Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom

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ABSTRACT

Quarter and cow risk factors associated with the development of clinical mastitis (CM) during lactation were investigated during a 12-mo longitudinal study on 8 commercial Holstein-Friesian dairy farms in the southwest of England. The individual risk factors studied on 1,677 cows included assessments of udder and leg hygiene, teat-end callosity, and hyperkeratosis; body condition score; and measurements of monthly milk quality and yield. Several outcome variables for CM were used for statistical analysis, which included use of generalized linear mixed models. Significant covariates associated with an increased risk of CM were increasing parity, decreasing month of lactation, cows with very dirty udders, and quarters with only very severe hyperkeratosis of the teat-end. Thin and moderate smooth teat-end callosity scores were not associated with an increased risk for CM. Cows that recorded a somatic cell count >199,000 cells/mL and a milk protein percentage <3.2 at the first milk recording after calving were significantly more likely to develop CM after the first 30 d of lactation. There was no association between cow body condition score and incidence of CM. Of the cases of CM available for culture, 171 (26.7%) were confirmed as being caused by *Escherichia coli* and 121 (18.9%) confirmed as being caused by *Streptococcus uberis*. Quarters with moderate and very severe hyperkeratosis of the teat-end were at significantly increased risk of clinical *E. coli* mastitis before the next visit. Quarters with very severe hyperkeratosis of the teat-end were significantly more likely to develop clinical *Strep. uberis* mastitis before the next visit. There were strong trends within the data to suggest an association between very dirty udders (an increased risk of clinical *E. coli* mastitis) and teat-ends with no callosity ring present (an increased risk of clinical *Strep. uberis* mastitis). These results highlight the importance of individual quarter-

and cow-level risk factors in determining the risk of CM associated with environmental pathogens during lactation.

Key words: clinical mastitis, risk factor, hyperkeratosis, udder hygiene score

INTRODUCTION

Despite extensive research into risk factors for the development of bovine mastitis, the most recent published work suggests that the mean incidence rate of clinical mastitis (CM) in the United Kingdom has increased to greater than 50 cases per 100 cows per year (Bradley et al., 2007). A report from the Dairy Information System (DAISY, University of Reading, UK) has estimated the cost of an average case of CM to be £177 (Esslemont and Kossaiati, 2002). In addition to the cost of disease, CM is a common cause of mortality in adult dairy cows with a recent study reporting a fatality in 2.2% of cases (Bradley and Green, 2001).

Much of the previous work that has been conducted has concentrated on risk factors for CM at the herd level (Schukken et al., 1990; Barkema et al., 1999; Peeler et al., 2000); for example, those factors that may increase the exposure of a herd to environmental pathogens (e.g., poor cubicle bed management) or increase the host susceptibility to environmental pathogens (e.g., inadequate nutrition). A recent study of cow-level risk factors investigated IMI with *Streptococcus uberis* and *Staphylococcus aureus* (Zadoks et al., 2001).

Quarter-level risk factors reported for CM include previous bacterial infection (Zadoks et al., 2001; Green et al., 2002), teat position (Faull et al., 1983; Zadoks et al., 2001), and teat-end hyperkeratosis. Hyperkeratosis (HK) of the teat-end is a histological term referring to an increase in the thickness (hyperplasia, callus) of the stratum corneum (keratin layer) at the teat-end and is a nonspecific response to a chronic stimulus. A recent study (Neijenhuis et al., 2001) found that within a data set of 2,157 cows in 15 herds, quarters with CM had a greater degree of teat-end callosity (TEC) than healthy quarters within infected cows, and cows

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Table 1. Descriptive summary of the study herds at the beginning of the study period

Herd	Herd size, n	Mean parity	Calving pattern	Lactating cow housing	Average 305-d milk yield, L/cow	Incidence rate of clinical mastitis ¹	Geometric mean bulk milk SCC, cells/mL
1	92	3.4	Nonseasonal	Straw yards	7,442	0.70	152
2	226	3.4	Nonseasonal	Cubicles (sand)	10,938	1.38	148
3	198	3.3	Nonseasonal	Cubicles (chopped straw)	7,711	0.56	175
4	151	3.0	Seasonal ²	Cubicles (chopped straw)	8,535	0.72	76
5	266	3.1	Nonseasonal	Cubicles (sawdust)	8,370	0.69	233
6	218	2.4	Seasonal ³	Cubicles (paper)	9,966	0.78	157
7	146	2.7	Nonseasonal	Cubicles (chopped straw)	7,537	0.72	191
8	71	3.8	Nonseasonal	Cubicles (chopped straw)	8,343	0.94	151

¹Calculated as cases per cow-year.²Autumn and spring.³Autumn.

with CM paired with healthy herd mates also had more TEC, particularly when mastitis had occurred between the second and fifth months of lactation. A study of 22,593 quarters investigating risk factors for *Staph. aureus* IMI (Zadoks et al., 2001) found that extremely callused teat-ends were significantly associated with a higher rate of *Staph. aureus* IMI, although TEC was not associated with increased risk of *Strep. uberis* IMI.

Cow-level risk factors for CM include breed (Brolund, 1985; Elbers et al., 1998), lactation number (Zadoks et al., 2001), leaking milk between milkings (Schukken et al., 1990; Elbers et al., 1998; O'Reilly et al., 2006), periparturient disease (Peeler et al., 1994), and cow cleanliness (Ward et al., 2002). Within the current literature, there is little direct evidence to support the theory that cows in apparent negative energy balance are at increased risk of developing CM, although cows with a butter fat:protein ratio of >1.5 were associated with an increased risk for mastitis and other periparturient disease (Heuer et al., 1999). Twinning, dystocia, retained fetal membranes, and lameness before service increased the risk of a CM episode before service in a previous study (Schukken, 1989; Peeler et al., 1994), possibly because of concurrent ketosis and deficiency in vitamin E or selenium (Peeler et al., 1994). In an observational study of 4 UK dairy herds housed on straw yards (Ward et al., 2002), farms with the lowest incidence rate of CM had the cleanest cows and the most satisfactory bed management. Cow and cubicle cleanliness was reported as important in the models for *E. coli* CM in a previous study (Schukken et al., 1991).

The purpose of this study was to investigate quarter and cow risk factors for CM under UK field conditions, particularly those related to cow hygiene, teat-end damage, and energy balance.

MATERIALS AND METHODS

Herd Selection

A convenience sample of 8 commercial Holstein-Friesian dairy herds was selected from a central milk-recording database (National Milk Records, Chippenham, UK). Inclusion criteria included location (within a 2-h drive of the School of Veterinary Science, University of Bristol, Langford, UK), a high incidence rate of CM (>0.5 cases per cow year), available monthly milk quality and individual cow SCC data, and likely compliance to data recording and sampling. None of the herds farmed under organic conditions. Herd characteristics are summarized in Table 1.

Visit Protocol

The cohort of farms was visited every month to collect quarter and cow-level data over a period of 12 mo (June 2004 to May 2005) for a total of 96 herd visits. At each visit, milking cows were observed during one milking and the number of cows in milk on the day of the visit was recorded. A Dictaphone (a portable sound-recording device) was employed to capture data at each visit and the information later transcribed onto a paper and electronic database system. All animals were initially identified by freeze brand and linked with the animal's ear tag from milk recording information. When deciding on methods for collecting animal risk factor data, methods that were easily reproducible in a clinical commercial setting were chosen. Although scoring procedures had to be rapid, teat-end scoring was performed in detail. For all scoring assessments, standardized procedures were produced including laminated photographs to promote consistency. All measurements were made by one researcher.

Explanatory Variables

Hygiene scores were collected as each cow entered the parlor, and scores were recorded alongside the animal's freeze-brand number. Cow udder and leg hygiene were assessed and scores were collected using a 4-point scale described previously (Schreiner and Ruegg, 2002). An udder hygiene score (**UHS**) or leg hygiene score (**LHS**) of 1 referred to no contamination of the skin of the rear of the udder or the hind limb between the hock and coronary band. A score of 2 was slightly dirty (2–10% of the area covered in dirt), a score of 3 moderately dirty (10–30% of the area covered in dirt), and a score of 4 indicated caked-on dirt (>30% of these areas completely covered in dirt).

Immediately following cessation of milking and removal of the cluster apparatus but before the application of postmilking teat disinfection, all 4 teats of each cow in milk during the visit were examined using a light source. An assessment of TEC thickness and roughness were made using the 8-point scale described for research purposes (Neijenhuis et al., 2000). A score N described a teat-end with no ring; a score 1A, 1B, and 1C described a thin, moderate, or thick smooth callosity ring, respectively; and a score 2A, 2B, 2C, and 2D described a thin, moderate, thick, or extreme (i.e., severe HK) rough callosity ring, respectively.

Cow BCS was measured using a 5-point scale, which has been described previously (Edmonson et al., 1989) and was performed visually from behind the cow on exit from the parlor.

Milk recording data were downloaded from National Milk Records (www.nmr.co.uk) following written permission from the farmers and imported into herd management software (Interherd, NMR Agrisoft, UK). This provided current parity, previous calving date, monthly SCC, monthly recorded butter fat and milk protein percentages, yield information, and DIM at each data collection visit.

Outcome Variables

Farmers were requested to take milk samples from and record all cases of CM (defined before the study as milk changes and/or swelling of the udder with or without signs of systemic illness in the cow) that occurred during the study period for bacteriological analysis before treatment. Following training, sampling was performed in accordance with a written standard operating procedure, using a supplied kit; all cases of CM were recorded using a standard format. All milk samples collected were frozen, batched, and submitted for microbiological analysis at an accredited laboratory (Compton Paddock Laboratories, Newbury, Berkshire,

UK) and analyzed using standard laboratory methods for the microbiological analysis of milk (National Mastitis Council, 1999). Ten microliters of secretion was inoculated onto blood agar and Edward's agar, and 100 μ L of secretion was inoculated onto MacConkey agar to enhance the detection of Enterobacteriaceae (National Mastitis Council, 1999). Plates were incubated at 37°C and read at 24, 48, and 72 h. Organisms were identified by gross colony morphology and Gram stain and further confirmatory techniques as necessary (Quinn et al., 1994). If a pathogen was isolated, it was recorded as an infection regardless of the number of colony-forming units. The presence of more than 3 bacterial species was considered a contaminated result; more than one major pathogen was considered a mixed etiology with both organisms causal.

Clinical mastitis was investigated as an outcome at the quarter level in the following ways: first for *first* cases of CM in lactation before the next scheduled farm visit and second for *all* cases of CM before the next scheduled farm visit. The latter outcome was investigated for all pathogens and repeated for *E. coli* and *Strep. uberis* alone; these pathogens were associated with most of the CM in the study. In addition to these outcomes, the incidence of CM was assessed by stage of lactation; cases arising <30 d or >30 DIM. Thirty DIM was used as an approximate cut off to differentiate CM cases likely to have arisen from dry-period IMI (<30 d in lactation) or IMI during lactation (Green et al., 2002).

Statistical Analysis

All data were entered into a database (Access, Microsoft Corp., Redmond, WA) and checked for incorrect entries. Covariates to be included were individually assessed using chi-square tests or ANOVA (Petrie and Watson, 1999) as appropriate, using Excel (Microsoft Corp.) and Minitab 13.30 (Minitab Inc., State College, PA). Generalized linear mixed models were specified as described previously (Goldstein, 1995) using MLwiN (Rasbash et al., 1999). Response variables were "first quarter case of CM before the next visit," "quarter case of CM before the next visit" (for all cases, for *E. coli* alone, and for *Strep. uberis* alone), and "quarter case of CM before the next visit >30 d in lactation." Random effects were included for "quarter" (level 2) and "cow" (level 3) to account for the correlated nature of the data; repeated measures within quarters and quarters within cow. "Herd" was included as a fixed effect. Parity and stage of lactation were investigated as potential confounding covariates and included in the final models. Covariates with a trend toward significance ($P < 0.25$) were initially carried forward for inclusion into

Figure 1. Distribution of udder (black bars) and leg (gray bars) hygiene scores in all cow months. Udder hygiene scores: 1 = completely free or very little dirt; 2 = slightly (2–10% of the area) covered in dirt; 3 moderately (10–30% of the area) covered in dirt; 4 = covered (>30% of the area) with caked-on dirt.

subsequent models. All variables were tested before final model selection to ensure that all potential predictors were examined in light of the full model structure, because including or excluding covariates in the model sometimes allowed other covariates to change in statistical significance and biological interpretation. Biologically plausible interactions of significant covariates were tested and remained in the model if significant ($P < 0.05$).

The models took the general form

$$Y_{ijk} \sim \text{Binary outcome (probability } \pi_{ijk})$$

$$\text{logit}(\pi_{ijk}) = \text{intercept} + \beta_1 \text{herd}_k + \beta_2 p_k + \beta_3 \text{lm}_{ijk} + \beta_4 X_{ijk} + \beta_5 X_{jk} + \beta_6 X_k + v_k + u_{jk},$$

where the subscripts i , j , and k denote the i th sample time, the j th quarter, and the k th cow, respectively; Y_{ijk} = the outcome variable in the i th sample time, the j th quarter of the k th cow; π_{ijk} = the fitted probability of outcome; β_{1-6} = coefficients associated with each covariate; herd_k = covariate herd; p_k = covariate parity (parity 1, 2, or >3); lm_{ijk} = covariate lactation month (lactation mo 1, 2, 3, 4, and 5 compared with 6 and greater) at the i th sample time for the j th quarter of the k th cow; X = explanatory covariates associated with i th sample time, j th quarter, or k th cow level; v_k = random effect to reflect residual variation for cow; and u_{jk} = random effect to reflect residual variation for quarter.

For the final models, the significance probability was set at $P < 0.05$. Model fit was assessed using plots of

accumulated level 1 and standardized level 2 residuals as described previously (Green et al., 2004).

RESULTS

Description of the Risk Factor Data

A total of 1,677 cows were recruited to the study from the 8 herds selected to participate, and a total of 61,959 quarter measurements were made during the 12-mo study period. A total of 14,641 UHS and LHS were available for analysis (Figure 1). The majority of UHS were score 1 (free of contamination, 65%), with 5% of UHS recorded as score 4 (heavily contaminated). Five percent of LHS were recorded as score 1, and more than 70% of LHS scored as 3 or 4 (contaminated or heavily contaminated).

During the study, 55,271 quarters were available for TEC assessment. Of these, 619 were not categorized because of that quarter being dry or because severe teat-end trauma precluded categorization; 1,285 scores were missed during the milking attended. Therefore, 53,367 quarter TEC measurements were assigned to the scores outlined in the method (Figure 2). Only 7% of TEC scores were classified as N (no ring). The majority of TEC scores were score 1A and 1B (thin and moderate smooth callosity ring); score 2D (extreme thickening, severe HK) was present in 1% of all teats scored during the study period.

A total of 14,074 cow BCS were available for analysis (Figure 3). The BCS distribution was not normal; the data were positively skewed, with only 25% of cows scored at BCS >2.5 during study visits.

Figure 2. Distribution of teat-end callosity scores in all quarter months. Scores: N = normal teat end; 1A = thin smooth callosity ring; 1B = moderate smooth callosity ring; 1C = thick smooth callosity ring; 2A = thin rough callosity ring; 2B = moderate rough callosity ring; 2C = thick rough callosity ring; 2D = extreme rough callosity ring (severe hyperkeratosis of the teat end).

Table 2. All cases of clinical mastitis by major and minor pathogens identified from samples submitted (n = 640)

Mastitis diagnosis	n	%
<i>Escherichia coli</i>	171	26.7
<i>Streptococcus uberis</i>	121	18.9
Yeast	17	2.7
Coagulase-positive staphylococci including <i>Staphylococcus aureus</i>	20	3.1
<i>Bacillus</i> spp.	8	1.3
<i>Aerococcus</i> spp.	3	0.5
<i>Klebsiella</i> spp.	3	0.5
<i>Proteus</i> spp.	2	0.3
<i>Pseudomonas</i> spp.	2	0.3
<i>Streptococcus dysgalactiae</i>	2	0.3
<i>Strep other</i>	2	0.3
<i>Arcanobacterium pyogenes</i>	1	0.2
<i>Enterobacter</i> spp.	1	0.2
Enterococci	1	0.2
<i>Lactococcus</i> spp.	1	0.2
CNS	29	4.5
<i>Corynebacterium</i> spp.	15	2.3
Mixed etiology	40	6.3
No growth	201	31.4
Contaminated	0	0.0

Description of the Bacteriology Data

A total of 929 quarter cases of CM were recorded in the 8 herds, of which 640 (69%) were sampled and available for analysis. The environmental pathogens *E. coli* and *Strep. uberis* were isolated in 171 (26.7%) and 121 (18.9%) of cases sampled, respectively (Table 2). The third most prevalent major mastitis pathogens were the coagulase-positive staphylococci (including *Staph. aureus*) (20 isolates, 3.1%), followed by yeasts (2.7%). Minor pathogens (CNS and *Corynebacterium* spp.) were identified in 44 cases (6.8%). A diagnosis of “mixed etiology” was made in 40 cases, only one of which involved 3 major mastitis pathogens. No contaminated samples were recorded. A diagnosis of “no growth” was made in 201 cases (31.4%) for which a sample was available for culture.

The distribution of all cases of CM and CM caused by *E. coli* and *Strep. uberis* by cow parity, lactation month, UHS, and TEC score is displayed in Table 3.

Models for CM in Lactation

A summary of the models for CM in lactation is shown in Table 4. Quarters recording a teat-end score of 2D had increased odds to develop a first case of CM before the next visit and increased odds to develop a quarter case of CM before the next visit, compared with all other TEC scores. There was no association between thin and moderate TEC thickness scores and risk of CM. Cows with a UHS of 4 had increased odds to develop a first quarter case of CM before the next

visit and increased odds to develop a case of CM before the next visit, compared with UHS 1 and 2. Cows in the first month of lactation had increased odds to develop a first case of CM in lactation before the next visit and to develop a case of CM before the next visit, compared with lactation mo 6 and above. This trend continued with lactation mo 2 to 5. Both parity 1 and parity 2 animals had decreased odds for a first quarter case and decreased odds for all quarter cases of CM before the next visit, compared with parity 3 cows and older; in addition, the odds were reduced for parity 1 compared with parity 2 animals.

The odds of a quarter case of CM after the first 30 DIM were significantly increased with an SCC >199,000 cells/mL ≤30 DIM, very severe HK of the teat-end (score 2D), and a low first test-day milk protein percentage (<3.2%). The odds of a quarter case of CM after the first 30 DIM were significantly decreased in parity 1 and 2 animals compared with parity 3 cows and older.

Models for Pathogen-Specific CM

The models for pathogen-specific (*E. coli* and *Strep. uberis*) quarter cases of CM before the next visit in lactation are summarized in Tables 5 and 6, respectively. The odds for a quarter case of *E. coli* CM were significantly higher in the first 6 mo of lactation, compared with lactation mo 7 and greater. Moderate and very severe HK of the teat-end (scores 2B and 2D) were also associated with increased odds for a quarter case of *E. coli* CM. There was a strong trend for UHS 3 and 4 to be associated with an increased risk for a quarter case of *E. coli* CM. The odds of a first quarter case of *E. coli* CM significantly decreased in parity 1 animals compared with parity 2 cows and older.

Figure 3. Distribution of BCS in all cow months.

Table 3. Distribution of all quarter clinical mastitis cases before the next visit by selected study variables

Variable		Quarter case of clinical mastitis			
		1			0
		All cases (n = 929)	<i>E. coli</i> (n = 171)	<i>Strep. uberis</i> (n = 121)	(n = 61,030)
Parity	1	131 (14.7)	22 (12.9)	16 (13.3)	16,330 (27.1)
	2	177 (19.9)	41 (24.1)	26 (21.7)	13,450 (22.3)
	3	170 (19.1)	38 (22.4)	18 (15.0)	10,143 (16.8)
	>3	411 (46.3)	69 (40.6)	60 (50.0)	20,402 (33.8)
	Missing ¹	40	1	1	705
Lactation month	1	125 (14.1)	23 (13.5)	22 (18.3)	5,055 (8.4)
	2	106 (11.9)	18 (10.6)	15 (12.5)	4,974 (8.2)
	3	94 (10.6)	16 (9.4)	9 (7.5)	4,902 (8.1)
	>3	563 (63.4)	113 (66.5)	74 (61.7)	45,394 (75.3)
	Missing	41	1	1	704
UHS ²	1	485 (59.7)	79 (55.2)	60 (56.6)	37,699 (65.3)
	2	183 (22.5)	33 (23.1)	30 (28.3)	12,369 (21.4)
	3	95 (11.7)	22 (15.4)	12 (11.3)	5,377 (9.3)
	4	49 (6.0)	9 (6.3)	4 (3.8)	2,307 (4.0)
	Missing	117	28	15	3,278
TEC ³	N	64 (8.6)	10 (7.0)	15 (15.0)	4,002 (7.5)
	1A	195 (26.1)	34 (23.9)	28 (28.0)	16,414 (30.8)
	1B	250 (33.5)	46 (32.4)	32 (32.0)	19,613 (36.8)
	1C	32 (4.28)	4 (2.8)	4 (4.0)	2,346 (4.4)
	2A	79 (10.6)	14 (9.9)	9 (9.0)	5,706 (10.7)
	2B	61 (8.2)	16 (11.3)	5 (5.0)	2,805 (5.3)
	2C	26 (3.5)	5 (3.5)	3 (3.0)	1,229 (2.3)
	2D	35 (4.7)	13 (9.5)	4 (4.0)	510 (1.0)
	Missing	187	19	21	6,506

¹These quarter cases of clinical mastitis were missing relevant information and were discarded from the final models.

²Udder hygiene score: 1 = completely free or very little dirt; 2 = slightly (2–10% of the area) covered in dirt; 3 moderately (10–30% of the area) covered in dirt; 4 = covered (>30% of the area) with caked-on dirt.

³Teat-end callosity score: N = normal teat end; 1A = thin smooth callosity ring; 1B = moderate smooth callosity ring; 1C = thick smooth callosity ring; 2A = thin rough callosity ring; 2B = moderate rough callosity ring; 2C = thick rough callosity ring; 2D = extreme rough callosity ring (severe hyperkeratosis of the teat end).

The odds for a quarter case of *Strep. uberis* CM were significantly increased in the first months of lactation (compared with lactation mo 7) and very severe HK of the teat-end (2D) only. There was a strong trend for TEC score N to be associated with an increased risk for a quarter case of *Strep. uberis* CM. The odds of a quarter case of *Strep. uberis* CM in lactation were significantly decreased in parity 1 animals compared with parity 2 cows and older.

DISCUSSION

This prospective longitudinal study has shown that individual cow factors are important in influencing the risk of CM during lactation, and these factors indicate a differing susceptibility to CM between animals.

Very severe HK of the teat-end was clearly associated with an increased risk of CM in the 8 herds studied. Although very severe HK was associated with a significantly higher risk of CM in all the lactation models, it was notable that there was no association between thin and moderate HK of the teat end or increased TEC

thickness and the risk of overall CM. These data suggest that only severe disruption to the normal anatomy and physiology of the teat orifice is clearly associated with increased risk of bacterial colonization of the streak canal and development of CM. These findings contrast with a study by Neijenhuis et al. (2001) who reported that small increases in TEC score were significantly associated with an increased risk of CM when assessing quarters within or between different cows. The data in the present study indicated that it was generally only the very severe HK category that was important in determining the risk of overall CM and that smaller teat changes in intermediate TEC categories appeared to be less important. In this study, moderate HK was only identified to significantly increase the risk of *E. coli* CM, again contrasting with the findings presented by Neijenhuis et al. (2001), which showed that clinical cases of *E. coli* mastitis in early lactation tended to occur in cows with lower rather than higher TEC scores during lactation. There was a strong trend within the data for an association between teat-ends with no callosity ring and the risk of *Strep. uberis* CM, although

Table 4. Summary of the significant terms ($P < 0.05$) for clinical mastitis in the final lactation models

Variable	Coefficient	SEM	Odds ratio	Confidence interval	
				2.5%	97.5%
First quarter case of clinical mastitis before the next visit					
Parity 1	−0.879	0.137	0.42	0.32	0.55
Parity 2	−0.363	0.123	0.70	0.54	0.89
(reference = parity 3 and above)					
Lactation mo 1	1.051	0.134	2.86	2.19	3.74
Lactation mo 2	0.866	0.141	2.38	1.79	3.15
Lactation mo 3	0.707	0.149	2.03	1.51	2.73
Lactation mo 4	0.756	0.146	2.13	1.59	2.85
Lactation mo 5	0.373	0.171	1.45	1.03	2.04
(reference 6 and above)					
TEC ¹ 2D	0.983	0.287	2.67	1.50	4.74
(reference = TEC N to 2C)					
UHS ² 4	0.421	0.181	1.52	1.06	2.19
(reference = UHS 1 and 2)					
Quarter case of clinical mastitis before the next visit					
Parity 1	−1.023	0.14	0.36	0.27	0.48
Parity 2	−0.388	0.124	0.68	0.53	0.87
(reference = parity 3 and above)					
Lactation mo 1	0.876	0.121	2.40	1.89	3.06
Lactation mo 2	0.778	0.123	2.18	1.70	2.78
Lactation mo 3	0.617	0.13	1.85	1.43	2.40
Lactation mo 4	0.626	0.13	1.87	1.44	2.43
Lactation mo 5	0.311	0.147	1.36	1.02	1.83
(reference = mo 6 and above)					
TEC 2D	0.825	0.276	2.28	1.50	3.96
(reference = TEC N to 2C)					
UHS 4	0.362	0.169	1.43	1.02	2.01
(reference = UHS 1 and 2)					
Quarter case of clinical mastitis before the next visit > 30 d in milk					
Parity 1	−1.005	0.168	0.37	0.26	0.51
Parity 2	−0.367	0.143	0.69	0.52	0.92
(reference = parity 3 and above)					
CalvSCC ³ >199,000	0.569	0.114	1.77	1.41	2.22
TEC 2D	0.786	0.278	2.19	1.26	3.83
(reference = TEC N)					
CalvPro ⁴ <3.2%	0.308	0.122	1.36	1.07	1.74
(reference = CalvPro >3.2%)					

¹Teat-end callosity score: N = normal teat end; 1A = thin smooth callosity ring; 1B = moderate smooth callosity ring; 1C = thick smooth callosity ring; 2A = thin rough callosity ring; 2B = moderate rough callosity ring; 2C = thick rough callosity ring; 2D = extreme rough callosity ring (severe hyperkeratosis of the teat end).

²Udder hygiene score: 1 = completely free or very little dirt; 2 = slightly (2–10% of the area) covered in dirt; 3 moderately (10–30% of the area) covered in dirt; 4 = covered (>30% of the area) with caked-on dirt.

³The first test-day SCC.

⁴The first test-day protein percentage.

this was nonsignificant in the final model. This is in agreement with findings reported by Neijenhuis et al. (2001) and may suggest that parakeratosis or thickening of the teat-end reduces the risk for clinical mastitis due to this pathogen, although the reasons remain unclear. The prevalence of HK observed in these study herds differed from that reported by Neijenhuis et al. (2001), who found that 38% of lactating quarters in 15 herds scored rough compared with 22% of quarters from 8 herds in our study. These conflicting reports suggest that the relationship between TEC scores and CM is not straightforward and that individual herd factors may influence the relationship. More research in this area would be worthwhile.

The findings in this study have shown a clear association between cows with very dirty udders and an increased risk of overall CM in lactation. There were strong trends for UHS to be associated with an increased risk for *E. coli* CM within the final models and for *Strep. uberis* CM in the univariate analysis, although this is likely to be an issue with a lack of power in the data. These findings may suggest that environmental hygiene is less important for the acquisition of *Strep. uberis* CM in these herds but further research into the importance of hygiene and the risk of different pathogens that cause environmental mastitis may be worthwhile. Hygiene scoring differed from the approach taken in a previous UK paper that scored the flanks and tails in addition to

Table 5. Summary of the terms for *Escherichia coli* clinical mastitis in the final lactation model

Variable	Coefficient	SE	Odds ratio	Confidence interval	
				2.5%	97.5%
Quarter case of <i>E. coli</i> clinical mastitis before the next visit					
Parity 1 (reference = parity 2 and above)	−0.838	0.295	0.43	0.24	0.78
Lactation mo 1	1.312	0.295	3.71	2.06	6.70
Lactation mo 2	0.828	0.33	2.29	1.18	4.43
Lactation mo 3	0.778	0.333	2.18	1.12	4.24
Lactation mo 4	1.049	0.303	2.85	1.56	5.23
Lactation mo 5	0.537	0.354	1.71	0.84	3.47
Lactation mo 6 (reference = mo 7 and above)	1.122	0.294	3.07	1.70	5.53
TEC ¹ 2B	0.721	0.308	2.06	1.11	3.81
TEC 2C ²	0.217	0.520	1.24	0.44	3.51
TEC 2D (reference = TEC N to 2A)	1.794	0.427	6.01	2.56	14.13
UHS ³ 3 and 4 ² (reference = UHS 1 and 2)	0.446	0.242	1.56	0.96	2.53

¹Teat-end callosity score: N = normal teat end; 1A = thin smooth callosity ring; 1B = moderate smooth callosity ring; 1C = thick smooth callosity ring; 2A = thin rough callosity ring; 2B = moderate rough callosity ring; 2C = thick rough callosity ring; 2D = extreme rough callosity ring (severe hyperkeratosis of the teat end).

²These covariates were nonsignificant at the 95% level but displayed a strong trend.

³Udder hygiene score: 1 = completely free or very little dirt; 2 = slightly (2–10% of the area) covered in dirt; 3 moderately (10–30% of the area) covered in dirt; 4 = covered (>30% of the area) with caked-on dirt.

udders and legs (Ward et al., 2002). The simpler system used in this study and described in a recent US study (Schreiner and Ruegg, 2002) allowed large numbers of animals to be scored rapidly. Work done by the same authors concluded that it was more likely that udder and/or leg (which will come into contact with the udder when the cow lies down) hygiene score correlated most with mastitis risk as measured by SCC (Schreiner and Ruegg, 2003). Poor cow hygiene is a common problem in many housed dairy herds, and the cleanliness of cows provides a useful indicator of the environmental challenge and is elementary to food safety and quality

assurance schemes (Hughes, 2001). Hygiene scoring is a useful tool to indicate when cows may be too dirty but practical recommendations to keep cows clean are likely to be related to a combination of management factors (Green et al., 2007). Inorganic bedding materials such as sand have been shown to have significantly lower numbers of bacteria compared with organic bedding (Hogan et al., 1989), and a recent study found coliforms and *Klebsiella* spp. to be more numerous when cows were bedded on sawdust but *Streptococcus* spp. to be more numerous when cows were bedded on sand (Zdanowicz et al., 2004). The differences in management

Table 6. Summary of the terms for *Streptococcus uberis* clinical mastitis in the final lactation model

Variable	Coefficient	SEM	Odds ratio	Confidence interval	
				2.5%	97.5%
Quarter case of <i>Strep. uberis</i> clinical mastitis before the next visit					
Parity 1 (reference = parity 2 and above)	−0.884	0.339	0.41	0.21	0.81
Lactation mo 1	1.359	0.317	3.89	2.06	7.34
Lactation mo 2	1.095	0.335	2.99	1.53	5.84
Lactation mo 3 ¹	0.558	0.370	1.75	0.83	3.66
Lactation mo 4 (reference = mo 5 and above)	1.245	0.318	3.47	1.84	6.56
TEC ² N ¹	0.621	0.323	1.86	0.98	3.55
TEC 2D (reference = TEC 1A to 2C)	1.464	0.592	4.32	1.32	14.13

¹These covariates were nonsignificant at the 95% level but displayed a strong trend.

²Teat-end callosity score: N = normal teat end; 1A = thin smooth callosity ring; 1B = moderate smooth callosity ring; 1C = thick smooth callosity ring; 2A = thin rough callosity ring; 2B = moderate rough callosity ring; 2C = thick rough callosity ring; 2D = extreme rough callosity ring (severe hyperkeratosis of the teat end).

style of the 8 herds studied may have confounded the investigation of pathogen-specific risk factors because of differing bacterial populations present in lactating cow housing. In practice, however, this is often the case with herds using many different types of bedding material as well as different qualities and grades of the same material (e.g., sawdust and wood shavings).

Cows that recorded an SCC >199,000 cells/mL and a milk protein percentage <3.2 at ≤ 30 DIM were at greater risk of developing CM after the first month of lactation. Milk quality data from monthly milk samples are routinely recorded in many dairy herds and with the increasing availability of the Internet, these data are now readily available to veterinary practitioners and consultants. The use of first test-day information such as butter fat percentage, protein percentage, and butter fat to protein ratio may be useful indirect measurements of energy status in dairy cows. Several studies have investigated the relationship between milk composition and energy balance in early lactation (Reist et al., 2002; Friggens et al., 2007). Changes in energy balance alter metabolite concentrations, which are thought to impair neutrophil function (Suriyasathaporn et al., 2000) although the exact mechanisms may be unclear (Perkins et al., 2001; Scalia et al., 2006). In a study that investigated first test-day milk recording data as predictors of disease in lactation (Heuer et al., 1999), cows with a butter fat to protein ratio of greater than 1.5 at the first milk recording after calving had an increased odds for CM in the subsequent lactation. Data from the current study suggest that milk protein may be more important than butter fat: protein ratio with respect to mastitis in UK dairy herds.

Despite an association between milk protein percentage and risk of CM, this study was not able to demonstrate a significant relationship between BCS and risk of CM. This is in agreement with the study conducted by Zadoks et al. (2001). It may be that BCS is too historic or too imprecise to use as an effective proxy for changing metabolic status within multi-level models that use clinical disease as an outcome, and further research is required to investigate this. The distribution of BCS data within the 8 Holstein-Friesian study herds does suggest that this cow sample contained a greater proportion of thin cows compared with other studies; for example, Berry et al. (2007), which combined data from Holstein-Friesian and Jersey cows, and this could be due to nutritional management and production differences of the herds enrolled into this study.

Part of the largest variation seen in SCC concentration is thought to be due to infection of the gland with bacteria (Sordillo et al., 1997). Therefore, cows that have calved with an increased SCC will be at greater risk of CM (Table 4), either because they are in the

early stages of active infection or because a subclinical infection may become clinical again given the right circumstances.

In the final lactation models, significant independent variables associated with the risk of CM included parity of the cow and month of lactation. Several previous studies have shown that the incidence rate of CM is lower (Milteneburg et al., 1996; Barkema et al., 1998; Bradley and Green, 2001; Zadoks et al., 2001) and the plot of SCC and DIM much flatter (Scheepers et al., 1997) in parity 1 cows compared with older animals. Periparturient heifers are less likely to have succumbed to a previous case of CM and therefore are unlikely to be persistently infected and record recurrent CM cases. Younger cows may also be housed, fed, and milked in a separate group away from the main herd to allow heifers to acclimate to the ration and cubicles on the unit; consequently, younger cows may be managed to a higher standard. It may also be that resistance to IMI declines with increasing age, as older animals often have concurrent health issues such as lameness. There were strong trends for the incidence of *Strep. uberis* CM to increase with increasing parity, perhaps reflecting persistence of this pathogen within the udder. Stage of lactation was associated with an increased risk for CM in this study and in particular, the risk for *E. coli* mastitis was as high in mo 6 of lactation as it was in mo 1, reinforcing the opportunistic nature of infection if exposure to the organism in the environment is not kept to a minimum. Previous studies have reported an increased incidence rate of CM in early lactation (Milteneburg et al., 1996; Barkema et al., 1998; Elbers et al., 1998; Bradley and Green, 2001) due to a likely combination of dry period infections (Bradley and Green, 2000) and susceptibility of cows in early lactation (Oliver and Sordillo, 1988).

During this study, particular emphasis was placed on observational measurements including hygiene scoring, teat-end scoring, and body condition scoring as these parameters can be modified or improved to allow prevention of disease. These methods of assessment are also simple to perform and noninvasive, and their use is encouraged within the current industry drive for herd health planning.

CONCLUSIONS

Individual cow factors such as increased teat-end roughness (particularly very severe HK) and heavily contaminated udders increase the risk of CM in lactation. In addition, cows recording an SCC >200,000 cells/mL or a milk protein percentage of <3.2 at the first test-day postcalving were more likely to record a case of CM after the first 30 DIM. Cow hygiene and TEC score also influence the risk of pathogen-specific

CM, particularly clinical *E. coli* mastitis compared with clinical *Strep. uberis* mastitis.

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