

Cow factors affecting the risk of clinical mastitis

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The objective of the present study was to identify cow risk factors associated with development of clinical mastitis (CM) in subsequent stages of lactation. A total of 3,309 lactations from spring-calving Holstein-Friesian cows were included in the analysis; parity number ranged from one to three, inclusive. A generalised estimating equations approach with a logit link function was used to account for the binary nature of the data and the unequal number of repeated records per cow. The dependent variable was the probability of developing CM in the subsequent stage of lactation. Independent variables included in the model were chosen using stepwise selection; herd, year of birth, month of calving, parity, period of lactation and previous CM history significantly affected the probability of CM. Two-way interactions between parity and period of lactation and between parity and incidence of CM in the previous lactation were also included in the model. A greater probability of developing CM is expected in cows that experienced CM in the previous lactation and/or previously within the same lactation. The probability of CM occurring in cows that experienced at least one case of CM in the previous lactation was 0.92 to 3.75 times that of a cow that experienced no CM in the previous lactation. It is possible to predict the probability of an animal developing CM in the subsequent stage of lactation when information is available on the parity and month of calving of the animal and its previous history of CM.

Keywords: Clinical mastitis; history; odds; probability

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Introduction

Clinical mastitis (CM) is a considerable cost to the dairy farmer and dairy industry. The costs associated with CM include reduced milk production, increased culling, increased labour, increased treatment costs and milk which cannot be used for human consumption. An additional cost of inferior udder health is consumer perception regarding animal welfare as well as the impact of using antibiotics in animals on their efficacy for human health. Therefore, future breeding and management decisions should incorporate an element of mastitis control.

In order to effectively control CM, the factors affecting the risk of an animal developing CM should be assessed. The probability of an animal developing CM in the future can be calculated from prediction equations, providing easily interpretable data allowing the farmer to make a more informed culling decision.

Several studies (Bartlett *et al.*, 1992; Elbers *et al.*, 1998; Barkema *et al.*, 1999) have investigated the herd-level risk factors associated with incidence of CM in a herd. Fewer studies have investigated cow-level factors associated with the development of CM. The risk of developing CM is greatest in early lactation (Bunch *et al.*, 1984; Houben *et al.*, 1993), and increases with parity (Bunch *et al.*, 1984; Lucey and Rowlands, 1984; Houben *et al.*, 1993) and level of milk production (Bunch *et al.*, 1984; Schukken *et al.*, 1991; Houben *et al.*, 1993). Houben *et al.* (1993) reported that the risk of a cow developing CM in the subsequent month of lactation is also a function of number of cases of CM in the previous lactation, number of clinical cases in the previous months of the current lactation, and the occurrence of CM in the current month.

Houben *et al.* (1993) reported that the risk of contracting CM was 4.8 times greater if the animal experienced CM in the previous stage of lactation. Rowlands, Lucy and Russell (1986) reported that CM occurred in 38% of cows that experienced CM in the previous lactation, as opposed to 23% of those that had not. Rowlands *et al.* (1986) attributed this to either an increased susceptibility to further outbreaks or prolongation of a subclinical infection through the dry period and into the next lactation.

The objective of the present study was to investigate and quantify the cow risk factors associated with an animal developing CM in the future. Solutions derived for the risk factors may be incorporated into a prediction equation to calculate the probability of infection with CM in future stages of lactation.

Materials and Methods

The data were from three research herds in the south of Ireland and records were available from 1988 to 2000, inclusive. A potential 2,119 Holstein-Friesian cows had data on somatic cell counts and/or incidence of CM. Following the removal of parities greater than three, a total of 3,944 lactations from 1,878 cows remained. Only lactations with a valid spring (January to May, inclusive) calving date and a milk test-day record after 150 days in milk were subsequently retained. The final dataset consisted 3,309 lactation records from 1,636 cows.

Diagnosis and definition of clinical mastitis
Monthly quarter milk samples were collected in an aseptic manner from all cows. These samples were subjected to a range of analyses that included Californian Mastitis Testing (CMT). The presence of

CM was identified visually based on a positive CMT score. Clinical mastitis was also detected routinely by the stockman at milking time when foremilk was inspected before cluster attachment. Clinical mastitis in this study was therefore defined as present (1) if the milk from at least one udder quarter exhibited a positive CMT score or was recorded as clinical by the herdsman, otherwise absent (0).

Only days in milk between calving and 305-days post-partum were included in the analysis. Each lactation was divided into ten periods (t); 0–30, 31–60 . . . 271–305. A lactation only received a record for a given period if a milk test day record was also available within that period. If at least one case of CM was observed within a period then that period received a value of 1; otherwise a value of 0 was given. These values were summed to give the number of cases of CM up to a given period within the same lactation; these were subsequently grouped into four groups, 0, 1, 2, ≥ 3 cases of CM up to a given period. The presence or absence of CM in an entire lactation (days 0 to 305) was also coded as 1 or 0 and the number of cases of CM per lactation was coded as 0, 1, 2, and ≥ 3 cases of CM per lactation.

First parity animals had no data on incidence of CM pre-calving and therefore received a zero for presence of CM in the previous lactation. Although the accuracy of the prediction equation for first parity animals may have been augmented with the inclusion of information on the nulliparous animals, these data were not available. Similarly, no value for incidence of CM in the previous period of lactation was available for CM in the first 30 days of lactation; therefore, CM in the previous period and number of cases of CM in the previous periods received a value of zero.

A dataset was also created excluding information on the first period of lactation.

In this dataset the number of cases of CM in the previous periods was given a value of zero for the second period of lactation. An additional sub set of data was created whereby only the first period of lactation was retained; data from the first period were included in predicting the probability of CM in subsequent periods.

Effect of incidence of clinical mastitis on reappearance

Because the effect of CM in one lactation on the incidence of CM in the subsequent lactation can only be assessed if the animal has a subsequent lactation, some selection bias may exist between lactations. Therefore, a separate analysis was carried out to investigate the effect of CM in one lactation on the reappearance of the animal in the data set in the subsequent lactation. Reappearance was coded as 1 (reappeared) or 0 (did not reappear).

Statistical analysis

The effect of CM on reappearance in the subsequent lactation was investigated using logistic regression. The logistic regression model was fitted using PROC GENMOD in SAS (SAS Institute, 2003). A separate analysis was carried out for reappearance in lactation two and lactation three. The dependent variable was reappearance and the independent variable was incidence of CM in the previous lactation. The significance of CM in the previous lactation in determining the reappearance rate was determined using the likelihood ratio test on nested models.

The regression analysis on the incidence of CM utilised generalised estimating equations (Liang and Zeger, 1986) with a logit link function to account for the repeated records per cow on this binary trait. The generalised estimating equations were fitted using PROC GENMOD in SAS (SAS Institute, 2003). An exchangeable correlation structure was

fitted between records within cow in the present study.

The dependent variable was the risk of CM in period $t + 1$ of the current lactation; the probability of CM being equal to one was modeled. Factors considered for possible inclusion in the model were herd, year of birth, year of calving, month of calving, parity, period of lactation (t), the presence or absence of CM in the current period (t), the number of clinical cases up to the previous period ($t-1$) within the same lactation, and the number of cases in the previous lactation. Age at calving, nested within parity, was created as a covariate. The variables created for possible inclusion in the model were chosen on perceived biological importance and the possible availability of data on each variable nationally. For the initial construction of the multiple regression model only lactations with an observation for each of the possible confounding variables were retained. This was to avoid the effect of missing values on variables selected, through the stepwise algorithm, for inclusion in the multiple regression model.

The model was progressively built up from a univariate analysis to a multiple regression analysis. Whether or not to include variables in the model was decided using a stepwise forward selection ($P < 0.20$) followed by backward elimination ($P > 0.10$); the significance of each term was determined using the likelihood ratio test on nested models. Two-way interactions with a biologically meaningful interpretation were tested between the main effects that remained in the model; the level of significance for inclusion was set at $P < 0.10$ also based on the likelihood ratio test. Multicollinearity among independent variables in the final model was tested with principal component analysis and by determining the variance inflation factor associated with each independent variable.

The model based regression coefficients and standard errors are presented in the present study. Odds ratios (OR) adjusted for the effects of the other variables in the model, were calculated as the exponent of the regression coefficients.

Transition probabilities of developing CM in the future were calculated from the regression coefficients as follows:

$$P_{(t+1)} = \frac{e^{(\alpha + \sum_{i=1}^k \hat{\beta}_i X_i)}}{1 + e^{(\alpha + \sum_{i=1}^k \hat{\beta}_i X_i)}}$$

where

$P_{(t+1)}$ = conditional probability of contracting CM in period ($t+1$) of lactation
 α = intercept (in the present study the intercept is summed with the average effect of herd and the average effect of year or birth)

$\hat{\beta}_i X_i$ = predicted regression coefficient ($\hat{\beta}_i$) for the corresponding level of fixed effect (X_i), including interactions.

Results

Herd, year of birth, month of calving, parity, period (t) of lactation, the presence or absence of CM in current (t) period, the number of clinical cases up to the previous ($t-1$) period within the same lactation, and the number of cases of CM in the previous lactation were all significantly associated with incidence of CM in the subsequent ($t+1$) period of lactation. Year of calving and age at calving, nested within parity, had no significant effect. Significant two-way interactions existed between parity and period of lactation and between parity and incidence of CM in the previous parity. No collinearity existed between any of the variables included in the final model.

The odds of a cow reappearing in the second lactation following at least one case of CM in the first lactation was significantly

($P < 0.001$) lower (OR = 0.70) than if no CM was observed in the first lactation (i.e., there was a greater probability of a cow being culled prior to second calving if she experienced CM in first lactation). Similarly the odds of reappearing in the third lactation was significantly ($P < 0.05$) lower (OR = 0.84) if the cow developed CM in the second lactation compared to if the cow did not develop CM in the second lactation.

Of the lactation records with at least one case of CM, the average number of cases of CM per lactation were 1.23, 1.44, and 1.37 for lactations one, two, and three, respectively. The regression coefficients and odds ratios for all effects included in the final model across all three lactations are summarised in Table 1 when all data were included in the analysis.

Table 1. Estimates of regression coefficients (s.e.) and odds ratios (OR) from the multiple regression model for lactation numbers one to three for all data

Independent variable	Lactation 1		Lactation 2		Lactation 3	
	Estimate	OR	Estimate	OR	Estimate	OR
Intercept	-1.82 (0.335)		-1.82 (0.335)		-1.82 (0.335)	
Parity	0.00	1.00	-0.60 (0.147)	0.55	-0.60 (0.156)	0.55
Month of Calving						
January	0.00	1.00	0.00	1.00	0.00	1.00
February	0.14 (0.101)	1.15	0.14 (0.101)	1.15	0.14 (0.101)	1.15
March	-0.18 (0.125)	0.84	-0.18 (0.125)	0.84	-0.18 (0.125)	0.84
April	-0.81 (0.246)	0.45	-0.81 (0.246)	0.45	-0.81 (0.246)	0.45
May	-0.39 (0.411)	0.68	-0.39 (0.411)	0.68	-0.39 (0.411)	0.68
Period of lactation†						
1	0.00	1.00	0.00	1.00	0.00	1.00
2	-2.57 (0.230)	0.08	-1.33 (0.220)	0.26	-1.48 (0.244)	0.23
3	-2.73 (0.252)	0.07	-2.04 (0.281)	0.13	-2.19 (0.292)	0.11
4	-2.85 (0.265)	0.06	-2.18 (0.297)	0.11	-2.17 (0.311)	0.11
5	-3.06 (0.289)	0.05	-2.25 (0.303)	0.11	-2.68 (0.377)	0.07
6	-3.43 (0.339)	0.03	-2.32 (0.312)	0.10	-3.21 (0.489)	0.04
7	-3.63 (0.375)	0.03	-2.85 (0.379)	0.06	-2.59 (0.413)	0.08
8	-3.11 (0.302)	0.05	-2.80 (0.383)	0.06	-2.62 (0.424)	0.07
9	-2.67 (0.263)	0.07	-2.84 (0.404)	0.06	-2.11 (0.375)	0.12
10	-2.78 (0.314)	0.06	-1.86 (0.311)	0.16	-1.40 (0.334)	0.25
No. of CM‡ events in previous lactation						
0	0.00	1.00	0.00	1.00	0.00	1.00
1			-0.09 (0.208)	0.92	0.70 (0.210)	2.02
2			0.74 (0.329)	2.10	0.65 (0.325)	1.92
≥3			1.13 (0.243)	3.10	1.32 (0.295)	3.75
No. of CM‡ events in previous periods of the current lactation						
0	0.00	1.00	0.00	1.00	0.00	1.00
1	0.91 (0.144)	2.48	0.91 (0.144)	2.48	0.91 (0.144)	2.48
2	1.47 (0.253)	4.35	1.47 (0.253)	4.35	1.47 (0.253)	4.35
≥3	1.83 (0.304)	6.21	1.83 (0.304)	6.21	1.83 (0.304)	6.21
CM‡ previous period						
No	0.00	1.00	0.00	1.00	0.00	1.00
Yes	1.47 (0.156)	4.37	1.47 (0.156)	4.37	1.47 (0.156)	4.37

† Period 1 to 10 of lactation represent 0 to 30, 31 to 60, 61 to 90, 91 to 120, 121 to 150, 151 to 180, 181 to 210, 211 to 240, 241 to 270 and 271 to 305 days post-calving, respectively.

‡ CM = clinical mastitis.

A significantly ($P < 0.01$) lower probability of CM existed in the second and third lactations compared to the first lactation. Although, month of calving significantly ($P < 0.001$) affected incidence of CM, only April-calving cows had significantly lower odds of developing CM than January calving cows. Nevertheless, there was a tendency for later calving cows to be at a lower risk of contracting CM than cows calving early in the year. The probability of developing CM was greatest in the first 30 days post-calving compared to later in lactation; the odds of developing CM in the period of 151 to 180 days of lactation was 0.03 to 0.10 times that of developing CM in the first 30 days post partum across all lactations.

The odds of contacting CM in the subsequent period of lactation was greater if the cow experienced CM in the previous lactation and/or in the previous periods of the current lactation. The odds of developing CM in any period of lactation was over 4 times greater if a case of CM was experienced in the immediately prior period of lactation, compared to the absence of a case of CM in the previous period.

Table 2 summarises the regression coefficients and odds ratios for all effects in the final model across all three lactations without the first period of lactation included in the analysis. Excluding the first 30 days of lactation in the analysis reversed the signs of the regression coefficients for the effect of lactation number. The odds of infection in the second lactation was greater than that in the first lactation; the odds of infection in the third lactation was not significantly different from one. The trend among the remaining odds ratios were similar to the analysis which contained all data.

Discussion

The objective of this study was to investigate the risk factors associated with CM, thereby facilitating prediction of the probability of future incidence of CM. The odds of CM increased with stage of lactation and were greatest in cows that had previously experienced CM, either in the previous lactation and/or earlier in the current lactation.

In agreement with the present study, Rupp and Boichard (2000) also reported a reduced hazard ratio of a cow developing CM as calving month changed from January to May. However, Chassagne, Barnouin, and Chacornac (1998) reported a higher odds (not significant) of infection in cows calving between March and May (inclusive) compared to cows calving between December and February (inclusive). The degree of exposure to pathogens is influenced more by month of calving in outdoor-grazing dairy systems than in indoor-feeding dairy systems operated in other countries. Milk production in Ireland is based on compact, seasonal calving, grass-based systems (Dillon *et al.*, 1995). The date of turnout to grass by day was mid-February to mid-March in the three research herds included in this study. Under this system, cows calving late in the calving season are turned out to grass immediately post-calving. A lower incidence of CM in outdoor grazing systems has been reported compared to confinement systems of milk production (Washburn *et al.*, 2002) owing to the relatively cleaner environment and the lower level of contact between cows grazing outdoors. Therefore, differences between the results in the present study and other international studies which were based on cows fed indoors may be expected.

The lower odds of CM in the second lactation compared to the first lactation

Table 2. Estimates of regression coefficients (s.e.) and odds ratios (OR) from the multiple regression model for lactation numbers one to three with the first period of lactation excluded from the data

Independent variable	Lactation 1		Lactation 2		Lactation 3	
	Estimate	OR	Estimate	OR	Estimate	OR
Intercept	-1.45 (0.644)		-1.45 (0.644)		-1.45 (0.644)	
Parity	0.00	1.00	0.63 (0.283)	1.89	0.52 (0.301)	1.68
Month of Calving						
January	0.00	1.00	0.00	1.00	0.00	1.00
February	0.02 (0.134)	1.02	0.02 (0.134)	1.02	0.02 (0.134)	1.02
March	-0.43 (0.179)	0.65	-0.43 (0.179)	0.65	-0.43 (0.179)	0.65
April	-1.28 (0.414)	0.28	-1.28 (0.414)	0.28	-1.28 (0.414)	0.28
May	-0.22 (0.540)	0.80	-0.22 (0.540)	0.80	-0.22 (0.540)	0.80
Period of lactation [†]						
2	0.00	1.00	0.00	1.00	0.00	1.00
3	-0.21 (0.318)	0.81	-0.75 (0.315)	0.48	-0.74 (0.333)	0.48
4	-0.32 (0.329)	0.73	-0.89 (0.331)	0.41	-0.72 (0.350)	0.49
5	-0.54 (0.349)	0.59	-0.96 (0.336)	0.38	-1.24 (0.410)	0.29
6	-0.90 (0.390)	0.41	-1.04 (0.346)	0.36	-1.75 (0.512)	0.17
7	-1.11 (0.422)	0.33	-1.57 (0.408)	0.21	-1.13 (0.445)	0.32
8	-0.59 (0.361)	0.56	-1.51 (0.41)	0.22	-1.18 (0.455)	0.31
9	-0.14 (0.329)	0.87	-1.56 (0.429)	0.21	-0.67 (0.411)	0.51
10	-0.27 (0.371)	0.77	-0.60 (0.346)	0.55	0.02 (0.374)	1.02
No. of CM [‡] events in previous lactation						
0	0.00	1.00	0.00	1.00	0.00	1.00
1			-0.02 (0.265)	0.98	0.60 (0.266)	1.83
2			0.83 (0.400)	2.29	0.72 (0.404)	2.06
≥3			1.05 (0.284)	2.86	1.09 (0.368)	2.97
No. of CM [‡] events in previous periods of the current lactation						
0	0.00	1.00	0.00	1.00	0.00	1.00
1	0.99 (0.143)	2.70	0.99 (0.143)	2.70	0.99 (0.143)	2.70
2	1.45 (0.253)	4.27	1.45 (0.253)	4.27	1.45 (0.253)	4.27
≥3	1.77 (0.307)	5.87	1.77 (0.307)	5.87	1.77 (0.307)	5.87
CM [‡] previous period						
No	0.00	1.00	0.00	1.00	0.00	1.00
Yes	1.46 (0.155)	4.31	1.46 (0.155)	4.31	1.46 (0.155)	4.31

[†] Period 2 to 10 of lactation represent 31 to 60, 61 to 90, 91 to 120, 121 to 150, 151 to 180, 181 to 210, 211 to 240, 241 to 270 and 271 to 305 days post-calving, respectively.

[‡] CM = clinical mastitis.

reported in the present study contrasts with most previous studies. However, most previous studies failed to account for the cow's previous history of CM. Houben *et al.* (1993), after accounting for previous mastitis history, reported significantly lower odds of CM in the second lactation compared to the first lactation; differences in odds of CM between second and

third lactation were not significant. However, Houben *et al.* (1993) did not include the first 30.5 days of lactation in their analysis.

The change in sign of regression coefficients for parity following the removal of records from the first period of lactation (Table 2) suggests that the majority of cases of CM in first lactation animals

occur around parturition; 58%, 38% and 42% of observed cases of CM occurred in the first 30 days of lactation in the first, second, and third lactation. Across all lactations the odds of CM was greatest in the first 30 days of lactation and decreased thereafter until the sixth month when it subsequently increased again. This pattern corroborates Houben *et al.* (1993) who showed an almost consistent decrease in the odds of infection as lactation progressed. Suriyasathaporn *et al.* (2000) reported a significantly higher risk of CM in early and mid lactation compared to late lactation; differences in risk of CM between early and mid lactation were not significant. Similarly, Miltenburg *et al.* (1996) documented 39.1% and 25.4% of all CM cases reported occurring during the first month of lactation in first lactation cows and all cows, respectively. The increased incidence of CM in early lactation may be attributable to changes in both immune function (Kehrli, Nonnecke, and Roth, 1989b) and nonspecific host defense mechanisms (Kehrli, Nonnecke, and Roth, 1989a) in the peripartum dairy cow.

Cows which experienced three or more cases of CM in the previous lactation were more likely to develop CM in the current lactation. The odds of CM in the current lactation for a cow that experienced three or more cases of CM in the previous lactation varied from 2.86 to 3.75 times that if the cow did not experience CM in the previous lactation. This is similar to the odds ratio of 2.9 reported by Houben *et al.* (1993) for cows with three or more cases of CM in the previous lactation compared to a cow that experienced no CM. These results also concur with other studies which showed that cows with CM in the preceding lactation have a higher probability of CM in the current lactation (Bunch *et al.*, 1984; Rowlands *et al.*, 1986;

Firat, 1993; Rupp, Beaudreau, and Boichard, 2000). Firat (1993) reported that cows with CM in the preceding lactation were twice as susceptible to CM in the current lactation than cows without CM in the preceding lactation. Similarly, Rowlands *et al.* (1986) reported that CM occurred in 38% of cows that experienced CM in the previous lactation compared to 23% if they did not. The increased susceptibility of cows to reoccurrence of CM in a subsequent lactation may be attributable to either an increased susceptibility to future outbreaks or through a subclinical infection persisting through the dry period. Care should be taken, however, in the interpretation of the absolute values of the odds ratios reported since the existence of CM in a lactation may predispose that animal to a greater probability of culling, thereby not appearing in the subsequent lactation. Rupp *et al.* (2000) documented a lower odds of reappearance in the second lactation if high lactation-average somatic cell score was observed in the first lactation.

In agreement with the present study Houben *et al.* (1993) reported a significantly higher odds of CM in cows that experienced one or more cases of CM earlier in lactation. This may be attributable to previous CM persisting at a subclinical level. The odds of infection with CM in any stage of lactation in cows that experienced CM in the preceding stage of lactation was 4.4 times greater than if the animal did not experience CM in the preceding stage of lactation; this is very similar to the odds ratio (4.8) reported by Houben *et al.* (1993) under similar circumstances. Zadoks *et al.* (2001) also reported a higher incidence rate of *Streptococcus uberis* and *Staphylococcus aureus* in udder quarters that had recovered from prior CM infections compared to quarters that had not experienced CM.

Zadoks *et al.* (2001) also reported a higher rate of *Staphylococcus aureus* infection in quarters where previous *aureus* infections occurred in other quarters within the same cow. Elbers *et al.* (1998) reported that from their study on 1,103 quarter cases of CM, 42 udder quarters had one recurrent case of CM in the same quarter and 5 cows had two recurrent cases in the same quarter.

Conclusions

Although derived from a limited study size on three research herds, the regression coefficients reported in the present study may be used to predict, albeit with some caution, the probability of future CM. For example, using the regression coefficients from Table 1 the probability of a second lactation cow that calves in February, developing CM in the fourth period of lactation (121 to 150 days in milk) after already experiencing one case of CM in the previous lactation and another case of CM in immediately previous period of the current lactation is:

$$\frac{e^{(-1.82-0.60+0.14-2.18-0.09+1.47)}}{1+e^{(-1.82-0.60+0.14-2.18-0.09+1.47)}} = 0.045$$

Thus, there is a 4.5% probability of contracting CM in the fourth period of the second lactation; the probability is reduced to 1.1% if the animal experienced no case of CM in the preceding period of lactation. Probability calculations like these can be incorporated into individual farmer reports created from the national database to provide more detailed information to the farmer and allowing for better management and culling decisions. However, deviations from the calculated probabilities may exist between farms, given the significance of farm in the model of analysis in the present study. Therefore,

incidence of CM may be different on farms adopting different management practices (e.g., milking equipment, milking techniques, winter housing).

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