Figure 3), and showed smaller differences between heifers stratified based on their SCC_{el} classes (Figures 3 and 5). This selection procedure resulted in smaller regression coefficients of LnSCC_{el} on LnSCC (Table 5); the effects of LnSCC_{el} (at DIM 9.5) were reasonably constant at 0.1 over the whole lactation, and significant. Similarly, the estimated OR was in the range of 1.1 to 1.2 and significant, except for the last 30-d DIM interval (Table 5).

Season was significantly associated with test-day SCC in all but 3 DIM intervals, and a higher MY was always associated with lower SCC (Table 3). Black Holstein-Friesian heifers tended to have lower LnSCC compared with the other breeds, even though the models corrected for MY (Table 3). The effect of LnSCC_{el} on LnSCC seemed to be the same within all breeds, be-

Figure 2. Proportion of SCC/DIM in early lactation in 2000 from 14,766 heifers exceeding the indicated SCC in early lactation (SCC_{el}) thresholds ($\times 1000$ cells/mL).

Figure 3. Geometric mean SCC ($\times 1000$ cells/mL) during first lactation from 14,234 (full dataset) dairy heifers, stratified by SCC in early lactation (SCC_{el}, $\times 1000$ cells/mL), 0 to 50 ($\square$), 51 to 200 ($\blacksquare$), 201 to 500 ($\triangle$), 501 to 1000 ($\blacktriangle$), and >1000 cells/mL ($\times$).

cause the interaction between LnSCC_{el} and breed was nonsignificant.

The distribution of the variance over the levels of the data hierarchy was assessed in the null models (no fixed effects): between 8.5 (346 to 365 DIM) and 14.2% (256 to 285 DIM) of the variation in LnSCC resided at the herd level, leaving the majority of the variance at the heifer and test level.

In all models, the quadratic term for SCC_{el} was significant, indicating that the association between LnSCC and LnSCC_{el} was nonlinear. The models without the quadratic term were however, based on inspection of graphs of both the simple and more elaborate models, a very good approximation of the models with the quadratic term. Therefore, results are presented only for models without the quadratic term.

DISCUSSION

In this study, monthly milk-recording data collected through the DHI program were used to study the effect of elevated SCC measured in the first 2 wk after calving on SCC in subsequent months of the first lactation. Elevated SCC_{el} resulted in elevated test-day SCC, and a higher probability of test-day SCC exceeding 200,000 cells/mL. However, the impact of SCC_{el} depended on

the day it was measured in the period called “early lactation”.

Composite milk SCC data are used worldwide as a proxy for udder health at the cow level (Schukken et al., 2003). In the first 2 wk after calving SCC changes rapidly. Dilution due to increasing production can contribute to the decrease of SCC (Schepers et al., 1997), but it also suggests spontaneous cure of transient IMI caused by CNS (Oliver and Mitchell, 1983). The SCC_{el} pattern described in our study was similar to that seen in other studies using composite milk samples (Dohoo, 1993; Laevens et al., 1997). Somatic cell counts are elevated shortly after calving (Dohoo and Meek, 1982) and care should be taken in estimating the prevalence of infected heifers in that period using a certain SCC threshold. This is also reflected by the rapidly changing proportion of animals exceeding several thresholds per DIM in this study. If 200,000 cells/mL was chosen to classify heifers as being infected in early lactation, then at 5 DIM, 43% of all heifers would be regarded as subclinically infected, whereas this would have been 24 and 19% at 9 and 14 DIM, respectively. Dohoo (1993) warned about using composite SCC before 9 DIM to estimate the prevalence of infected heifers as this would lead to an overestimation of infected animals. Thus, according to the study of Dohoo (1993), stating that

Table 3. Final multilevel linear regression models per 30-d DIM interval describing log-transformed SCC (LnSCC; $\times 1000$ cells/mL) during the course of first lactation (between 15 and 365 DIM) in 2000 and 2001 from 14,234 dairy heifers from 3264 herds (full dataset).

Variables	30-d DIM interval											
	15 to 45			46 to 75			76 to 105			106 to 135		
	β	SE	P^1	β	SE	P^1	β	SE	P^1	β	SE	P^1
Intercept	4.259	0.043		4.274	0.040		4.233	0.039		4.331	0.040	
LnSCC ^{2,10,11}	0.364	0.008	<0.001	0.310	0.008	<0.001	0.264	0.008	<0.001	0.244	0.009	<0.001
DIM ^{3,10,11}	0.024	0.004	<0.001	0.023	0.003	<0.001	0.017	0.004	<0.001	0.011	0.004	<0.001
MY ^{4,10}	-0.014	0.002	<0.001	-0.016	0.002	<0.001	-0.014	0.002	<0.001	-0.021	0.002	<0.001
Test-season			<0.001			<0.001			<0.001			0.001
Apr–Jun	Ref.	...		Ref.	...		Ref.	...		Ref.	...	
Jan–Mar	-0.141	0.034		-0.193	0.029		-0.140	0.027		-0.100	0.025	
Jul–Sep	-0.205	0.043		-0.146	0.042		-0.033	0.041		-0.004	0.040	
Oct–Dec	-0.186	0.032		-0.183	0.029		-0.114	0.027		-0.097	0.030	
Breed			NS			NS			NS			NS
Breed 1 ⁵	Ref.	...		Ref.	...		Ref.	...		Ref.	...	
Breed 2 ⁶	-0.091	0.046		-0.099	0.045		0.007	0.047		0.001	0.049	
RHF ⁷	-0.076	0.039		-0.090	0.038		-0.032	0.040		-0.071	0.041	
BHF ⁸	-0.103	0.037		-0.084	0.036		-0.023	0.038		-0.032	0.040	
LnSCC $\times$ DIM ⁹	0.013	0.003	<0.001	0.021	0.003	<0.001	0.018	0.003	<0.001	0.019	0.003	<0.001
	136 to 165			166 to 195			196 to 225			226 to 255		
	β	SE	P^1	β	SE	P^1	β	SE	P^1	β	SE	P^1
Intercept	4.411	0.038		4.430	0.037		4.500	0.036		4.565	0.036	
LnSCC ^{2,10,11}	0.223	0.008	<0.001	0.212	0.008	<0.001	0.197	0.008	<0.001	0.192	0.008	<0.001
DIM ^{3,10,11}	0.012	0.004	<0.001	0.018	0.004	<0.001	0.012	0.003	<0.001	0.011	0.003	<0.001
MY ^{4,10}	-0.020	0.002	<0.001	-0.021	0.002	<0.001	-0.024	0.002	<0.001	-0.022	0.002	<0.001
Test-season			0.001			<0.001			NS			NS
Apr–Jun	Ref.	...		Ref.	...		Ref.	...		Ref.	...	
Jan–Mar	-0.086	0.023		-0.087	0.024		-0.077	0.028		-0.065	0.038	
Jul–Sep	0.034	0.034		0.058	0.029		0.012	0.024		-0.007	0.022	
Oct–Dec	-0.061	0.035		0.046	0.036		0.020	0.034		0.036	0.030	
Breed			NS			NS			NS			0.004
Breed 1 ⁵	Ref.	...		Ref.	...		Ref.	...		Ref.	...	
Breed 2 ⁶	-0.021	0.048		-0.047	0.048		-0.016	0.047		-0.012	0.047	
RHF ⁷	-0.076	0.040		-0.083	0.041		-0.068	0.040		-0.104	0.039	
BHF ⁸	-0.094	0.039		-0.110	0.039		-0.101	0.038		-0.132	0.038	
LnSCC $\times$ DIM ⁹	0.019	0.003	<0.001	0.016	0.003	<0.001	0.013	0.003	<0.001	0.015	0.003	<0.001
	256 to 285			286 to 315			316 to 345			346 to 365		
	β	SE	P^1	β	SE	P^1	β	SE	P^1	β	SE	P^1
Intercept	4.619	0.037		4.636	0.043		4.713	0.059		4.882	0.092	
LnSCC ^{2,10,11}	0.178	0.008	<0.001	0.170	0.009	<0.001	0.150	0.011	<0.001	0.151	0.017	<0.001
DIM ^{3,10,11}	0.013	0.003	<0.001	0.011	0.004	<0.001	0.011	0.005	0.003	0.014	0.007	0.002
MY ^{4,10}	-0.024	0.002	<0.001	-0.029	0.003	<0.001	-0.026	0.003	<0.001	-0.026	0.005	<0.001
Test-season			0.031			<0.001			0.003			NS
Apr–Jun	Ref.	...		Ref.	...		Ref.	...		Ref.	...	
Jan–Mar	-0.101	0.040		-0.070	0.043		-0.041	0.054		-0.130	0.079	
Jul–Sep	0.039	0.022		0.104	0.026		0.113	0.043		0.034	0.068	
Oct–Dec	0.038	0.027		0.030	0.030		0.023	0.044		-0.050	0.068	
Breed			0.002			NS			NS			NS
Breed 1 ⁵	Ref.	...		Ref.	...		Ref.	...		Ref.	...	
Breed 2 ⁶	0.007	0.047		0.016	0.053		-0.013	0.064		0.097	0.100	
RHF ⁷	-0.105	0.040		-0.077	0.044		-0.099	0.052		-0.121	0.080	
BHF ⁸	-0.123	0.038		-0.107	0.042		-0.138	0.049		-0.128	0.077	
LnSCC $\times$ DIM ⁹	0.012	0.003	<0.001	0.015	0.003	<0.001	0.012	0.004	<0.001	0.020	0.006	0.004

¹Bonferroni corrected P -values (original values multiplied by 12).²Log-transformed SCC in early lactation (between 5 and 14 DIM) ($\times 1000$ cells/mL milk).³Day of assessment of LnSCC.⁴Daily milk yield (kg milk).⁵Belgian White-Blue double-purpose heifers and heifers of unknown breed.⁶Meuse-Rhine-Yssel, Red, Red and White, Jersey.⁷Red Holstein-Friesian.⁸Black Holstein-Friesian.⁹Interaction term between LnSCC and DIM.¹⁰Centered by subtracting the mean value (LnSCC = 4.7; DIM = 9.5; MY = 22.6). See text for details.¹¹ P -values correspond to test of both main effect and interaction.

Figure 4. Regression coefficients for log-transformed SCC in early lactation (LnSCC_{cel}, × 1000 cells/mL) per 30-d DIM interval measured at 7 (□), 9.5 (■), and 12 (△) DIM in early lactation. Estimates are based on the final multilevel linear regression models describing SCC (× 1000 cells/mL) during first lactation (between 15 and 365 DIM) in 2000 and 2001 from 14,234 dairy heifers from 3264 herds.

more than 27% of all heifers in the present study were infected in early lactation using an infection threshold of 200,000 cells/mL would be an overestimation. On the other hand, Barkema et al. (1999) concluded that quarter-milk SCC was applicable from 2 DIM to determine the IMI status in an udder quarter and that quarter-milk SCC of bacteriological negative quarters was as low as 42,000 cells/mL. Consequently, a heifer without infected quarters should have an equally low com-

posite milk SCC. A heifer with one quarter infected with a minor pathogen would most likely never have a composite milk SCC >200,000 cells/mL, due to dilution by the noninfected quarters, which would lead to an underestimation of infected heifers in early lactation using the 200,000 cells/mL threshold, contrary to that discussed by Dohoo (1993). No bacteriological cultures were done in this study, making it impossible to determine exactly the prevalence of infected heifers in early

Table 4. Regression coefficients for log-transformed SCC in early lactation (LnSCC_{cel}) and the interaction between LnSCC_{cel} and DIM per 30-d DIM interval based on the final multilevel logistic regression models describing logit(SCC200) during first lactation (between 15 and 365 DIM) from 14,234 dairy heifers from 3264 herds (full dataset).

30-d DIM interval	LnSCC _{cel}			LnSCC _{cel} × DIM	
	β	SE	OR ¹	β	SE
15 to 45	0.622	0.024	1.863	0.027	0.008
46 to 75	0.537	0.023	1.711	0.038	0.008
76 to 105	0.469	0.023	1.598	0.029	0.008
106 to 135	0.409	0.023	1.506	0.029	0.008
136 to 165	0.394	0.022	1.483	0.024	0.008
166 to 195	0.337	0.023	1.401	0.025	0.008
196 to 225	0.340	0.021	1.404	0.028	0.007
226 to 255	0.326	0.021	1.385	0.027	0.007
256 to 285	0.294	0.021	1.342	0.025	0.007
286 to 315	0.292	0.023	1.339	0.027	0.008
316 to 345	0.279	0.027	1.322	0.018	0.009
346 to 365	0.261	0.040	1.298	0.025	0.013

¹OR = Odds ratio.

Figure 5. Geometric mean SCC ($\times 1000$ cells/mL) during first lactation from 7807 heifers with a second test-day SCC $\leq 50,000$ cells/mL, stratified by SCC in early lactation (SCC_{el}, $\times 1000$ cells/mL), 0 to 50 ($\square$), 51 to 200 ($\blacksquare$), 201 to 500 ($\triangle$), 501 to 1000 ($\blacktriangle$), and >1000 cells/mL ($\times$).

lactation. More studies on the dynamics of quarter- and composite milk SCC in association with IMI status of quarters and animals in early lactation should be conducted to reach better conclusions.

A high proportion of IMI caused by CNS is cleared once heifers start lactating (Oliver and Mitchell, 1983);

SCC in recovered quarters will take some time to return to a normal level. This may be the reason why the impact of SCC_{el} on SCC in the rest of the lactation depended on the day it was assessed. A value of SCC_{el} that is still high 2 wk after calving will have a larger impact on future SCC. It could be hypothesized that a

Table 5. Regression coefficients of log-transformed SCC in early lactation (LnSCC_{el}) per 30-d DIM interval based on the final multilevel linear and logistic regression models describing LnSCC ($\times 1000$ cells/mL) and logit(SCC200) during first lactation (between 76 and 365 DIM) from 7807 dairy heifers from 2827 dairy herds (data subset).

30-d DIM interval	Linear regression models			Logistic regression models		
	β	SE	P^1	β	SE	OR ²
76 to 105	0.109	0.010	<0.001	0.155	0.052	1.167
106 to 135	0.100	0.011	<0.001	0.173	0.047	1.189
136 to 165	0.109	0.011	<0.001	0.192	0.042	1.211
166 to 195	0.118	0.011	<0.001	0.133	0.043	1.142
196 to 225	0.110	0.011	<0.001	0.185	0.039	1.203
226 to 255	0.118	0.011	<0.001	0.185	0.037	1.203
256 to 285	0.104	0.012	<0.001	0.118	0.037	1.125
286 to 315	0.108	0.013	<0.001	0.128	0.039	1.137
316 to 345	0.086	0.016	<0.001	0.162	0.046	1.176
346 to 365	0.079	0.026	0.038	0.086	0.072	1.090

¹Bonferroni corrected P -values (original values multiplied by 10), corresponding to test of both main effect and interaction.

²OR = Odds ratio.

heifer with an elevated SCCel at 5 DIM may have had quarters recovering from CNS infections resulting in a more or less normal SCCel level within the next few days. This hypothesis is supported by a study, done on a Californian dairy with low prevalence of major mastitis pathogens, which investigated the association between IMI in early lactation and SCC in the first 5 DHI test periods in heifers. Subclinical infections with minor pathogens had no effect on average SCC (Kirk et al., 1996). On the other hand, it could be hypothesized that a heifer with an elevated SCCel at 14 DIM in our study either suffered from a persistent IMI caused by a major pathogen already present before calving or had been infected early postpartum. Intramammary infections caused by major mastitis pathogens most likely do not cure as easily as IMI caused by CNS (Oliver and Mitchell, 1983), resulting in elevated SCCel until 14 DIM and after. However, more studies are needed to elucidate these findings as no bacteriological cultures were done in this study.

A high SCC shortly after calving resulted in elevated test-day SCC in the subsequent months, which is in accordance with the findings of Coffey et al. (1986). In that study, SCC was examined during the first and subsequent lactations by classes of SCC in the initial days of first lactation to determine if heifers with initially low SCC were at increased risk of subsequent infections. However, they concluded that the initial rank of SCC classes (<100,000, 100,000 to 400,000, and >400,000 cells/mL) was maintained throughout the remainder of first and subsequent lactation. Rupp and Boichard (2000) concluded that heifers with the highest initial SCC had the highest probability of clinical mastitis.

A high SCCel shortly after calving also increased the probability of having elevated SCC (>200,000 cells/mL) during the ongoing first lactation. This threshold was used as a cut-off value for subclinical IMI. Although every threshold has its advantages and disadvantages (Schukken et al., 2003), 200,000 cells/mL is commonly accepted as the threshold for IMI (Hillerton, 1999). Our interpretation was that heifers with an elevated SCCel, on average, remained more at risk for subsequent subclinical IMI. Coffey et al. (1986) have found that heifers starting their lactation with a higher SCC were more at risk for acquiring new IMI, with a higher prevalence of IMI caused by major pathogens. However, as we had chosen to estimate the impact of SCCel per 30-d DIM interval, rather than using the data set as a whole and to model the test-day SCC as repeated measures, we were not able, using the 200,000 cells/mL threshold, to distinguish between existing IMI (current and preceding SCC >200,000 cells/mL) or new infections (current SCC >200,000 cells/mL and preceding SCC $\leq$ 200,000

cells/mL). We were, therefore, unable to determine whether heifers with elevated SCCel were more at risk of new IMI or whether they were persistently infected.

The results from our study highlight the importance of reducing the prevalence of elevated SCCel in dairy heifers, especially in the second part of the period defined as early lactation. Farmers should therefore focus on prevention of IMI before calving rather than on cure of existing IMI after calving, because the effect of elevated SCCel was still present and significant, although to a lesser extent, in heifers with a second test-day SCC $\leq$ 50,000 cells/mL. In other words, of a heifer with an elevated SCCel (e.g., 750,000 cells/mL) but a healthy udder at the second test-day measurement (e.g., 45,000 cells/mL), still had higher SCC throughout the lactation, and was more at risk for IMI than a heifer starting with a low SCCel and a similar low second test-day SCC.

In a recent study, we investigated the distribution of the variance of SCCel over the herd and heifer level using multilevel regression analysis. We concluded that focusing more on differences between heifers than between herds in targeting prevention is probably the best approach, because heifers within the same herd responded differently to the same management practices (De Vliegher et al., 2004). This was supported by the findings from the present study, and, although the distribution was not as extreme as in our earlier paper, 10% of the variation resided at the herd level, leaving more room for improvement at the heifer and test level.

As test-day records collected through the DHI program were used in our study, some heifers suffering from clinical mastitis in early lactation were missed as probably no samples for SCC measurement were collected at the particular time, or at least not from the affected quarter. Therefore, we expect that the negative effect of elevated SCCel on SCC during the rest of the lactation calculated in this study is an underestimation of the true effect. An additional underestimation could be suspected from the fact that from the originally selected 14,766 heifers only 14,234 were available for further analyses. Some of the more than 500 "missing" heifers could have been culled because of udder health problems in early lactation. However, the geometric mean SCCel of the 14,243 heifers was larger than the geometric mean SCCel from the 14,766 heifers. The heifers that were missing were therefore most probably culled for other reasons or because the farm stopped doing business. In addition, some heifers might have been sold to farmers that were not involved in the DHI program.

Current research looks at the effect of elevated SCCel on production and culling hazard. Combining the results from such studies will enable us to estimate the

economical losses for herds with a high prevalence of heifers suffering from elevated SCCel.

CONCLUSIONS

A large proportion of Belgian heifers had SCCel that suggested they had an IMI during the peripartum period. An elevated SCCel had a negative effect on udder health throughout the first lactation. The magnitude of the effect was dependent upon the time at which SCCel was measured. Elevated SCCel in the first part of the period defined as early lactation led to a reduced increase in test-day SCC than if the SCCel was elevated later. It could be hypothesized that the prevention of IMI prepartum rather than the cure of existing IMI at the time of calving is needed, as a negative effect of elevated SCCel was still present in a subgroup of heifers having a very low SCC ($\leq 50,000$ cells/mL) at the second test-day measurement.

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REFERENCES

- Barkema, H. W., H. Deluyker, Y. H. Schukken, and T. J. G. M. Lam. 1999. Quarter-milk somatic cell count at calving and at the first six milkings after calving. *Prev. Vet. Med.* 38:1-9.
- Coffey, E. M., W. E. Vinson, and R. E. Pearson. 1986. Somatic cell counts and infection rates for cows of varying somatic cell count in initial test of first lactation. *J. Dairy Sci.* 69:552-555.
- De Vlieghe, S., H. Laevens, H. W. Barkema, I. Dohoo, H. Stryhn, G. Opsomer, and A. de Kruif. 2004. Management practices and heifer characteristics associated with early lactation somatic cell counts of dairy heifers in Belgium. *J. Dairy Sci.* 87:937-947.
- Dohoo, I. R. 1993. An evaluation of the validity of individual cow somatic cell counts from cows in early lactation. *Prev. Vet. Med.* 16:103-110.
- Dohoo, I. R., S. W. Martin, and H. Stryhn. 2003. *Veterinary Epidemiologic Research*. 1st ed., AVC, Inc., Charlottetown, PEI, Canada.
- Dohoo, I. R., and A. H. Meek. 1982. Somatic cell counts in bovine milk. *Can. Vet. J.* 23:119-125.
- Dohoo, I. R., E. Tillard, H. Stryhn, and B. Faye. 2001. The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. *Prev. Vet. Med.* 50:127-144.
- Hillerton, J. E. 1999. Redefining mastitis based on somatic cell count. *Bull. Int. Dairy Fed.* 345:4-6.
- Kirk, J. H., J. C. Wright, S. L. Berry, J. P. Reynolds, J. P. Maas, and A. Ahmadi. 1996. Relationship of milk culture status at calving with somatic cell counts and milk production of dairy heifers during early lactation on a Californian dairy. *Prev. Vet. Med.* 28:187-198.
- Laevens, H., H. Deluyker, Y. H. Schukken, L. De Meulemeester, R. Vandermeersch, E. De Muelenaere, and A. de Kruif. 1997. Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative cows. *J. Dairy Sci.* 80:3219-3226.
- McDermott, J. J., and Y. H. Schukken. 1994. A review of methods used to adjust for cluster effects in explanatory epidemiological studies of animal populations. *Prev. Vet. Med.* 18:155-173.
- Myllys, V. 1995. Staphylococci in heifer mastitis before and after parturition. *J. Dairy Res.* 62:51-60.
- Neave, F. K., F. H. Dodd, R. G. Kingwill, and D. R. Westgarth. 1969. Control of mastitis in the dairy herd by hygiene and management. *J. Dairy Sci.* 52:698-707.
- Oliver, S. P., M. J. Lewis, B. E. Gillespie, and H. H. Dowlen. 1992. Influence of prepartum antibiotic therapy on intramammary infections in primigravid heifers during early lactation. *J. Dairy Sci.* 75:406-414.
- Oliver, S. P., M. J. Lewis, B. E. Gillespie, H. H. Dowlen, E. C. Jaenicke, and R. K. Roberts. 2003. Prepartum antibiotic treatment of heifers: Milk production, milk quality, and economic benefit. *J. Dairy Sci.* 86:1187-1193.
- Oliver, S. P., and B. A. Mitchell. 1983. Intramammary infections in primigravid heifers near parturition. *J. Dairy Sci.* 66:1180-1183.
- Pankey, J. W., P. A. Drechsler, and E. E. Wildman. 1991. Mastitis prevalence in primigravid heifers at parturition. *J. Dairy Sci.* 74:1550-1552.
- Rasbash, J., W. Browne, H. Goldstein, M. Yang, I. Plewis, M. Healy, G. Woodhouse, D. Draper, I. Langford, and T. Lewis. 2000. *A User's Guide to MLwiN*. 2nd ed. Institute of Education, London, UK.
- Roberson, J. R., L. K. Fox, D. D. Hancock, and C. C. Gay. 1994. Coagulase-positive Staphylococcus intramammary infections in dairy heifers. *J. Dairy Sci.* 77:958-969.
- Rupp, R., F. Beaudeau, and D. Boichard. 2000. Relationship between milk somatic cell counts in the first lactation and clinical mastitis occurrence in the second lactation of French Holstein cows. *Prev. Vet. Med.* 46:99-111.
- Rupp, R., and D. Boichard. 2000. Relationship of early first lactation somatic cell count with risk of subsequent first clinical mastitis. *Livest. Prod. Sci.* 62:169-180.
- Schepers, A. J., T. J. G. M. Lam, Y. H. Schukken, J. B. M. Wilmink, and W. J. A. Hanekamp. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. *J. Dairy Sci.* 80:1833-1840.
- Schukken, Y. H., D. J. Wilson, F. Welcome, L. Garrison-Tikofsky, and R. N. Gonzalez. 2003. Monitoring udder health and milk quality using somatic cell counts. *Vet. Res.* 34:579-596.