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Dynamics of somatic cell counts and intramammary infections across the dry period

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ABSTRACT

The objectives of this research were to study the relationship between somatic cell count (SCC) and intramammary infection (IMI) across the dry period and the risk of subclinical mastitis at the first dairy herd improvement (DHI) test of the subsequent lactation. A secondary objective was to determine SCC test characteristics for diagnosis of IMI at both the cow and quarter levels. A total of 218 cows from a university herd were enrolled at dry-off. Duplicate quarter milk samples were collected from all quarters at dry-off, calving and on the day of the first DHI test. Somatic cell count status across the dry period was defined based on the comparison of quarter SCC from dry-off and the post-calving sampling periods and comparison of composite SCC from DHI samples from the last test and first test of the following lactation. Of new IMI detected from post-calving milk samples ($n = 45$), 46.7, 26.7 and 11% were caused by CNS, *Streptococci* and Gram-negative bacteria, respectively. Of cured IMI at post-calving ($n = 91$), 61.5, 23.1 and 9.9% had CNS, *Streptococci* and Coryneforms isolated from dry-off milk samples. The most frequent microorganisms related to cured IMI were CNS (33%). Of chronically infected quarters across the dry period ($n = 10$), only one had the same species of pathogen isolated from dry-off and post-calving samples. The sensitivity of a SCC threshold of 200,000 cells/mL for detection of subclinical IMI was 0.64, 0.69 and 0.65 for milk samples obtained at dry-off, post-calving and first DHI test, respectively. The specificity was 0.66, 0.84 and 0.93 for milk samples obtained at dry-off, post-calving and first DHI test, respectively. Quarters with SCC $\geq$ 200,000 cells/mL at both dry-off and post-calving sampling periods were 20.4 times more likely to be subclinically infected by a major pathogen (rather than being uninfected) and 5.6 times more likely to be subclinically infected by a minor pathogen (rather than being uninfected) at the first DHI test than quarters with SCC $<$ 200,000 cells/mL at both periods. Cows with SCC greater than 200,000 cells/mL at both the last and the first DHI test between lactations produced 9.1 kg less milk on the first DHI test day than the average milk production of cows with SCC less than 200,000 cells/mL at both periods.

1. Introduction

Monitoring of somatic cell count (SCC) dynamics across the dry period to predict udder health status and make management decisions has been suggested (Whist and Østeras, 2006). Monthly SCC values obtained from dairy herd improvement (DHI) associations are often used to monitor subclinical mastitis and allow the estimation of epidemiologic measures of disease frequency such as prevalence and incidence (Laevens et al., 1997; Ruegg, 2003). However, little research has been directed toward monitoring changes in SCC between test days of subsequent lactations. Cook et al. (2002) used DHI SCC data from a convenience sample of 145 Wisconsin dairy herds and

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reported estimated mean dry-cow new infections (proportion of cows with a SCC < 200,000 cells/mL at the last DHI test before dry-off and SCC ≥ 200,000 cells/mL at the first test of the subsequent lactation), and mean dry-cow cures (proportion of cows with a SCC ≥ 200,000 cells/mL at the last DHI test before dry-off and SCC < 200,000 cells/mL at the first test of the subsequent lactation) as 22.4 and 62.9%, respectively. Whist and Østeras (2006) used a large number of small herds in Norway to study the association between future composite SCC in current lactations and composite SCC obtained from test days at calving or prior to drying-off. The use of SCC from milk samples obtained at the last test day prior to drying-off in the first lactation explained 20% of composite SCC variance occurring in the second lactation. Likewise, SCC from the last test day prior to drying-off in the second lactation explained approximately 16% of the composite SCC variance in the third lactation. However, the relationship between these changes and quarter-level intramammary infections (IMIs) and SCC has not been reported. Reduced milk production as a result of IMI is one of the most important causes of economic losses attributed to mastitis (Blosser, 1979; Wilson et al., 1997) and no studies have reported the association between SCC status across the dry period and milk production in early lactation.

A SCC threshold of 200,000 cells/mL has been utilized to reduce diagnostic error when detecting IMI (Dohoo and Leslie, 1991; Schepers et al., 1997; Djabri et al., 2002). For herds in which contagious pathogens (such as *Streptococcus agalactiae* and *Staphylococcus aureus*) were among the causative pathogens, the epidemiological sensitivity of that threshold has ranged from 0.73 to 0.89 with estimated specificities of 0.75 – 0.90 (McDermott et al., 1982; Dohoo and Leslie, 1991; Schepers et al., 1997). The proportion of IMI caused by contagious pathogens has decreased and increased emphasis has been placed on the importance of IMI caused by minor and environmental pathogens (such as coagulase-negative *Staphylococci* (CNS) and *Streptoccus* spp.) (Wilson et al., 1997; Godden et al., 2003; Cook et al., 2005). Minor pathogens often induce less intense SCC responses (Sheldrake et al., 1983; Barkema et al., 1999; Schukken et al., 2003); therefore, the performance of SCC as diagnostic test might be expected to have different characteristics for herds that have controlled major contagious pathogens.

The primary objective of this study was to determine the relationship between SCC and IMI across the dry period and the risk of subclinical mastitis at the first DHI test of the subsequent lactation in a modern dairy herd that used intramammary dry-cow treatment and had a very low prevalence of mastitis caused by contagious pathogens. The secondary objective was to determine epidemiological test characteristics of SCC for diagnosis of IMI at both the cow and quarter level.

2. Material and methods

2.1. Enrolment of animals and sampling strategy

Enrollment and sampling have been previously described (Pantoja et al., 2009). Briefly, multiparous cows from the University of Wisconsin's dairy herd that were dried off from August 2005 to January 2007 were enrolled at dry-off (n = 218). Each cow could only be enrolled once into the experiment. The herd was composed of 285 lactating Holsteins which were milked twice daily, produced 33.2 kg of milk per cow per day and had a bulk tank SCC of 240,000 cells/mL.

Duplicate quarter milk samples were collected from all quarters at dry-off before administration of dry-cow therapy (DCT), post-calving (median, 6 DIM; range, 2–9 DIM), and on the day of the first DHI test (median, 18 DIM; range, 5–42 DIM). No first test DHI milk samples were collected before post-calving milk samples. All four quarters received intramammary DCT, which consisted of 1,000,000 units of procaine penicillin and 1 g of dihydrostreptomicin (QuarterMaster; Pfizer Animal Health, Kalamazoo, MI) followed by administration of an intramammary internal teat sealant which contained 4 g of bismuth subnitrate (Orbeseal; Pfizer Animal Health). After the subsequent parturition and on the day of the first DHI test, foremilk quarter milk samples (from all quarters) were collected before the afternoon milking.

2.2. Bacteriology and somatic cell count

Microbiological procedures were as prescribed by NMC (1999) and have been previously described (Pantoja et al., 2009). Briefly, 10 μL of each milk sample were streaked onto one quarter of a blood agar plate and incubated at 37 °C up to 48 h. An IMI was defined as the presence of 3 or more colonies of the same type and the presence of ≥3 dissimilar colony types was considered contaminated. Identification of bacterial species was as defined by NMC (1999). Final identification of microorganisms was performed using the appropriate API tests (API 20E, API 20Strep and API Staph; bioMerieux-Vitek Inc., Hazelwood, MO) according to the manufacturer's instructions. Isolates identified with API confidence levels greater than 0.90 were considered identified mastitis pathogens at species level. Otherwise, they were identified at genus level. Quarter SCC was determined using the Direct Cell Counter (DeLaval, Illinois). Data from DHI were obtained electronically.

2.3. Statistical analysis

2.3.1. Definitions used for analysis

Infection status was defined at both the quarter and cow level. A quarter was considered infected (IMI) when the same mastitis pathogen was isolated from both duplicate milk samples obtained on the same sample day. A cow was considered infected when at least one quarter was classified as having an IMI. Isolation of a mastitis pathogen from only one of the duplicate milk samples was considered non-significant growth (NSG). Isolation of the same two pathogens from both duplicate milk samples was considered to be a mixed IMI unless one or both organisms had been recovered from another sampling period. When one of the duplicate samples was contaminated but no organisms were recovered from the other sample the quarter was coded as no growth (NG).

Outcomes of quarters with non-significant growth were combined with NG for analysis. When both duplicate milk samples were contaminated the data were considered missing.

Quarter SCC status across the dry period was defined based on comparison of quarter SCC between the date of dry-off and the post-calving sampling period using the following definitions: (1) *Estimated chronic infection* (*QECHRI*): milk samples obtained from the same quarters that had SCC $\geq$ 200,000 cells/mL at both the dry-off and post-calving sampling periods; (2) *Estimated new infection* (*QENEWI*): milk samples obtained from the same quarters that had a SCC $<$ 200,000 cells/mL at the dry-off sampling period but had a SCC $\geq$ 200,000 cells/mL at post-calving sampling period; (3) *Estimated cured infections* (*QECURI*): milk samples that had a SCC $\geq$ 200,000 cells/mL at the dry-off sampling period and a SCC $<$ 200,000 cells/mL at post-calving sampling period; (4) *Estimated uninfected* (*QEUNIN*): milk samples obtained from the same quarters that had SCC $<$ 200,000 cells/mL at both the dry-off and post-calving sampling periods.

Cow SCC status across the dry period (composite SCC) was defined based on comparisons of DHI test day SCC between the last test date and the first test date in the subsequent lactations using the following definitions: (1) *Estimated chronic infection* (*CECHRI*): milk samples obtained from the same cows that had composite SCC $\geq$ 200,000 cells/mL at both the last DHI test of the previous lactation and the first DHI test of the subsequent lactation sampling periods; (2) *Estimated new infection* (*CENEWI*): milk samples obtained from the same cows that had a composite SCC $<$ 200,000 cells/mL at the last DHI test of the previous lactation sampling period but had a SCC $\geq$ 200,000 cells/mL at the first DHI test of the subsequent lactation sampling period; (3) *Estimated cured infections* (*CECURI*): milk samples that had a composite SCC $\geq$ 200,000 cells/mL at the last DHI test of the previous lactation sampling period and a SCC $<$ 200,000 cells/mL at the first DHI test of the subsequent lactation sampling period; (4) *Estimated uninfected* (*CEUNIN*): milk samples obtained from the same cows that had composite SCC $<$ 200,000 cells/mL at both the last DHI test of the previous lactation and the first DHI test of the subsequent lactation sampling periods.

Quarter IMI status across the dry period was defined based on comparison of quarter IMI between the date of dry-off and the post-calving sampling periods using the following definitions: (1) *Chronic IMI*: milk samples obtained from the same quarters that had an IMI diagnosed at both the dry-off and post-calving, regardless of the presence of the same pathogen at both sampling periods; (2) *New IMI*: milk samples obtained from the same quarters that were uninfected at the dry-off sampling period but infected at the post-calving sampling period; (3) *Cured IMI*: milk samples from infected quarters at the dry-off sampling period and uninfected at the post-calving sampling period; (4) *Uninfected*: milk samples obtained from the same quarters that were uninfected at both the dry-off and post-calving sampling periods.

Cow IMI status across the dry period was defined based on comparisons of milk samples collected at dry-off and the first DHI test day in the subsequent lactations using the following definitions: (1) *Chronic IMI*: milk samples obtained from the same cows that had at least one infected quarter at both dry-off and the first DHI test of the subsequent lactation, regardless of the presence of the same pathogen at both sampling periods; (2) *New IMI*: milk samples obtained from the same cows that were uninfected at dry-off sampling period but infected at the first DHI test of the subsequent lactation sampling period; (3) *Cured IMI*: milk samples obtained from the same cows that were infected at dry-off and uninfected at the first DHI test of the subsequent lactation sampling period; (4) *Uninfected*: milk samples obtained from the same cows that were uninfected at both dry-off and the first DHI test of the subsequent lactation sampling periods.

Microbiological results were used as the gold standard for determination of epidemiological test characteristics of SCC status. Sensitivity and specificity were calculated for each sampling period using SCC thresholds of 50, 100, 150, 200, 250 and 300×10^3 cells/mL for both quarter and composite milk samples. Sensitivity of quarter SCC was calculated as the number of infected quarters with SCC greater than the selected threshold divided by the total number of infected quarters. Specificity of quarter SCC was calculated as the number of uninfected quarters with SCC less than the selected threshold divided by the total number of uninfected quarters. Positive predictive values were calculated as the number of infected quarters with SCC greater than the selected threshold divided by the total number of quarters with SCC exceeding the selected threshold. Negative predictive values were calculated as the number of uninfected quarters with SCC less than the selected threshold divided by the total number of quarters with an SCC less than the selected threshold.

The sensitivity of DHI composite SCC was defined as the number of infected cows with DHI SCC greater than the selected threshold divided by the total number of cows with at least 1 quarter with an IMI. The specificity of DHI composite SCC to predict IMI was defined as the number of uninfected cows with SCC less than the selected threshold divided by the total number of cows with no quarters with IMI. The predictive value of a positive DHI composite SCC was defined as the number of cows with at least 1 quarter with an IMI that had SCC greater than the selected threshold divided by the total number of cows with SCC greater than the selected threshold. The predictive value of a negative DHI composite SCC was defined as the number of cows with no quarters with IMI that had SCC less than the selected threshold divided by the total number of cows with SCC less than the selected threshold. *Enterococcus* spp., *Aerococcus* spp. and *Micrococcus* spp. were defined as Strep-like mastitis pathogens. Major pathogens isolated from milk samples collected at the first DHI test were defined as *Streptococcus* spp., Strep-like bacteria and Gram-negative bacteria. Minor pathogens were defined as CNS, Yeasts, *Corynebacterium bovis* and *Arcanobacter pyogenes*.

2.3.2. Analysis at the quarter level

Initially, chi-square tests and logistic regression (Pagano and Gauvreau, 2000) were used to assess

associations between the probability of developing subclinical mastitis at the first DHI test of the subsequent lactation (outcome variable) and individual explanatory variables. Univariate analyses were performed using PROC FREQ and PROC LOGISTIC of SAS (SAS Institute, 2008). Explanatory variables which were associated with the outcome variable ($P < 0.25$), as well as their interaction terms, were used to perform multivariate analysis. Final models were constructed according to forward and backward variable selection procedures and biological significance of variables. The models' goodness of fit was assessed using chi-square tests (Littell et al., 2006).

The relationship between the probability of developing subclinical mastitis (IMI) at the first DHI test sampling period and explanatory variables (Table 1) was assessed using a logistic regression mixed model (Littell et al., 2006) (model 1):

$$\text{Logit}\,(\pi_{ijkl}) = \alpha + \beta(\text{dry period length}_i) + \text{SCCstatus}_j + \text{Calv season}_k + \text{Parity}_l + \delta_i$$

where π is the probability of developing subclinical mastitis (IMI) at the first DHI test of the subsequent lactation (yes = 1, no = 0), α is the intercept, dry period length$_i$ is the length of the dry period for the cow i, SCCstatus$_j$ is the SCC status (j = QECHRI, QENEWI, QECURE, QEUNIN), Calv season$_k$ is the calving season (k = Fall, Winter, Spring, Summer), Parity$_l$ is the parity status (l = 2, 3, 4 and >4) and δ_i is a random term relative to the effect of cow i, used to model the covariance between observations on each quarter within each cow (Barkema et al., 1997). Logistic regression with a random effect was performed using PROC GLIMMIX of SAS (SAS Institute, 2008).

The relationship between IMI status at the first DHI test sampling period (nominal response variable) and explanatory variables (Table 1) was assessed using a baseline category logit model (Hartzel et al., 2001) (model 2).

$$\log\left(\frac{\pi_{klmn}}{\pi_{klmn,\text{uninfected}}}\right) = \alpha_j + \beta_j(\text{dry period length}_{kj}) + \text{SCCstatus}_{lj} + \text{Calv season}_{mj} + \text{Parity}_{nj} + \delta_{kj}.$$

where π is the probability of IMI caused by a j pathogen group at the first DHI test of the subsequent lactation, j = 1 for IMI caused by a major pathogen, j = 2 for IMI caused by a minor pathogen, j = 3 for an uninfected quarter (response baseline category), α_j is the intercept, dry period length$_{kj}$ is the length of the dry period for the cow k, SCC status$_{lj}$ is the SCC status (l = QECHRI, QENEWI, QECURI and QEUNIN), Calv season$_{mj}$ is the calving season (m = Fall, Winter, Spring and Summer), Parity$_{nj}$ is the parity status (n = 2, 3, 4 and >4) and δ_{kj} is a random term relative to the effect of cow k, used to model the covariance between observations on

Table 1
Description of variables used in the statistical models.

Variables	Abbreviation	Levels	Type – unit
Probability of IMI at the first DHI test	PIMI	Yes No	Categorical
Probability of IMI caused by grouped pathogens at the first DHI test	PIMIgrouped	IMI caused by major pathogens IMI caused by minor pathogens Uninfected	Categorical nominal
Quarter SCC status across the dry period	SCCstatus	QECHRI[a] QENEWI[b] QECURI[c] QEUNIN[d]	Categorical
Cow SCC status across the dry period	SCCstatus	CECHRI[e] CENEWI[f] CECURI[g] CEUNIN[h]	Categorical
Parity	Parity	2 3 4 >4	Categorical
Calving season	Cseason	Summer (June–August) Fall (September–November) Winter (December–February) Spring (March–May)	Categorical
Milk yield at the last DHI test	Milkylast		Continuous (kg)
Milk yield at the first DHI test	Milkyfirst		Continuous (kg)
Length of the dry period	Dry-period length		Continuous (days)

[a] Quarters with SCC ≥ 200,000 cells/mL at dry-off and post-calving.
[b] Quarters with SCC ≥ 200,000 cells/mL at dry-off and <200,000 cells/mL at post-calving.
[c] Quarters with SCC < 200,000 cells/mL at dry-off and ≥200,000 cells/mL at post-calving.
[d] Quarters with SCC < 200,000 cells/mL at dry-off and calving.
[e] Cows with SCC ≥ 200,000 cells/mL at the last and first DHI test between lactations.
[f] Cows with SCC ≥ 200,000 cells/mL at the last test and <200,000 cells/mL at the first DHI test.
[g] Cows with SCC < 200,000 cells/mL at the last test and ≥200,000 cells/mL at the first DHI test.
[h] Cows with SCC < 200,000 cells/mL at the last and first DHI test between lactations.

each quarter within each cow (Barkema et al., 1997). The baseline category logit model was constructed using PROC GLIMMIX of SAS (SAS Institute, 2008). The PROC GLM (SAS Institute, 2008) was used to test the equality of quarter mean SCC from samples collected at dry-off, post-calving and first DHI test. The Tukey–Kramer test (Pagano and Gauvreau, 2000) was used to assess differences between individual means. The PROC NPAR1WAY (SAS Institute, 2008) was used to perform a nonparametric comparison of means (Wilcoxon test) to assess the equality of mean quarter SCC grouped by microbiological outcome (IMI caused by CNS, *Streptococci*, Gram-negative pathogen, Coryneforms, other pathogen or no growth). When SCC was used as a response variable, SCC was transformed to somatic cell scores (SCS) according to the formula SCS = log2 (SCC/100) + 3 (Shook, 1993).

2.3.3. Analysis at the cow level

The relationship between milk yield at the first DHI test (continuous response variable) and explanatory variables (Table 1) was assessed using an analysis of variance (ANOVA) model (Kleinbaum et al., 2007) (model 3):

$$Y_{ijkl} = \alpha + \beta(\text{dry period length}_i) + \text{SCCstatus}_j + \text{Calv season}_k + \text{Parity}_l + \varepsilon_{ijkl}$$

where Y is the mean milk production on the first DHI test day of the subsequent lactation, α is the intercept, dry period length$_i$ is the length of the dry period for the cow i, SCCstatus$_j$ is the SCC status (j = CECHRI, CENEWI, CECURE, CEUNIN), Calv season$_k$ is the calving season (k = Fall, Winter, Spring, Summer), Parity$_l$ is the parity status (l = 2, 3, 4 and >4) and ε_{ijkl} is a residual term, assumed $\varepsilon_{ijkl} \sim N(0, \sigma^2_\varepsilon)$. The ANOVA was performed using PROC GLM of SAS (SAS Institute, 2008). The PROC GLM (SAS Institute, 2008) was used to perform an analysis of variance to compare the mean SCS from DHI samples collected at the last test of the previous lactation and the first test of the subsequent lactation. The level of statistical significance was set at 0.05 for all analyses.

3. Results

Of cows (n = 218 cows, 861 quarters) initially enrolled, complete data were obtained from 205 cows (809 quarters). Data from 13 cows (52 quarters) were not used because the cows left the herd (n = 11 cows, 44 quarters) or died (n = 2 cows, 8 quarters) before completion of the study. One cow died immediately after calving as a result of toxic mastitis. Less than 1.5% of all duplicate quarter milk samples collected at dry-off (n = 1618), post-calving (n = 1618), and at the first DHI test (n = 1618) were contaminated.

Of enrolled cows with complete data (n = 205), 60.9, 19.5, 9.3 and 10.2% were of parity 2, 3, 4 and greater than 4, respectively. The distribution of parturitions (n = 205) in seasons of the year was: Summer (18.6%); Fall (32.7%); Winter (35.6%) and Spring (12.3%). The dry period had a mean length of 68 days, which was close to the industry standards. The average SCS in the previous lactation was 2.6 (SD = 1.3) and milk yields for the last DHI test in the previous lactation and first DHI test in the current lactation were 24.7 kg (SD = 5.8) and 36.8 kg (SD = 8.8), respectively.

3.1. Microbiological results

Coagulase-negative *Staphylococci* were the most prevalent pathogens causing IMI at all sampling periods. The distribution of microbiological results for samples collected at dry-off (n = 804), calving (n = 808) and the first DHI test (n = 805) was no growth (88–93%), CNS (3–7%), *Streptococci* and Strep-like bacteria (2–3%), Gram-negative (≤1%) and others (1–2%). No *Streptococcus agalactiae* and *S. aureus* were ever isolated. Of all quarters from which an IMI was diagnosed at dry-off (n = 102), 91 (89.2%) did not have an IMI present at post-calving (cured IMI). Forty-five quarters (6.4%) which did not have an IMI present at dry-off had an IMI detected post-calving (new IMI) and 10 quarters (9.8%) had an IMI present at both dry-off and post-calving (chronic IMI) (Table 2).

Of quarters from which IMI was caused by CNS isolated from dry-off milk samples (n = 64), microbiological results of post-calving quarter milk samples were: different species of CNS (n = 2); *Streptococci* (n = 1); Gram-negative (n = 1) and bacteriologically negative (n = 60). Of quarters from which IMI was caused by *Streptococci* isolated from dry-off milk samples (n = 23), microbiological results of post-calving quarter milk samples were: different species of *Streptococci* (n = 2); Gram-negative (n = 1); mixed IMI (n = 1); CNS (n = 1) and bacteriologically negative (n = 17). Of quarters from which IMI was caused by *Corynebacterium* spp. or *A. pyogenes* isolated from dry-off milk samples (n = 10), microbiological results of post-calving quarter milk samples were: same species of pathogen (*C. bovis*; n = 1) or bacteriologically negative (n = 9 quarters). Of quarters from which IMI was caused by Gram-negative pathogens (n = 2) isolated from dry-off milk samples, all post-calving milk samples were bacteriologically negative. Of quarters from which IMI was caused by mixed pathogens (n = 3) isolated from dry-off milk samples, all post-calving milk samples were bacteriologically negative.

The distribution of pathogens detected in new post-calving IMI (isolated in the post-calving milk samples but not the dry-off samples from the same quarter) was different (P = 0.04) from the distribution of pathogens detected in cured infections (isolated in the dry-off milk samples but not post-calving). Coagulase-negative *Staphylococci* and *Streptococci* were the most frequent pathogens detected in new post-calving IMI (Table 3). The most frequent microorganisms related to cured IMI were CNS (66%, Table 3). Of the 10 quarters defined as QECHRI, only 1 had the same species of pathogen isolated from dry-off and post-calving samples (*C. bovis*). Two quarters had IMI caused by the same pathogen genus (CNS) at dry-off and post-calving, but not the same species. Two quarters had CNS isolated from dry-off milk samples and different pathogens isolated from post-calving milk samples (*Streptococci* and *E. coli*, respectively); two quarters had a streptococcal IMI at both dry-off and post-calving sampling periods (different species) and three quarters had a streptococcal IMI at dry-off and a subsequent IMI caused by *Klebsiella* spp., CNS and mixed

Table 2
Prevalence of intramammary infection (IMI) and estimated number of quarters and cows with chronic, new and cured IMI across the dry period, estimated using somatic cell count (SCC) and bacteriological examination of milk.

	Estimated prevalence of IMI				Status across the dry period					
	Last DHI test	Dry-off	Post-calving (days 2–9)	First DHI test	Chronic IMI[a]	ECHRI[b]	New IMI[c]	ENEWI[d]	Cured IMI[e]	ECURI[f]
Quarter level										
Bacteriology[g]										
N (%)		102 (12.8%)	55 (6.9%)	68 (8.5%)	10 (9.8%)		45 (6.4%)		91(89.2%)	
Total		804	808	805	102		702		102	
Quarter SCC[h]										
N (%)		302 (37.4%)	156 (19.3%)	92(11.4%)		76(25.2%)		79(15.6%)		225 (74.5%)
Total		807	808	806		302		505		302
Cow level										
Bacteriology[i]										
N (%)		71 (34.6%)		43(21.1%)	22 (31.0%)		21(15.7%)		49 (69.0%)	
Total		205		204	71		134		71	
DHI SCC[j]										
N (%)	55 (27.0%)			48(23.5%)		18 (32.7%)		30(20.1%)		37 (67.3%)
Total	204			204		55		149		55

[a] Quarter which were defined as infected at dry-off and post-calving or cows that were infected both dry-off and first DHI test of the subsequent lactation.
[b] Quarters with SCC ≥ 200,000 cells/mL at dry-off and post-calving or cows with SCC ≥ 200,000 cells/mL at the last and first DHI tests of two successive lactations.
[c] Quarters defined as uninfected at dry-off and infected at post-calving or cows uninfected at dry-off and infected (at least one quarter) at the first DHI test of the subsequent lactation.
[d] Quarters with SCC < 200,000 cells/mL at dry-off and SCC ≥ 200,000 cells/mL at post-calving or cows with SCC < 200,000 cells/mL at the last DHI test and SCC ≥ 200,000 cells/mL at the first DHI test of the subsequent lactation.
[e] Quarters defined as infected at dry-off and uninfected at post-calving or cows infected at dry-off and uninfected at the first DHI test of the subsequent lactation.
[f] Quarters with SCC ≥ 200,000 cells/mL at dry-off and SCC < 200,000 cells/mL at post-calving or cows with SCC ≥ 200,000 cells/mL at the last DHI test and SCC < 200,000 cells/mL at the first DHI test of the subsequent lactation.
[g] Estimated IMI defined based on isolation of mastitis pathogens from quarter milk samples.
[h] Estimated IMI defined based on quarter milk samples with SCC ≥ 200,000 cells/mL.
[i] Estimated IMI defined based on cows from which mastitis pathogens were isolated from at least one quarter milk sample.
[j] Estimated IMI defined based on composite milk samples with SCC ≥ 200,000 cells/mL.

Table 3
Microorganisms related to new and cured IMI across the dry period.

Microbiological results	New IMI[a]	Cured IMI[b]
Gram-negative		
Enterobacter sakazaki	2 (4.4%)	
Escherichia coli	1 (2.2%)	2 (2.2%)
Serratia marscense	1 (2.2%)	
Klebsiella oxytoca	1 (2.2%)	
Gram-positive		
Arcanobacter pyogenes		3 (3.3%)
Corynebacterium bovis	3 (6.7%)	5 (5.5%)
Corynebacterium renale		1 (1.1%)
Coagulase-negative Staphylococci	21 (46.7%)	60 (65.9%)
Strepcococcus no ID[c]		5 (5.5%)
Streptococcus dysgalactiae		3 (3.3%)
Streptococcus uberis	7 (15.6%)	1 (1.1%)
Enterococcus spp.	3 (6.7%)	1 (1.1%)
Aerococcus viridians	2 (4.4%)	5 (5.5%)
Micrococcus spp.		2 (2.2%)
Bacillus spp.	1 (2.2%)	
Yeast	1 (2.2%)	
Mixed infection	2 (4.4%)	3 (3.3%)
Total IMI	45 (100%)	91 (100%)

[a] Intramammary infection diagnosed in the same quarter from post-calving milk samples but not from dry-off milk samples.
[b] Intramammary infection diagnosed in the same quarter from dry-off milk samples but not from post-calving milk samples.
[c] API results obtained with < 90% of confidence at species level.

IMI (*Aerococcus viridans* and *Enterococcus durans*) at post-calving.

3.2. Somatic cell count and patterns of estimated subclinical mastitis across the dry period

The mean SCS was greatest for quarter milk samples obtained at dry-off, decreased for milk samples obtained post-calving and was least for milk samples obtained at the first DHI test (Table 4). The SCS of composite milk samples collected from the last DHI test of the previous lactation was not different from the SCS of samples collected on the first DHI test day ($P = 0.09$; Table 4). For all sampling periods, milk samples from uninfected quarters had mean SCS less than quarters infected with CNS, *Streptococci* and Gram-negative bacteria (Table 5). Except from dry-off milk samples, SCS from quarters infected with Coryneform bacteria were not different from SCS from uninfected quarters. Quarters infected with Gram-negative pathogens had greater SCS than quarters infected with CNS at the first DHI test sampling period ($P = 0.02$; Table 5).

At the quarter-level, the prevalence of subclinical mastitis at dry-off ($P < 0.01$), post-calving ($P < 0.01$) and first DHI test ($P = 0.05$), was always greater when estimated by use of a SCC threshold than IMI based on isolation of pathogens (Table 2). The proportion of quarters classified as having chronic, new or cured IMI across the dry period were

Table 4
Somatic cell count (cells/mL × 1000) of milk samples by sampling period.

	Last DHI[a]			Dry-off[b]			Calving[c]			First DHI[d]		
	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Quarter SCC[e]				372.0	715.0	807	275.4	782.3	808	203.9	658.2	806
Quarter SCS[f]				3.3[a]	2.2	807	2.7[b]	1.9	808	1.6[c]	2.1	806
DHI SCC[g]	263.2	524.2	204							301.6	727.6	204
DHI SCS[h]	3.1[a]	1.8	204							2.8[a]	2.7	204

Means within a row with different superscript letters (a–c) differ ($P < 0.05$).
[a] Milk samples collected at the last DHI test of the previous lactation.
[b] Milk samples collected at dry-off.
[c] Milk samples collected 2–9 days post-calving.
[d] Milk samples collected at the first DHI test of the subsequent lactation.
[e] Individual quarters somatic cell count.
[f] Individual quarters somatic cell score.
[g] Composite DHI somatic cell count.
[h] Composite DHI somatic cell score.

different when estimated by use of a SCC threshold, as compared to IMI based on recovery of pathogens ($P < 0.01$). A greater proportion of chronically infected quarters and new IMI across the dry period was found when estimated using a SCC threshold, as compared to microbiological examination of milk, whereas the proportion of cured IMI was less when estimated by quarter SCC.

At the cow level, the prevalence of subclinical mastitis at the end of lactation was not different ($P = 0.09$) when estimated using DHI SCC from the last DHI test and microbiological examination of milk from samples collected at dry-off. At the first DHI test of the subsequent lactation, the prevalence of subclinical mastitis was not different when estimated by using DHI SCC and microbiological examination of milk ($P = 0.47$). The proportion of cows with chronic, new and cured IMI across the dry period was not different when estimated using DHI SCC threshold, as compared to IMI based on recovery of pathogens ($P > 0.33$).

The sensitivity of a SCC threshold of 200,000 cells/mL for detection of subclinical IMI in quarter milk samples was similar (0.64–0.69) at dry-off, post-calving and the first DHI test sampling periods. The specificities of that same threshold were 0.66 (dry-off), 0.84 (post-calving) and 0.93 (first DHI test) (Table 6). As compared to the use of individual quarter SCC, the use of composite milk DHI SCC samples resulted in reduced sensitivities and specificities for detection of IMI at all sampling periods (Tables 6 and 7). However, positive predictive values tended to be greater when estimated using composite DHI SCC, as compared to individual quarter SCC.

Table 5
Somatic cell score by pathogen group for microbiologically positive quarter milk samples.

Pathogen	Dry-off[a]		Calving[b]		First test[c]	
	Mean	*N*	Mean	*N*	Mean	*N*
Gram-negative	6.86[ab]	2	6.67[a]	7	6.00[b]	12
Mixed	5.70	3	4.77	3		0
Streptococci	5.19[b]	28	5.81[a]	15	5.52[ab]	21
Coryneforms[d]	4.79[ab]	10	4.00[ab]	4	4.17[abc]	3
Staphylococci	4.46[a]	59	4.84[a]	24	3.84[a]	26
No growth	3.03[c]	702	2.48[b]	753	1.30[c]	737
Others		0	6.01	2	6.46	6
Total	3.25	804	2.66	808	1.59	805

Means within a column with different superscript letters (a–c) differ ($P < 0.05$).
[a] Milk samples collected at dry-off.
[b] Milk samples collected 2–9 days post-calving.
[c] Milk samples collected at the first DHI test of the subsequent lactation.
[d] *Corynebacterium* spp. and *Arcanobacter pyogenes*.

3.3. Risk of subclinical mastitis in the subsequent lactation and milk production

3.3.1. Quarter-level analysis

Quarters which were classified as QECHRI across the dry period were 11.8 times more likely to be subclinically infected (presence of IMI) at the first DHI test than quarters which were classified as QEUNIN across the dry period (model 1; odds ratio, 11.8; confidence interval for odds ratio, 4.8–29.5) (Table 8).

Table 6
Sensitivity, specificity, positive predictive value (PPV) and negative predicted value (NPV) of quarter milk somatic cells count used to detect subclinical intramammary infection.

Period	Threshold (cells/mL × 1000)	Sensitivity	Specificity	PPV	NPV
Dry-off	50	0.94	0.37	0.18	0.98
	100	0.88	0.52	0.21	0.97
	150	0.76	0.60	0.22	0.95
	200	0.64	0.66	0.22	0.93
	250	0.51	0.72	0.21	0.91
	300	0.49	0.76	0.23	0.91
Post-calving	50	0.89	0.43	0.10	0.98
	100	0.78	0.67	0.14	0.98
	150	0.76	0.78	0.20	0.98
	200	0.69	0.84	0.24	0.97
	250	0.61	0.87	0.26	0.97
	300	0.57	0.89	0.27	0.97
First DHI[a]	50	0.82	0.75	0.23	0.98
	100	0.69	0.87	0.31	0.97
	150	0.66	0.92	0.41	0.97
	200	0.65	0.93	0.46	0.97
	250	0.60	0.95	0.49	0.96
	300	0.58	0.95	0.51	0.96

[a] First DHI test of the subsequent lactation.

Table 7
Sensitivity, specificity, positive predictive value (PPV) and negative predicted value (NPV) of composite milk somatic cells count used to detect subclinical intramammary infection.

Period	Threshold (cells/mL × 1000)	Sensitivity	Specificity	PPV	NPV
Last DHI[a]	50	0.86	0.40	0.43	0.84
	100	0.63	0.63	0.47	0.76
	150	0.51	0.69	0.47	0.73
	200	0.40	0.80	0.51	0.72
	250	0.34	0.86	0.56	0.71
	300	0.30	0.88	0.57	0.71
First DHI[b]	50	0.81	0.51	0.31	0.91
	100	0.65	0.70	0.37	0.88
	150	0.58	0.78	0.42	0.87
	200	0.47	0.82	0.41	0.85
	250	0.40	0.86	0.44	0.84
	300	0.37	0.88	0.44	0.84

[a] Last DHI test of the previous lactation.
[b] First DHI test of the subsequent lactation.

Quarters which were classified as QECHRI across the dry period were 24 times more likely to be subclinically infected by a major pathogen (rather than uninfected) and 5 times more likely to be subclinically infected by a minor pathogen (rather than uninfected) at the first DHI test than quarters which were classified as QEUNIN across the dry period (model 2, $P < 0.01$, Table 8). Quarters which were defined as QENEWI were 4 times more likely to be subclinically infected by a minor pathogen (rather than being uninfected), than quarters which were defined as QEUNIN across the dry period (model 2, $P < 0.01$).

3.3.2. Cow level analysis

Composite milk SCC status across the dry period (CECHRI, CENEWI, CECURI and CEUNIN) was associated with milk yield at the first DHI test ($P < 0.01$, model 3, Table 8). Cows estimated as CECHRI or CENEWI produced 9.2 and 4.9 kg less milk on the first DHI test day than the average milk production of CEUNIN, respectively.

4. Discussion

4.1. Risk of IMI at the first DHI test based on SCC status across the dry period

Results of our study suggest that quarters with SCC ≥ 200,000 cells/mL at dry-off and post-calving were much more likely to be subclinically infected at the first DHI test of the subsequent lactation. Major pathogens (mostly environmental *Streptococci*) were the main organisms related to these subclinical infections. In contrast, minor pathogens were the main organisms related to quarters defined as QENEWI across the dry period. Quarters defined as QENEWI were 4 times more likely to be infected by minor pathogens than quarters defined as QEUNIN mostly due to IMI caused by CNS. Even though parity was not statistically significant in the full models, we did not remove this variable from the final models due to its importance as a risk factor associated with the development of clinical mastitis (Pantoja et al., 2009) and suggest that parity information be used along with SCC when making individual cow decisions.

4.2. Dynamics of IMI across the dry period

In agreement with previous research (Oliver and Mitchell, 1983; Smith et al., 1985; Green et al., 2005), most pathogens causing IMI at dry-off did not survive through the dry period. One exception was IMI caused by *Corynebacterium* spp., in which a single quarter had *C. bovis* isolated from both the dry-off and post-calving samples. In a previous study, approximately two-thirds ($n = 35$) of quarters from which *Corynebacterium* spp. were isolated before calving ($n = 55$) the same species was also cultured from milk samples obtained at dry-off (Green et al., 2005). Although several previous studies (Godden et al., 2003; Cook et al., 2005) have demonstrated that CNS were the most prevalent pathogens causing IMI at dry-off, the proportion of specific pathogens causing new IMI during the dry period has varied among studies (Dingwell et al., 2002; Godden et al., 2003; Cook et al., 2005). Similar to another Wisconsin study (Cook et al., 2005), most of the new IMIs (46.7%) that occurred in our study were caused by CNS. In contrast, Dingwell et al. (2002) and Godden et al. (2003) found a greater proportion of new IMI present at post-calving sampling periods caused by environmental *Streptococci* than IMI caused by CNS. Green et al. (2005) reported that *Escherichia coli* was isolated at least once from 12% ($n = 113$) of all quarters ($n = 957$) that were sampled 3 times during the late dry to calving period, but only 2% of those isolates were isolated twice and 0.01% 3 times from the same quarter. However, care should be taken when comparisons are made among studies that used different sampling methodologies and definitions of IMI. In our study, we did not obtain dry period secretions and all quarters received DCT and an internal teat sealant after dry-off. Likewise, microbiological procedures vary among studies. For example, the definition of IMI caused by CNS was growth of 500 CFU/mL from a single milk sample (Dingwell et al., 2002; Cook et al., 2005), as compared to 300 CFU/mL recovered from both duplicate milk samples used in our study. We enrolled cows that received DCT in all quarters because this management practice is widely used in the target population of dairy herds to which results of this study can be extrapolated. Rodrigues et al. (2005) reported that 93.3% of Wisconsin herds enrolled in a milk quality program used DCT in all quarters of all cows. Therefore, interpretation of the results of this study should be conditional to cows that received DCT and an internal teat sealant.

The similarities between SCC status across the dry period, prevalence of IMI at dry-off and pathogen profiles observed in this study and previous recent data support the generalization of our results to a broader population of Wisconsin herds. Cook et al. (2002) reported CENEWI and CECURI from 145 Wisconsin herds as 22.4 and 62.9%, which was similar to results from our study (CENEWI: 20.1% and CECURI: 67.3%). Likewise, the prevalence of IMI and the proportion of IMI caused by CNS at dry-off that were observed in the present study (12.8 and 8%, respectively), were very similar to that reported by Cook et al. (2005) for three commercial herds in Wisconsin.

Table 8
Results of the three final statistical models for which the response variables were the probability of IMI at the first DHI test (model 1), the probability of IMI caused by grouped pathogens (model 2) and milk production (model 3).

Explanatory variables	Coefficient	Standard error	*P*-value	Odds ratio[a]	95% C.I.[b]	
					LCL	UCL
Model 1						
Intercept	−3.10	0.57				
SCCstatus			<0.01			
QECHRI[c]	2.47	0.47		11.84	4.75	29.54
QECURI[d]	0.40	0.47		1.49	0.64	3.45
QENEWI[e]	1.12	0.48		3.07	1.20	7.83
QEUNIN[f]	Reference					
Parity			0.61			
2	−0.03	0.55		0.97	0.33	2.87
3	−0.53	0.60		0.59	0.18	1.90
4	0.22	0.65		1.25	0.35	4.46
>4	Reference					
δ (random term)	1.29	0.40				
Model 2						
Intercept (major pat)	−4.91	0.81				
Intercept (minor pat)	−2.96	0.72				
SCCstatus			<0.01			
QECHRI[c] (major)	3.19	0.60		24.43	7.42	79.10
QECHRI[c] (minor)	1.62	0.63		5.04	1.45	17.54
QECURI[d] (major)	0.91	0.58		2.48	0.83	7.43
QECURI[d] (minor)	−0.17	0.62		0.84	0.25	2.83
QENEWI[e] (major)	0.60	0.74		1.82	0.42	7.84
QENEWI[e] (minor)	1.41	0.60		4.10	1.28	13.17
QEUNIN[f] (major)	Reference					
QEUNIN[f] (minor)	Reference					
Parity			0.19			
2 (major)	1.16	0.75		3.18	0.72	14.01
2 (minor)	−1.04	0.72		0.36	0.09	1.46
3 (major)	−0.50	0.90		0.61	0.10	3.57
3 (minor)	−0.66	0.72		0.52	0.13	2.12
4 (major)	0.97	0.85		2.63	0.50	13.83
4 (minor)	−0.33	0.84		0.72	0.14	3.72
>4 (major)	Reference					
>4 (minor)	Reference					
δ (major)	1.23	0.56				
δ (minor)	1.81	0.57				
Model 3						
Intercept	38.80	2.20				
SCCstatus			<0.01			
CECHRI[g]	−9.18	2.34				
CECURI[h]	−1.52	1.70				
CENEWI[i]	−4.92	1.75				
CEUNIN[j]	Reference					
Parity			0.84			
2	−0.74	2.20				
3	0.27	2.37				
4	−1.61	2.68				
>4	Reference					

[a] Odds of developing subclinical mastitis at the first DHI test (model 1) and odds of IMI caused by a major pathogen (rather than being uninfected) at the first DHI test (model 2).
[b] Confidence interval for odds ratio (lower and upper confidence limits).
[c] Quarters with SCC ≥ 200,000 cells/mL at dry-off and post-calving.
[d] Quarters with SCC ≥ 200,000 cells/mL at dry-off and <200,000 cells/mL at post-calving.
[e] Quarters with SCC < 200,000 cells/mL at dry-off and ≥200,000 cells/mL at post-calving.
[f] Quarters with SCC < 200,000 cells/mL at dry-off and calving.
[g] Cows with SCC ≥ 200,000 cells/mL at the last and first DHI test between lactations.
[h] Cows with SCC ≥ 200,000 cells/mL at the last test and <200,000 cells/mL at the first DHI test.
[i] Cows with SCC < 200,000 cells/mL at the last test and ≥200,000 cells/mL at the first DHI test.
[j] Cows with SCC < 200,000 cells/mL at the last and first DHI test between lactations.

4.3. Characteristics of an SCC test threshold of 200,000 cells/mL to estimate IMI across the dry period

We collected milk samples immediately before drying off and in the early post-partum period (6 DIM, range = 2–9) and it is possible that physiologic responses unrelated to infection may have caused some quarters to exceed the SCC threshold. In one study (Barkema et al., 1999), the geometric mean SCC of cows with culture-negative quarters (milked twice a day) decreased from 588,000 cells/mL on the first day post-calving to 166,000 cells/mL on the third day after calving, suggesting that SCC status estimated from quarter samples obtained in the very early post-partum period may overestimate the proportion of IMI. The SCC of very low producing cows has been reported to increase at dry-off but the difference was not significant for cows producing more than 4 kg/day on the last DHI test (Bodoh et al., 1976). Milk production at the last test before dry-off was 24.7 kg/per cow per day (range = 11.8–42.3) for cows included in this study; thus, a dilution effect of milk on SCC was not likely to have influenced SCC performance used as a diagnostic test at dry-off.

Somatic cell counts' thresholds are used to make many management decisions. As with any diagnostic test, errors occur when exclusively depending on a single test (Schukken et al., 2003) and in order to minimize the amount of error, diagnostic test parameters such as sensitivity and specificity have been calculated for various SCC thresholds (Schepers et al., 1997). The choice of SCC threshold depends on the purpose of the test. Lowering the threshold increases sensitivity and consequently provides minimal false-negative results whereas raising the threshold increases specificity, providing minimal false-positive results (Dohoo et al., 2003).

We used a SCC threshold of 200,000 cells/mL, which has been considered optimal to reduce diagnostic error when detecting IMI (Dohoo and Leslie, 1991; Schepers et al., 1997; Djabri et al., 2002). Many DHI reports in the United States summarize SCC data using a threshold of 200,000 cells/mL. The use of this threshold was selected to ensure the direct applicability of the results of this study to commercial dairy farms. The use of a 200,000 cells/mL SCC threshold has resulted in sensitivities that range from 0.73 to 0.89 and specificities that range from 0.75 to 0.90 (McDermott et al., 1982; Dohoo and Leslie, 1991; Schepers et al., 1997). Two main aspects can explain the differences in diagnostic test characteristics observed between our study and previously reported data. First, previous studies were conducted in herds in which contagious pathogens (such as *S. agalactiae* and *S. aureus*) were among causative pathogens, in contrast to this herd where the distribution of subclinical pathogens tended to be skewed toward minor pathogens. Intramammary infections caused by minor pathogens (such as CNS) normally induce less intense SCC responses in milk than those caused by major pathogens (Sheldrake et al., 1983; Barkema et al., 1999; Schukken et al., 2003). Cows infected with minor pathogens have been reported to have composite SCC that ranged from 190,000 to 519,000 cells/mL as opposed to cows infected with major pathogens, which had SCC greater than 600,000 cells/mL (Sheldrake et al., 1983; Barkema et al., 1999; Schukken et al., 2003). In our study, the mean SCS for IMI caused by minor pathogens was very close to the threshold of 200,000 cells/mL (SCS = 4) at all sampling periods and even less than the threshold at the first DHI test (Table 5). As a consequence, an increased number of quarters or cows misclassified as uninfected (false negative results) can be expected and, in fact, it dramatically increased the specificity of the SCC test to detect IMI. Second, the lack of a true gold standard for diagnosis of IMI results in ambiguity regarding epidemiological characteristics of diagnostic tests for subclinical mastitis. Bacteriological examination of milk has limitations for diagnosing IMI and strategies such as the collection of duplicate milk samples, inoculation of greater milk volumes and the use of pre-incubation are often used to enhance the accuracy of this technique. We collected duplicate quarter milk samples to minimize the number of false positive microbiological results (quarters misclassified as infected). As a result, a reduction in sensitivity and an increase in specificity of SCC as diagnostic test for identifying IMI might be expected as compared to the use of single quarter milk samples. A greater volume of milk or a detection of fewer than 300 CFU/mL would also have increased the sensitivity of the SCC test for detection of IMI but also the number of false positive results. These characteristics might therefore explain why the prevalence of IMI at dry-off and post-calving and the proportion of new and chronic IMI across the dry period was always greater when estimated by using quarter SCC, as compared to microbiological examination of milk.

At the cow level (composite milk DHI SCC), further aspects need to be considered in interpreting the SCC test. Most cows (>63% for all screening periods) had only one infected quarter (based on microbiological examination of milk). Thus, fewer numbers of somatic cells in milk from the other uninfected quarters might have decreased the composite milk SCC, which also resulted in an increased number of cows misclassified as negative and decreased sensitivities of the DHI SCC test, as compared to individual quarter SCC. However, because of the influence of the prevalence of IMI on the predictive value of a positive SCC test (Dohoo et al., 2003), greater positive predictive values of the SCC test were observed at the cow level, as compared to individual quarter SCC (the estimated prevalence at the cow level was always greater than that estimated using individual quarter SCC). In this study, SCC of composite milk samples from the last DHI test of lactation were used to estimate the presence of IMI at the cow level whereas cow IMI status based on microbiological examination of milk was defined based on the isolation of mastitis pathogens from milk samples collected on the dry-off day. If IMI status changed (e.g. cows developed IMI or cured of IMI during this time interval) (median = 16 days; range = 0–32), the estimation of IMI at the cow level using DHI SCC threshold at the end of lactation would have been biased. While this is a weakness of the study, there is no indication that this period was a high-risk period for exposure to mastitis pathogens and it is unlikely that this gap influenced study outcomes.

The specificities of quarter SCC used for the diagnosis of IMI progressively increased from samples collected at

dry-off, to post-calving to the first DHI test. During the post-calving period the use of a 200,000 cells/mL threshold resulted in specificities that were greater than 90%. Negative predictive values were greater than 95% for post-calving and first DHI test samples, which indicates that quarters identified as uninfected by using SCC were likely truly uninfected. Therefore, characteristics of SCC as diagnostic test found in this study are probably typical of modern dairy herds that have successfully controlled contagious mastitis pathogens.

The classification of SCC status across the dry period can be a valuable tool for monitoring milk quality. In addition to identification of quarters with increased risk of subclinical and clinical mastitis in a subsequent lactation, the monitoring of SCC status across the dry period can also aid the identification of cows which will probably produce less milk due to the presence of IMI. Cows defined as CECHRI and CENEWI produced 9.2 and 4.9 kg less milk than the average milk production of cows defined as CEUNIN at the first DHI test, respectively. Further research is needed to assess interventions directed to groups or individual quarters and cows identified by using SCC status across the dry period.

5. Conclusions

Similar to other modern dairy farms, CNS were the most common pathogens related to new IMI that were not present at dry-off but isolated from samples collected from the post-calving sampling period. Most of the chronic IMI across the dry period were not caused by the same pathogen. Quarters and cows with SCC $\geq$ 200,000 cells/mL across the dry period had a greater risk of being subclinically infected by a major pathogen at the first DHI test and produced less milk than quarters and cows with SCC $<$ 200,000 cells/mL across the dry period. A SCC threshold of 200,000 cells/mL identified bacteriologically negative quarters and cows with a margin of error less than quarters and cows with IMI, in a herd with a very low prevalence of contagious pathogens and a large proportion of subclinical mastitis caused by CNS.

Conflict of interest statement

We authors state that there was not any kind of financial or personal relationship with other people or organizations that could inappropriately influence the results of our work.

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