The Influence of Cow Factors on the Incidence of Clinical Mastitis in Dairy Cows

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ABSTRACT

Many cow-specific risk factors for clinical mastitis (CM) are known. Other studies have analyzed these risk factors separately or only analyzed a limited number of risk factors simultaneously. The goal of this study was to determine the influence of cow factors on the incidence rate of CM (IRCM) with all cow factors in one multivariate model. Also, using a similar approach, the probability of whether a CM case is caused by gram-positive or gram-negative pathogens was calculated. Data were used from 274 Dutch dairy herds that recorded CM over an 18-mo period. The final dataset contained information on 28,137 lactations of 22,860 cows of different parities. In total 5,363 CM cases were recorded, but only 2,525 CM cases could be classified as gram-positive or gram-negative. The cow factors parity, lactation stage, season of the year, information on SCC from monthly test-day records, and CM history were included in the logistic regression analysis. Separate analyses were performed for heifers and multiparous cows in both the first month of lactation and from the second month of lactation onward. For investigating whether CM was caused by gram-positive or gram-negative pathogens, quarter position was included in the logistic regression analysis as well. The IRCM differed considerably among cows, ranging between 0.0002 and 0.0074 per cow-day at risk for specific cows depending on cow factors. In particular, previous CM cases, SCC in the previous month, and mean SCC in the previous lactation increased the IRCM in the current month of lactation. Results indicate that it is difficult to distinguish between gram-positive and gram-negative CM cases based on cow factors alone.

Key words: clinical mastitis, incidence rate, dairy cow

INTRODUCTION

Mastitis is one of the most frequent and costly diseases (e.g., Halasa et al., 2007). Many factors influence

the incidence of clinical mastitis. These factors may include herd (e.g., Schukken et al., 1991; Barkema et al., 1999; Nyman et al., 2007) and cow factors (e.g., Barkema et al., 1998; Suriyasathaporn et al., 2000; Olde Riekerink et al., 2007). In an individual herd, cow factors are responsible for the difference among cows in having clinical mastitis (CM). It may be worthwhile to know which specific cows have the highest risk for CM. With this information, the farmer may give these cows more attention. This would be particularly useful on farms with an automatic milking system (AMS) because no human is present during the milking process to check the milk visually for abnormalities (Hogeveen and Ouweltjes, 2003). When milking with an AMS, cows to be checked for CM are currently selected based on results of sensor measurements only.

The association between cow factors and CM has been studied frequently. Somatic cell count is widely considered to be one of the most important risk factors for CM, with both high SCC (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Green et al., 2004), and very low SCC associated with an increased risk of subsequent CM (Suriyasathaporn et al., 2000; Peeler et al., 2003; Green et al., 2004). Existing research has established a number of facts about CM. For example, CM most often occurs early in lactation (e.g., Miltenburg et al., 1996; Barkema et al., 1998; Svensson et al., 2006), heifers have the lowest incidence rate of CM (IRCM), except in the first week of lactation (Barkema et al., 1998), and cows that have had CM once have a greater risk for CM later during lactation (Houben et al., 1993; Lam et al., 1997; Zadoks et al., 2001).

Most studies investigating associations between possible cow factors and CM studied the effect of one or a limited number of cow factors; for example, the effect of parity, lactation stage, and SCC on subsequent CM (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000). In one study, the association between IRCM and several cow factors including also CM history was assessed (Houben et al., 1993). No study, however, has analyzed IRCM based on all known cow factors together in one multivariate model.

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Knowing the pathogen involved in a CM case would be very useful. Particularly, a distinction between gram-positive and gram-negative pathogens would be informative, because antimicrobial treatment is necessary when gram-positive bacteria are involved, whereas supportive treatment is more appropriate for gram-negative CM cases (Morin et al., 1998; Pyörälä and Pyörälä, 1998). Inclusion of cow factors may assist in the detection of specific pathogens, but the association between cow factors and the risk for pathogen-specific CM has not been studied frequently (Zadoks et al., 2001; de Haas et al., 2004). de Haas et al. (2002) recommended that other sources such as parity and lactation stage, in addition to SCC test-day records, should be used for a more accurate prediction of the pathogen involved.

Novel in the current study is that several cow-specific risk factors for CM that are readily available on most dairy farms are analyzed together using a unique dataset comprising 22,860 cows. The first objective was to investigate if there were differences in the IRCM among cows based on combined information of cow factors. The second objective was to investigate, based on combined cow factors, whether a CM case was caused by gram-positive or gram-negative pathogens.

MATERIALS AND METHODS

Herds and Data Collection

The data used in the present study were described in detail elsewhere (Barkema et al., 1998). Records on CM were collected from 300 dairy herds entering the study between December 1992 and June 1994. Each herd participated in the study for approximately 1.5 yr. Eight herds did not complete the study because farming activities ceased. All herds had an annual milk production quota between 300,000 and 900,000 kg, and had cows of the Holstein-Friesian or Dutch Friesian breeds. Lactating cows were housed in a free-stall barn during winter and milked in a double-herringbone or 2-sided tandem. During the study, farmers were instructed to collect milk samples from every quarter that had visible signs of CM. Farms were visited monthly to collect milk samples, to motivate for a correct milk sampling procedure, and to provide feedback to the farmer. The samples were taken before treatment, stored in a freezer at the farm (at approximately -20°C), and collected every 6 to 8 wk for bacteriological culture. Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990). In short, 0.01-mL samples of all milk samples was cultured. In each of the cultures, the number of colony-forming units of the bacterial species was counted. The contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) were considered

to cause IMI if 1 colony (100 cfu/mL) was isolated. Isolation of ≥ 200 cfu/mL of environmental mastitis pathogens (*Escherichia coli*, streptococci other than *Strep. agalactiae*, *Klebsiella* spp., and *Pseudomonas* spp.) or $\geq 1,000$ cfu/mL of *Corynebacterium bovis* or CNS was considered significant. Collected data contained information on cow identification, date of occurrence, infected quarter, and the outcome of the bacteriological culturing of the milk samples. At the end of the data collection period, farmers were asked to estimate how many cases of CM were not sampled and not recorded. If this number exceeded 10 and was $>25\%$ of the number of cases sampled, the herd was excluded from analysis of the CM data. In total, 18 herds were excluded from the analysis for this reason (Barkema et al., 1998). The Dutch national milk recording system (Nederlands Rundvee Syndicaat, Arnhem, the Netherlands) provided information from the 3- or 4-weekly milk production system, including cow identification, date of milk recording, date of calving, date of drying off, test-day milk yields (kg of milk, fat, and protein) and SCC (cells/mL) for all cows.

Data Preparation

Originally, the dataset consisted of 120,398 lactations from 39,764 cows with a total of 8,571 CM cases. Only lactations that had been recorded from calving onward were included in the dataset to ensure that no previous cases of CM had occurred within the lactation. For this reason 88,220 lactations were excluded. Lactations were included until dry-off or culling. Subsequently, lactations with no milk production information (in total 3,450 lactations) or a calving interval ≤ 320 or ≥ 600 d (in total, 591 lactations) were excluded from the dataset. Therefore, the final dataset consisted of 28,137 lactations from 22,860 cows with 201,708 test-day records and 5,363 CM cases. All cases of CM during dry-off were excluded. Intervals between pathogen-specific cases of CM in the same quarter had to be ≥ 14 d for a case to be included in the final dataset.

For this study, all CM cases were classified according to their gram status and divided in 3 groups: 1) gram-positive CM: *Strep. dysgalactiae*, *Strep. agalactiae*, *Strep. uberis*, other streptococci, *Staph. aureus*, CNS, *C. bovis*, and *Arcanobacterium pyogenes*; 2) gram-negative CM: *E. coli*, *Pseudomonas*, and *Klebsiella*; and 3) missing: no growth, contaminants, mold or fungi, yeast, and no samples taken. Mixed cultures containing 2 gram-positive pathogens were classified as gram-positive, and those containing 2 gram-negative pathogens as gram-negative. Mixed cultures containing a gram-positive and a gram-negative pathogen were classified as missing. In total, 2,491 gram-positive and 1,007 gram-

Table 1. Description of study variables with their abbreviation and different levels used for analysis

Variable	Abbreviation	Levels used in analysis
Dependent variables		
Clinical mastitis	CM	0 = no 1 = yes
Gram status of clinical mastitis case	GRAM	0 = gram-negative pathogen 1 = gram-positive pathogen
Independent variables		
Parity		1, 2, 3, ≥ 4
Month of lactation		1, 2, 3, ..., ≥ 8
First month of lactation subdivided per 5 days	MONTH1	1, 2, 3, ..., 6
Season		January–March April–June July–September October–December
SCC in previous month of the lactation ¹	SCC1	
Geometric mean SCC of all test-days in previous lactation ¹	SCC2	
Accumulated number of CM cases in the previous month of the lactation	MAST1	0, 1, 2
Accumulated number of CM cases in the month of lactation before the previous month of lactation	MAST2	0, 1, 2
Quarter position		1 = right front 2 = left front 3 = right rear 4 = left rear

¹The natural logarithm was used for these continuous variables.

negative CM cases were identified. For 1,865 CM cases, the gram status was missing.

Using information from literature and the expertise of the authors, cow-specific risk factors for IRCM were defined (Table 1). Only cow-specific risk factors, for which information is usually available on a farm, were included in this study. Of these, parity (Barkema et al., 1998) was known for each cow in the dataset, and lactation (Barkema et al., 1998; Green et al., 2004; Svensson et al., 2006) was divided into 30-d intervals. For every month in lactation, the binary trait having CM or not (1/0) was determined. From previous studies it was known that the IRCM is different in the first part of lactation (Houben et al., 1993; Barkema et al., 1998); therefore, in this study the first 30 d of lactation (**MONTH1**) were subdivided into 6 equal periods of 5 d each. Season of the year (Olde Riekerink et al., 2007) was determined for each month in lactation. The SCC from monthly test-day records (e.g., Beaudeau et al., 1998; Suriyasathaporn et al., 2000; Green et al., 2004) was also included. To determine the association between previous SCC and IRCM in the current month of lactation, SCC in the previous month in lactation (**SCC1**) was determined. Also, SCC of the previous lactation was included (Whist and Østerås, 2006; Green et al., 2007). This variable was defined as the geometric mean SCC from all available test-day records from the previous lactation (**SCC2**). The natural logarithm of both SCC1 and SCC2 was used for analysis. Clinical mastitis history at the cow level (Houben et al., 1993) was defined with 2 variables: the accumulated number

of CM cases in the previous month in lactation (**MAST1**) and the accumulated number of CM cases in the month in lactation before the previous month in lactation (**MAST2**). Because almost no cows with CM information from previous lactation were present in the dataset, no information on CM history from previous lactations was taken into account.

To prepare for the statistical analyses, 6 datasets were created (Table 2), including 4 to determine the IRCM (datasets 1 to 4). Datasets 1 and 2 were created for heifers and multiparous cows in the first 30 d of lactation. These datasets contained no information on SCC1, MAST1, and MAST2, and for heifers no information was available on SCC2. In these datasets, having CM was determined per 5 d. Datasets 3 and 4 were created for heifers and multiparous cows from the second month of lactation onward. In these datasets, having CM was determined per 30 d. Datasets 5 and 6 were created to predict the gram status of the CM cases. Dataset 5 included all heifers and dataset 6 all multiparous cows. In these datasets, all records with missing values for SCC1, SCC2, and quarter position were excluded (in total 973 cases). Dataset 5 contained 262 gram-positive and 102 gram-negative CM cases. Dataset 6 contained 1,526 gram-positive and 635 gram-negative CM cases. In these datasets, information on quarter position was also available.

Statistical Analysis

CM Detection in the First Month of Lactation. Statistical analyses were carried out to determine the

Table 2. Description of the 6 datasets created with the total number of lactations and clinical mastitis (CM) cases in each dataset

Dataset	Description	Lactations, n	CM cases, n	Variables in dataset ¹
1	All heifers in first 30 d of lactation	8,388	472	CM, MONTH1, season
2	All multiparous cows in first 30 d of lactation	19,749	1,145	CM, parity, MONTH1, season, SCC2
3	All heifers from the second month of lactation onward	8,121	526	CM, month in lactation, season, SCC1, MAST1, MAST2
4	All multiparous cows from the second month of lactation onward	19,109	3,220	CM, parity, month in lactation, season, SCC1, SCC2, MAST1, MAST2
5	All CM cases in heifers with known gram status		364	GRAM, month in lactation, season, SCC1, quarter position
6	All CM cases in multiparous cows with known gram status		2,161	GRAM, parity, month in lactation, season, SCC1, SCC2, quarter position

¹MONTH1 = first month of lactation subdivided in 6 equal 5-d periods; SCC1 = SCC in previous month of lactation; SCC2 = geometric mean SCC of all test-days in the previous lactation; MAST1 = accumulated number of CM cases in the previous month of the lactation; MAST2 = accumulated number of CM cases in the month of the lactation before the previous month of the lactation.

association between the independent variables and the IRCM in the first 30 d of lactation for heifers and multiparous cows (dataset 1 and 2), using SAS (PROC GENMOD) version 9.1 (SAS Institute Inc., Cary, NC). Because of the repeated measurements in the data, all datasets were analyzed using generalized estimating equations (GEE; Dohoo et al., 2003) with an exchangeable correlation matrix within herd, a binomial variance, and a logit link. All cow factors were analyzed using a backward stepwise procedure. For a categorical variable, all dummy variables were entered. Only variables at $P \leq 0.05$ in the likelihood ratio test were retained in the model. Goodness of fit of the model was assessed by judging the residuals. The residuals were plotted against the fitted values and judged for peculiarities (Dohoo et al., 2003).

To determine IRCM in a specific period during MONTH1 for multiparous cows, equation [1] was used:

$$\text{IRCM} = \frac{e^{\beta_0 + \beta_1 \times \text{parity} + \beta_2 \times \text{MONTH1} + \beta_3 \times \text{season} + \beta_4 \times \text{SCC2}}}{1 + e^{\beta_0 + \beta_1 \times \text{parity} + \beta_2 \times \text{MONTH1} + \beta_3 \times \text{season} + \beta_4 \times \text{SCC2}}} / 5 \quad [1]$$

where the outcome is the IRCM per cow-day at risk in a specific period during MONTH1; β_0 is the estimated intercept and the regression coefficients (log odds ratios) were estimated for parity (β_1), MONTH1 (β_2), season (β_3), and SCC2 (β_4). Because the data were ordered per 5 d, the IRCM was divided by 5 to calculate an IRCM per cow-day at risk. For heifers, the same model was used, except that the terms parity and SCC2 were omitted because they were not applicable.

CM Detection from the Second Month of Lactation Onward. The statistical analysis to determine the association between the independent variables and IRCM from the second month of lactation onward for heifers and multiparous cows (datasets 3 and 4) was

performed identical to the one described above for datasets 1 and 2. To determine IRCM in a specific month in lactation for multiparous cows, equation [2] was used:

$$\text{IRCM} = \frac{e^{\beta_0 + \beta_1 \times \text{parity} + \beta_2 \times \text{month} + \beta_3 \times \text{season} + \beta_4 \times \text{MAST1} + \beta_5 \times \text{MAST2} + \beta_6 \times \text{SCC1} + \beta_7 \times \text{SCC2}}}{1 + e^{\beta_0 + \beta_1 \times \text{parity} + \beta_2 \times \text{month} + \beta_3 \times \text{season} + \beta_4 \times \text{MAST1} + \beta_5 \times \text{MAST2} + \beta_6 \times \text{SCC1} + \beta_7 \times \text{SCC2}}} / 30 \quad [2]$$

where the outcome is the IRCM per cow-day at risk in a specific month of lactation; β_0 is the estimated intercept and the regression coefficients (log odds ratios) were estimated for parity (β_1), month in lactation (β_2), season (β_3), MAST1 (β_4), MAST2 (β_5), SCC1 (β_6), and SCC2 (β_7). In these analyses, biologically plausible 2-way interactions were also tested (parity by SCC1, season by SCC1, parity by month in lactation, month in lactation by SCC1). Because the data was ordered per 30 d, the IRCM was divided by 30 to calculate an IRCM per cow-day at risk. For heifers, the same model was used, except that the terms for parity and SCC2 were omitted, because they were not applicable.

Gram Status of the CM Cases. The analysis to determine the probability whether CM cases were caused by gram-positive or gram-negative pathogens was performed in a similar way, using GEE (Dohoo et al., 2003) with a binomial variance and a logit link. In these analyses, biologically plausible interactions were also tested (parity by SCC1, season by SCC1, parity by month in lactation, month in lactation by SCC1). Analyses were performed identically to the ones described above, using datasets 5 and 6.

To determine the probability that a CM case was caused by gram-positive pathogens for multiparous cows, equation [3] was used:

$$\text{GRAM} = \frac{e^{\beta_0 + \beta_1 \times \text{parity} + \beta_2 \times \text{month} + \beta_3 \times \text{season} + \beta_4 \times \text{SCC1} + \beta_5 \times \text{SCC2} + \beta_6 \times \text{quarter}}}{1 + e^{\beta_0 + \beta_1 \times \text{parity} + \beta_2 \times \text{month} + \beta_3 \times \text{season} + \beta_4 \times \text{SCC1} + \beta_5 \times \text{SCC2} + \beta_6 \times \text{quarter}}} \quad [3]$$

Table 3. Results of the analysis of cow-specific risk factors for the incidence of clinical mastitis in first month of lactation for heifers (dataset 1) and multiparous cows (dataset 2)¹

Variable	Heifers					Multiparous cows				
	β	SE	OR ²	95% CI for OR	P-value	β	SE	OR ²	95% CI for OR	P-value
Intercept	-6.141	0.250				-6.380	0.221			
Parity					NA ³					0.0417
2						Ref. ⁴	—	1.00		
3						-0.046	0.090	0.96	0.80–1.14	
≥4						0.146	0.073	1.16	1.00–1.34	
MONTH1					<0.0001					<0.0001
0 to 5 d	2.504	0.244	12.23	7.57–19.74		1.385	0.113	3.99	3.20–4.98	
6 to 10 d	1.244	0.264	3.47	2.07–5.82		0.162	0.142	1.18	0.89–1.55	
11 to 15 d	0.791	0.278	2.21	1.28–3.80		-0.034	0.118	0.97	0.77–1.22	
16 to 20 d	-0.003	0.325	0.99	0.53–1.89		-0.017	0.126	0.98	0.77–1.26	
21 to 25 d	0.337	0.295	1.40	0.79–2.50		-0.278	0.138	0.76	0.58–0.99	
26 to 30 d	Ref.	—	1.00			Ref.	—	1.00		
Season					0.0026					0.0030
January–March	Ref.	—	1.00			Ref.	—	1.00		
April–June	-0.318	0.175	0.73	0.52–1.03		0.011	0.103	1.01	0.83–1.24	
July–September	0.143	0.171	1.15	0.83–1.61		0.277	0.098	1.32	1.09–1.60	
October–December	0.304	0.140	1.36	1.03–1.78		0.242	0.093	1.27	1.06–1.53	
SCC2 ⁵					NA	0.222	0.039	1.25 ⁶	1.16–1.35	<0.0001

¹Estimated coefficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor.

²Odds ratio for having clinical mastitis in a specific period in the first month of lactation versus not having clinical mastitis.

³NA = not applicable.

⁴Ref. = reference category.

⁵SCC2 = geometric mean SCC of all available test-day records of the previous lactation.

⁶For an increase in 1 unit of natural logarithm of SCC.

where the outcome is the probability that a CM case was caused by gram-positive pathogens; β_0 is the estimated intercept and the regression coefficients (log odds ratios) were estimated for parity (β_1), month in lactation (β_2), season (β_3), SCC1 (β_4), SCC2 (β_5), and quarter position (β_6). For heifers, the same model was used, except that the terms for parity and SCC2 were omitted, because they were not applicable.

RESULTS

CM Detection in the First Month of Lactation

Results of the multivariate analysis of cow-specific risk factors in the first month of lactation for heifers and multiparous cows are given in Table 3. All cow-specific risk factors in the first month of lactation, described in Table 2, significantly contributed to the fit of the model. Heifers had a much higher IRCM in the first 5 d of lactation compared with other intervals [odds ratio (OR) = 12.2]. The values for OR after d 5 of lactation rapidly decreased to 1. Multiparous cows also had a high OR during the first 5 d of lactation (OR = 4.0), but not as high as for heifers. For both heifers and multiparous cows, season of the year was significantly associated with IRCM. Multiparous cows with a higher

SCC2 had an increased IRCM in the first month of lactation (OR = 1.25).

CM Detection from the Second Month of Lactation Onward

Results of the multivariate analysis for the second month of lactation onward for heifers are given in Table 4 and for multiparous cows in Table 5. All the cow-specific risk factors from the second month of lactation onward described in Table 2 significantly contributed to the fit of the model. Two interaction terms (parity $\times$ SCC1 and season $\times$ SCC1) were also associated with multiparous cow IRCM. Heifers and multiparous cows in the first months of lactation (but after the first month), during winter periods, with a CM history, and with higher SCC1 had the highest IRCM, and IRCM was highest for cows in higher parities and that had a higher SCC2 (Tables 4 and 5).

In Figure 1, the IRCM during the first 8 mo of lactation is presented for heifers and multiparous cows. Additionally, the IRCM in the first 30 d of lactation is presented separately. The IRCM in the first 10 d of lactation was higher for heifers than for multiparous cows. From d 10 of lactation onward, the IRCM for multiparous cows was higher than for heifers. For heif-

Table 4. Results of analysis of cow-specific risk factors for the incidence of clinical mastitis (CM) from second month of lactation onward for heifers (dataset 3)¹

Variable	β	SE	OR ²	95% CI for OR	P-value
Intercept	-7.167	0.267			
Month of lactation					<0.0001
2	1.008	0.161	2.74	2.00–3.76	
3	1.160	0.159	3.19	2.34–4.36	
4	0.919	0.169	2.51	1.80–3.49	
5	0.632	0.187	1.88	1.30–2.72	
6	0.672	0.191	1.96	1.35–2.84	
7	0.397	0.205	1.49	1.00–2.22	
≥8	Ref. ³	—	1.00		
Season					0.0210
January–March	Ref.	—	1.00		
April–June	-0.313	0.126	0.73	0.57–0.94	
July–September	-0.211	0.135	0.81	0.62–1.05	
October–December	0.053	0.134	1.05	0.81–1.37	
MAST1 ⁴					0.0081
0	Ref.	—	1.00		
1	0.910	0.262	2.49	1.49–4.16	
≥2	1.237	0.479	3.45	1.35–8.81	
MAST2 ⁵					0.0020
0	Ref.	—	1.00		
1	0.712	0.182	2.04	1.43–2.91	
≥2	1.056	0.289	2.88	1.63–5.07	
SCC1 ⁶	0.412	0.049	1.51 ⁷	1.37–1.66	<0.0001

¹Estimated coefficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor.

²Odds ratio for having clinical mastitis in a specific month of lactation vs. not having clinical mastitis.

³Ref. = reference category.

⁴MAST1 = accumulated number of CM cases in the previous month of the lactation.

⁵MAST2 = accumulated number of CM cases in the month of lactation before the previous month of the lactation.

⁶SCC1 = SCC in the previous month of the lactation.

⁷For an increase in 1 unit of natural logarithm of SCC.

ers and multiparous cows, the IRCM from the second month of lactation onward varied between 0.0002 and 0.0012 per cow-day at risk (Figure 1).

The IRCM increased with increasing values for SCC1 and SCC2 (Figure 2). For instance, while a specific multiparous cow with values for SCC1 and SCC2 of 100,000 cells/mL had an IRCM of 0.0013 per cow-day at risk, a multiparous cow with values of 1,000,000 cells/mL for both SCC1 and SCC2 had an IRCM of 0.0034 per cow-day at risk. The IRCM was lower for heifers than for multiparous cows: a specific heifer with an SCC1 of 1,000,000 cells/mL had an IRCM of 0.0011 per cow-day at risk (Figure 2).

The IRCM increased with increasing values for MAST1 and MAST2 (Figure 3). A specific multiparous cow with no CM history (MAST1 = 0, MAST2 = 0) had an IRCM of 0.0016 per cow-day at risk, whereas the same cow with 2 CM cases in the previous month of lactation (MAST1 = 2 and MAST2 = 0) would have an IRCM of 0.0045 per cow-day at risk. In a worst-case scenario (MAST1 = 2 and MAST2 = 2), the IRCM increased to 0.0074 per cow-day at risk (Figure 3).

Gram Status of the CM Cases

In total, 2,525 CM cases (in heifers and multiparous cows) with known gram status were analyzed. The causing pathogens were *Strep. dysgalactiae* (10%), *Strep. agalactiae* (0.6%), *Strep. uberis* (7%), other streptococci (8%), *Staph. aureus* (21.9%), CNS (5.4%), *E. coli* (26.7%), *A. pyogenes* (0.7%), *C. bovis* (2.7%), *Pseudomonas* (0.6%), and *Klebsiella* (1.5%). The remaining 14.9% of all CM cases were caused by mixed cultures containing 2 gram-positive or 2 gram-negative pathogens.

Results of the analysis to determine the gram status of the CM cases for heifers and multiparous cows are given in Table 6. From all cow-specific risk factors for heifers, described in Table 2, only season and SCC1 significantly contributed to the gram status of the CM cases. For multiparous cows only month in lactation, SCC1, and quarter position significantly contributed to the gram status of the CM cases (Table 6). During the first 6 mo of the year, the probability that a CM case in a heifer was caused by gram-positive pathogens was higher than in the last 6 mo of the year. Using the coefficients (Table 6), a CM case in a heifer (with a

Table 5. Results of analysis of cow-specific risk factors for the incidence of clinical mastitis (CM) from the second month of lactation onward for multiparous cows (dataset 4)¹

Variable	β	SE	OR ²	95% CI for OR	P-value
Intercept	-6.864	0.272			
Parity					
2	Ref. ³	—	1.00		0.0014
3	0.476	0.231	1.61	1.02–2.53	
≥4	0.766	0.209	2.15	1.43–3.24	
Month of lactation					<0.0001
2	1.405	0.071	4.07	3.54–4.68	
3	1.449	0.071	4.26	3.71–4.90	
4	1.209	0.075	3.35	2.90–3.88	
5	0.960	0.082	2.61	2.22–3.07	
6	0.889	0.080	2.43	2.08–2.85	
7	0.540	0.090	1.72	1.44–2.05	
≥8	Ref.	—	1.00		
Season					0.0007
January–March	Ref.	—	1.00		
April–June	-0.700	0.239	0.50	0.31–0.79	
July–September	-0.737	0.245	0.48	0.30–0.77	
October–December	0.030	0.210	1.03	0.68–1.55	
MAST1 ⁴					<0.0001
0	Ref.	—	1.00		
1	0.800	0.093	2.23	1.85–2.67	
≥2	1.138	0.258	3.12	1.88–5.17	<0.0001
MAST2 ⁵					
0	Ref.	—	1.00		
1	0.366	0.069	1.44	1.26–1.65	0.0159
≥2	0.600	0.130	1.82	1.41–2.35	
SCC1 ⁶	0.288	0.048	1.33 ⁸	1.21–1.47	<0.0001
SCC2 ⁷	0.142	0.030	1.15 ⁸	1.09–1.22	
Interaction 1					0.1005
Parity 2 × SCC1	Ref.	—	1.00 ⁸		
Parity 3 × SCC1	-0.068	0.047	0.93 ⁸	0.85–1.02	
Parity 4 × SCC1	-0.118	0.041	0.89 ⁸	0.82–0.96	
Interaction 2					0.1005
Jan–March × SCC1	Ref.	—	1.00 ⁸		
April–June × SCC1	0.090	0.047	1.09 ⁸	1.00–1.20	
July–Sept × SCC1	0.082	0.045	1.09 ⁸	1.00–1.19	
Oct–Dec × SCC1	0.002	0.041	1.00 ⁸	0.93–1.09	

¹Estimated coefficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor.

²Odds ratio for having clinical mastitis in a specific month of lactation vs. not having clinical mastitis.

³Ref. = reference category.

⁴MAST1 = accumulated number of CM cases in the previous month in lactation.

⁵MAST2 = accumulated number of CM cases in the month in lactation before the previous month in lactation.

⁶SCC1 = SCC in the previous month of the lactation.

⁷SCC2 = geometric mean SCC from all available test-day records of the previous lactation.

⁸For an increase in 1 unit of natural logarithm of SCC.

corresponding value of 100,000 cells/mL for SCC1) had a probability of being caused by gram-positive pathogens of 0.76 in the first, 0.80 in the second, and 0.61 in the third, and 0.54 in the last 3 mo of the year.

Location in the udder also matters. In multiparous cows, CM cases in front quarters had a higher probability of being caused by gram-positive pathogens (OR = 1.43) than those in rear quarters. Using the coefficients (Table 6), the probability that a CM case in a front quarter in a multiparous cow (in the second month of lactation with a corresponding value of 100,000 cells/mL for SCC1) was caused by gram-positive pathogens

was 0.68. The probability that an identical CM case in a rear quarter was caused by gram-positive pathogens was 0.60. In addition, higher values for SCC1 increased the probability that a CM case was caused by gram-positive pathogens.

DISCUSSION

The current study presents a cow-level risk study simultaneously analyzing all cow factors in 1 model to study their association with IRCM. Whereas most other studies investigated only some cow-specific risk factors

Figure 1. A) Incidence rate of clinical mastitis (CM) per cow-day at risk for a specific heifer (during last 3 mo of the year, no CM history, and SCC of 100,000 cells/mL in the previous month) and a specific multiparous cow (in third parity, during last 3 mo of the year, with no CM history, SCC of 100,000 cells/mL in the previous month, and geometric mean SCC of 100,000 cells/mL in the previous lactation) in different months of lactation; B) incidence rate for CM for the same cows in the first month of lactation.

Figure 2. The association between different values for SCC in the previous month (SCC1), geometric mean SCC in the previous lactation (SCC2), and the incidence rate of clinical mastitis (CM) per cow-day at risk for a specific cow (parity 3, fourth month of lactation, during last 3 mo of the year, and no CM history). Also, the association between different values of SCC1 and the incidence rate of CM for a specific heifer (fourth month of lactation, during last 3 mo of the year, and no CM history) is presented.

Figure 3. The incidence rate of clinical mastitis (CM) per cow-day at risk for a specific multiparous cow (parity 3, fourth month of lactation, during last 3 mo of the year, SCC of 150,000 cells/mL in the previous month in lactation, and a geometric mean SCC of 150,000 cells/mL in the previous lactation) in relation to different values for the accumulated number of CM cases in the previous month in lactation (MAST1) and the accumulated number of CM cases in the month in lactation before the previous month in lactation (MAST2).

for CM (e.g., Beaudreau et al., 1998; Suriyasathaporn et al., 2000; Olde Riekerink et al., 2007), our study is novel, because it combined the significant cow-specific risk factors mentioned in previous studies in a single model to determine the IRCM for specific cows. Our results indicate that a combination of all these cow-specific risk factors can give an indication for the risk of individual cows of having CM.

There were large differences in IRCM among cows; the IRCM ranged between 0.0002 and 0.0074 per cow-day at risk (Figures 1, 2, and 3). The results of our study correspond with the results of studies on some individual cow factors: cows in higher parities, at the beginning of lactation, and with higher values for SCC have the highest IRCM (e.g., Barkema et al., 1998; Beaudreau et al., 1998; Suriyasathaporn et al., 2000). Results from our study are also comparable with results from a previous study (Houben et al., 1993) that combined 4 cow-specific risk factors to detect CM.

Danish researchers reported that treatment of a CM case leading to a record of CM is determined by a series of cow and herd factors; some farmers treat cases with any signs of CM, whereas others will only record severe cases (Vaarst et al., 2002). In the current study, however, all farmers were informed in detail at the start of the study about recording CM, and a clear case defi-

Table 6. Results of the analysis of cow-specific risk factors for the prediction of the gram status of the clinical mastitis (CM) case in heifers (dataset 5) and multiparous cows (dataset 6)¹

Variable	Heifers					Multiparous cows				
	β	SE	OR ²	95% CI for OR	P-value	β	SE	OR ²	95% CI for OR	P-value
Intercept	-2.544	0.519				-0.874	0.249			
Month of lactation					NS ³					0.0032
1						-0.726	0.207	0.48	0.32–0.73	
2						-0.482	0.181	0.62	0.43–0.88	
3						-0.112	0.176	0.89	0.63–1.26	
4						-0.206	0.194	0.81	0.56–1.19	
5						-0.359	0.199	0.70	0.47–1.03	
6						-0.470	0.197	0.63	0.42–0.92	
7						0.082	0.259	1.09	0.65–1.80	
≥8						Ref. ⁴	—	1.00		
Season					0.0059					NS ³
January–March	Ref.	—	1.00							
April–June	0.215	0.435	1.24	0.58–2.91						
July–September	-0.717	0.406	0.49	0.22–1.08						
October–December	-0.987	0.381	0.37	0.18–0.79						
SCC1 ⁵	0.804	0.103	2.23 ⁶	1.82–2.74	<0.0001	0.377	0.037	1.46 ⁶	1.36–1.57	<0.0001
Quarter position					NS					0.0150
Front						0.356	0.112	1.43	1.15–1.78	
Rear						Ref.	—	1.00		

¹Estimated coefficients (β), standard error (SE) for the coefficient, odds ratio (OR), 95% confidence interval (CI) for OR, and significance level are given for each cow-specific risk factor.

²Odds ratio for being infected with a gram-positive pathogen vs. a gram-negative pathogen.

³NS = not significant variable for the prediction of the gram status of the CM case.

⁴Ref. = Reference category.

⁵SCC1 = SCC in the previous month of lactation.

⁶For an increase of 1 unit in natural logarithm of SCC.

nition was provided (Barkema et al., 1998). Of course, in a study with 300 participating herds it is impossible to state that each farm collected the samples in exactly the same way. Lam et al. (1993), however, determined that samples collected by farmers are a useful tool in epidemiologic studies on CM. Because of the information provided we are comfortable that recording of CM cases was as good as possible in a study of this size. A severity score was not present in the dataset. This information will be especially useful in detection systems for CM, when it is very serious when severe cases of CM are missed.

Somatic cell count is widely known to be an important risk factor for CM (e.g., Beaudeau et al., 1998; Suriyathaporn et al., 2000; Green et al., 2007). Results from our study indicate that SCC in the previous month (SCC1) and SCC in the previous lactation (SCC2) are significant cow-specific risk factors for an increased IRCM. The IRCM for a multiparous cow with different corresponding values for SCC1 and SCC2 ranged between 0.0011 and 0.0034 per cow-day at risk (Figure 2). From this we conclude that using SCC from monthly test-day records in AMS would improve the detection of CM. Our results also emphasize the importance of the cow-specific risk factor CM history for the risk of having CM later in lactation. With different values for MAST1 and MAST2 the IRCM ranged between 0.0016 and 0.0074 per cow-day at risk (Figure 3). Previous studies have found that previous CM cases and recovery from infections was a risk factor for reinfection (Houben et al., 1993; Zadoks et al., 2001). Therefore, recording CM and using this historical information in CM detection systems could be very useful.

In the literature, other cow factors that are usually available on a farm were mentioned that increase the IRCM. Diseases such as milk fever were associated with an increased IRCM (Østergaard et al., 2003), but this information was not available in our dataset. Milking speed (Waage et al., 1998; Klaas et al., 2005) and udder depth (Slettbakk et al., 1995; Klaas et al., 2004) are risk factors associated with a higher risk of CM. This information was available for the cows in our study. Information on udder depth, however, was available for only 70% of the cows in the dataset, and milking speed was only recorded for 40% of the cows. To prevent problems with removed records because of missing values, we decided not to include these risk factors in the analyses.

Many studies have been carried out to find risk factors for CM at the herd level (e.g., Schukken et al., 1991; Barkema et al., 1999; Nyman et al., 2007). We did not analyse these types of risk factors. In the model, the repeated herd term accounted for these factors. On a single farm, circumstances are equal for all cows and

therefore herd-level risk factors cannot distinguish the IRCM among cows. Probably, when there is no information about the individual cows, the herd-level incidence of CM might serve as a base value in detection systems. In a similar way for pathogen or gram status detection, it may be worthwhile to include the pathogen prevalence of a herd in detection systems as a base value. These base values will reflect the herd factors for CM or specific pathogens.

Because it is useful for treatment decisions to know the gram status of a CM case (Morin et al., 1998; Pyörälä and Pyörälä, 1998), we created with logistic regression a simple predictive model with a binary (positive or negative) outcome. It might still be useful, however, to predict the exact pathogen involved. Because of low incidence rates for most pathogens and a lot of missing values for SCC1, it was decided to predict the gram status of the CM cases. Knowing the gram status of previous CM cases could be an important risk factor for the gram status of the current CM case. Because of statistical problems, however, it was not possible to include this risk factor. The most probable reason is that there were almost no differences between the dependent (gram status of the current CM case) and the independent variable (gram status of the previous CM case).

Somatic cell count in the previous month (SCC1) is a significant variable in predicting the gram status of the CM cases for both heifers and older cows. Higher values for SCC1 increased the probability that CM cases were caused by gram-positive pathogens, which was in accordance with another study (de Haas et al., 2002). Other significant variables were different for heifers and multiparous cows (Table 6). One explanation for this difference could be that the dataset for heifers was relatively small (only 364 CM cases), whereas 2,161 cases were included for multiparous cows. We found that for specific heifers, the probability that a CM case is caused by a gram-positive pathogen ranged from 0.54 to 0.80 depending on the season of the year. This seasonal variation has been identified in other studies (Morin et al., 1998; Makovec and Ruegg, 2003; Olde Riekerink et al., 2007). For multiparous cows, rear quarters were more susceptible to gram-negative pathogens, possibly because rear quarters are more soiled and more susceptible to the environmental (and gram-negative) pathogens such as *E. coli* and *Klebsiella*. Results indicate that it is difficult to distinguish between gram-positive and gram-negative CM cases based on cow factors alone.

The current study indicates that differentiation can be made among cows in the risk of having CM based on a combination of cow factors. These differences among cows could be useful to aid automatic detection of CM.

On farms with an AMS, where the farmer is not present during the milking process, this will be particularly worthwhile. The number of false-positive warnings for CM based on sensor measurements of an AMS needs improvement (Hogeveen and Ouweltjes, 2003). For instance, in a recent study using electrical conductivity measurements of an AMS, a sensitivity of 56% and a specificity of 82% to detect CM were found (Mottram et al., 2007). Combining sensor information with the individual risk of having CM based on additional cow factors might reduce the number of these false-positive CM warnings generated by the AMS and improve the interpretation of the sensor outputs. Also, in previous studies a combination of sensor measurements and cow factors was presented for a better interpretation of the sensor outputs (de Mol and Woldt, 2001; Chagunda et al., 2006). A study should be conducted to quantify the possible decrease in the number of false-positive warnings for CM by combining sensor measurements with the individual risk of having CM based on cow factors. That study will have some special requirements: a dataset including both sensor information and information on cow factors is needed. Also, validation of the developed model is needed to assess the usefulness of combining the 2 information sources.

CONCLUSIONS

This study used an integrated analysis with several cow factors to determine the IRCM of dairy cows. All cow factors together (parity, month in lactation, season of the year, SCC in previous month, geometric mean SCC in previous lactation, and CM history) significantly influenced the risk of having CM. There was a large difference in IRCM among dairy cows. The IRCM ranged between 0.0002 and 0.0074 per cow-day at risk. Clinical mastitis history was an important factor in determining the IRCM. Therefore, registering CM cases and using this historical information would be very useful in detecting CM. Results indicate that additional cow factors should not be the sole criterion for differentiating between gram-positive and gram-negative CM cases.

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