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A prospective cohort study of the association between early lactation mastitis and presence of sole ulcers in dairy cows

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Abstract:	Objective: The objective of this study was to investigate the association between (sub)clinical mastitis in the first 30 days in milk (DIM) and the presence of sole ulcers (SU) later in lactation. Methods: Holstein cows and heifers were examined for presence of sole haemorrhage and SU before calving, in the first 14 days post-calving and in early lactation (after 30 DIM). Clinical mastitis (CM) episodes and SCC measurements were obtained from farm records. Multivariable logistic regression was used for data analysis. Results: Odds of SU in early lactation were 2.44 times greater (95% CI 0.97-5.54) in cows that had CM in the first 30 DIM compared to cows that did not have CM in the first 30 DIM. When cows that had SU pre-calving or at the calving check were excluded from the dataset, an association of CM in the first 30 DIM with later presence of SU was no longer statistically significant but the same numeric trend still existed (OR 2.25, 95% CI 0.81-5.34). The odds of SU in early lactation were 1.70 times greater in cows that had high SCC compared to cows that did not have high SCC in the first 100 DIM (95% CI 1.13 - 2.55). Conclusion: An association was found between CM in the first 30 DIM and presence of SU in early lactation (after 30 DIM). Elucidating the mechanism behind this relationship could improve our understanding of the aetiopathogenesis of both diseases and lead to new preventive strategies

A prospective cohort study of the association between early lactation mastitis and presence of sole ulcers in dairy cows

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ABSTRACT

Objective: The objective of this study was to investigate the association between (sub)clinical mastitis in the first 30 days in milk (DIM) and the presence of sole ulcers (SU) later in lactation.

Methods: Holstein cows and heifers were examined for presence of sole haemorrhage and SU before calving, in the first 14 days post-calving and in early lactation (after 30 DIM). Clinical mastitis (CM) episodes and SCC measurements were obtained from farm records. Multivariable logistic regression was used for data analysis.

Results: Odds of SU in early lactation were 2.44 times greater (95% CI 0.97-5.54) in cows that had CM in the first 30 DIM compared to cows that did not have CM in the first 30 DIM. When cows that had SU pre-calving or at the calving check were excluded from the dataset, an association of CM in the first 30 DIM with later presence of SU was no longer statistically significant but the same numeric trend still existed (OR 2.25, 95% CI 0.81-5.34). The odds of SU in early lactation were 1.70 times greater in cows that had high SCC compared to cows that did not have high SCC in the first 100 DIM (95% CI 1.13 - 2.55).

Conclusion: An association was found between CM in the first 30 DIM and presence of SU in early lactation (after 30 DIM). Elucidating the mechanism behind this relationship could improve our understanding of the aetiopathogenesis of both diseases and lead to new preventive strategies.

INTRODUCTION

Sole ulcers (SU) and clinical mastitis (CM) are two of the most important and prevalent diseases of dairy cattle (1,2). Each condition is painful (3,4) and has well-recognised effects on dairy farm profitability (5,6). A preliminary study carried out by our group found that cows were significantly more likely to develop SU in early lactation if they had clinical mastitis (CM) in the first 30 DIM (7).

Risk for occurrence of CM is greatest in the first 30 DIM (8,9). Incidence has been associated with reproductive disease (10) and reduced fertility (11,12) but associations with other diseases have not been widely explored. Lameness between calving and first service has been found to increase the risk of a CM episode in the same period (10) and both conditions have been associated with an elevated acute phase response, which is an indicator of systemic inflammation (13).

The occurrence of SU is highest in early- to mid-lactation (1,14). However, many of these lesions may originate from an initial insult around parturition (15). At this time the suspensory apparatus of the third phalanx (P3) undergoes increased laxity (16) and P3 can be displaced ventrally (17). This can result in compression and ischaemia of the corium underneath the tuberculum flexorium. Horn production becomes impaired and a full thickness defect develops over the following 8-12 weeks (18).

Our previous study (7) showed that cows that developed CM within the first 30 days of calving had almost four times higher odds of having a SU present at 8-10 weeks of lactation compared to cows that did not develop CM. The timing suggests the incidence of CM preceded the appearance of SU, but the study could not clearly show such cause-and-effect relationships.

Our main objective here was to investigate the association between CM in the first 30 DIM and presence of SU in dairy cows in early lactation (but after the first 30 DIM),

using a much larger dataset and more intensive recording of foot lesions than our previous study. Additionally, we aimed: a) to investigate whether these SU were new lesions that appeared after the episode of CM; b) to evaluate whether CM in the first 30 DIM was associated with the presence of both sole haemorrhage (SH) and SU, because SH is regarded as a precursor for the development of SU (19) and c) to investigate the association of somatic cell counts (SCC) in the first 30 DIM with the presence of SU in early lactation (but after 30 DIM), because individual SCC is an established proxy for both subclinical and clinical mastitis (20). Our null hypothesis was that there is no association between a case of CM in the first 30 days of lactation and having a SU present in early lactation (after 30 DIM).

MATERIALS AND METHODS

Data collection included 2,353 Holstein-Friesian cows and heifers from four farms and lasted from January 2019 until April 2020. The study was approved by the University of Liverpool Research Ethics Committee (VREC 466 and VREC 269). A convenience sample of four commercial dairy herds in North West England and North Wales were selected for their proximity to the School of Veterinary Science (University of Liverpool) and for their willingness to participate. Of the 2,353 cows enrolled, 132 were from Farm 1, 239 were from Farm 2, 1,550 were from Farm 3 and 432 were from Farm 4.

None of the farms farmed under organic conditions and all followed an all-year-round calving pattern. All farms routinely foot trimmed adult cows twice a year – at drying off and between 60-120 days calved - and used a footbath located in the exit lane of the milking parlour. All farms had rubber matting in the parlour and grooved concrete in the collecting yard, except for Farm 1 where a third of the collecting yard surface was

covered with rubber matting. All farms housed lactating cows in cubicles; Farm 1 bedded cubicles with mattresses and sawdust, the other 3 farms used sand.

All purebred Holstein cows and heifers with an expected calving date between March and December 2019 were eligible for enrolment. Each farm was visited weekly and data were collected from each animal three times: a) at a mean (± SD) interval of 55.91 ± 20.57 days before their expected calving date ('pre-calving check'), b) at a mean (± SD) interval of 5.43 ± 2.87 days post-calving ('calving check'), and c) at a mean (± SD) interval of 84.04 ± 13.86 days after calving ('early lactation check').

Cows were restrained in a foot trimming crush. Current parity and calving date were recorded. Body condition score was assessed on each occasion by research staff using a 1 to 5 scale with 0.25 increments (21).

All four feet were lifted in turn and examined by a qualified veterinarian. Over 90% of feet examinations were performed by the same person throughout the study. Superficial sole horn was trimmed to reveal sole lesions. Lesions were recorded and graded by severity based on the ICAR claw health atlas (22). Data collection was the same at all three time points except at the 'calving' check on farm 3 when only hind feet were inspected for lesions. All cows had routine foot-trimming conducted by farm or research staff at (or close to) the 'pre-calving' and 'early lactation' checks. Lame cows received additional foot trimming as necessary by farm staff.

Monthly milk recording data were downloaded from national milk recording agencies for all farms. They were imported into herd management software (TotalVet, SUM_IT). This provided monthly SCC and CM events during enrolment in the study. Cases of CM and treatments (as well as other health and treatment information) were recorded as part of routine farm management on all farms by farm staff and entered onto farm

management software. These data were exported in each case into Excel (Microsoft Corp., Redmond, WA).

Data was collected in Microsoft Access and checked for incorrect entries. Statistical analysis was carried out using R Studio (R Development Core Team 4.0., 2020).

To explore the relationship between CM and SU, independent variables were chosen based on repeatedly established associations or a logical biological relationship. The following were included due to an association with development of SU and other claw horn disruption lesions: farm (23), parity (14,24), body condition score (25,26), milk yield (24), previous occurrence of SU (27,28) and number of days since calving (28). Likewise, farm (20), milk yield (29) and parity (30) have been linked to the incidence of CM. Composite somatic cell count (SCC), uterine infection in the first 30 days postpartum and occurrence of other reproductive diseases (uterine torsion, retained foetal membranes and giving birth to twins) were also included in the initial analysis because they may be associated with systemic periparturient inflammation (31).

Clinical mastitis was separately considered for the first 30, 60 and 100 days, as well as between 30-60 and 60-90 days of lactation because there is an established link between incidence and stage of lactation (9).

To avoid excluding cows with missing milk yield data we chose to include the maximum recorded milk yield at any milk recording that occurred in the first 100 days of lactation. A cow was considered to have high SCC if she was recorded with a composite milk SCC >200,000 cells/ml (32).

To facilitate analysis and interpretation of results, independent variables were categorised as follows: - parity (1st, 2nd, >2nd), SCC (<200,000 cells/ml or >200,000 cells/ml) and BCS (<2.5, 2.5-3.0, >3.0; corresponding to what is generally accepted to be low, normal, or high BCS respectively). Number of DIM at the early lactation check

and maximum daily yield were fitted as continuous variables. Farm was treated as a categorical variable.

Model building

All regression analyses were performed using the glm function in the "stats" package of R (version 3.6.2). Univariable analysis was conducted using logistic regression for either SU or SU and SH. Days in milk at early lactation check (a continuous variable) was tested for log-linearity by plotting observed against predicted outcomes. Independent variables with $P < 0.2$ in univariable analysis were included in the multivariable model.

The following independent variables were offered to the models:- farm, parity, BCS at the early lactation check, DIM at early lactation check, presence of SU at either the pre-calving check or at calving, occurrence of clinical mastitis in the first 30 DIM and maximum daily yield recorded in the first 100 DIM.

The final multivariable model was selected based on the Akaike information criterion (AIC) using automated backwards stepwise selection via the MASS package (33). Once the simplest model that described the data was determined, independent variables were checked for multicollinearity and biologically plausible or previously reported two-way interactions were assessed. Potential two-way/first-order interaction terms were assessed for significance in the model using the Wald chi-squared test <0.05 and plotted.

The model fit was assessed using the Hosmer-Lemeshow test and by calculating the Pseudo R-squared.

To further understand the relationship between the dependent variable and explanatory variable of interest, we estimated the model-adjusted attributable fraction of the variable on the outcome using the AF package (34). This provided context to

any associations of interest by quantifying the proportion of cases that would not have occurred in the absence of the exposure (35).

To fully address our objectives, four separate multivariable regression models were built.

Model 1 (SU and CM)

Our first model investigated the association between CM in the first 30 DIM and presence of SU at the early lactation check.

Cows were excluded (n = 222, leaving 2,131 in the dataset) if they did not have foot lesion data recorded for the early lactation check (n = 221), or they were missing foot lesion data for either the pre-calving or calving check (n=4). In some cases (n = 38), animals were enrolled prior to parturition but did not subsequently calve because they aborted, died or were euthanised for health reasons. To avoid excluding records with missing covariables, a mean value was imputed for 9 cows missing BCS data at the early lactation check, 20 cows missing milk yield data for the first 100 DIM, and 8 cows missing SCC data. The outcome of interest (dependant variable) was the presence of a SU at the early lactation check.

Model 2 (New SU and CM)

A second model, excluding cows with a pre-existing SU, was fit in an attempt to investigate if the episode of CM occurred first and was followed by the later appearance of SU. Cows that had a SU recorded at either the pre-calving or calving check were excluded from the dataset; the outcome of interest in this model was the presence of a new SU at the early lactation check (n = 2,036).

Model 3 (SU/SH and CM)

Our third model included SH grades 2 (light pink lesion >2cm or dark red lesion <2cm) and 3 (dark red lesion >2cm or any size blue lesion) as well as SU as an outcome of interest (dependent variable) because SH are regarded as a precursor for the development of SU.

The same dataset was used as for Model 1 (n = 2,131).

Presence of SU or SH = 2 or 3 at pre-calving check or calving rather than presence of SU alone was also used as the relevant independent variable in this model.

Model 4 (SU and SCC)

As CM in the first 30 DIM and SCC over 200,000 cells/ml were correlated on univariate analysis ($P < 0.001$), a separate model was built to investigate the association of high SCC (>200,000 cells/ml) with the presence of SU at the early lactation check. SCC in the first 30 DIM and SCC in the first 100 DIM were both used as independent variables in separate versions of this model. As in Model 1, cows were excluded if they did not have early lactation foot lesion data, or they were missing foot lesion data for either the pre-calving or calving check. A further 8 cows were excluded as they had no SCC data recorded for the first 100 DIM. This left 2,101 cows available to fit the final model.

RESULTS

A total of 2,353 cows were recruited to the study from the 4 participating herds. Of these, 131 cows had SU in early lactation, and 49 cows had a first case of CM recorded in the first 30 DIM. Information regarding the datasets used in our analyses is presented in Table 1.

Of the 2,131 cows included in the analysis for Model 1, 8 cows had both CM recorded in the first 30 DIM and SU recorded in early lactation. The same dataset was used for Model 3. 19 cows had SU or SH = 2/3 in early lactation and CM in the first 30 DIM.

For Model 2, a further 153 cows were excluded because they were recorded as having SU at pre-calving or calving check. 6 cows had both CM recorded in the first 30 DIM and SU in early lactation.

Model 1

The following independent variables were retained in the model with SU in early lactation as the outcome of interest: farm, parity, BCS at early lactation check, DIM at early lactation check, presence of SU at pre-calving check or calving check and incidence of CM in the first 30 DIM. Results are presented in Table 2.

Of the cows that had CM in the first 30 DIM 16.3% had SU present in early lactation compared to only 5.9% of cows that did not have CM in the first 30 DIM in early lactation (P = 0.01). After correcting for farm, parity, BCS at early lactation check, DIM at early lactation check and presence of SU at pre-calving check or calving check, the odds of having SU in early lactation were 2.44 times greater for cows that had CM in the first 30 DIM compared to cows that did not have CM in the first 30 DIM (CI 0.97 - 5.54, P = 0.04). The attributable fraction of the risk of presence of SU in early lactation to CM in the first 30 DIM was 2.95% (CI -0.63 to 6.53%).

The odds of developing SU in early lactation were 7.56 times greater if cows had SU pre-calving or when freshly calved compared to cows that did not have SU pre-calving or when freshly calved (CI 4.46 - 12.76, P < 0.001).

Cows on farm 4 had significantly reduced odds of having SU present in early lactation (OR 0.27, CI 0.11-0.67, P = 0.004). Cows also had reduced odds of SU if they were in parity 2 (OR 0.12, CI 0.06-0.25, P < 0.001), had BCS category 2.5-3.0 (OR 0.45, CI 0.27-0.75, P = 0.002), or BCS category >3.0 (OR 0.32, CI 0.18-0.57, P < 0.001).

There was a significant association between DIM at early lactation check and presence of SU (OR 1.02, CI 1.0-1.04, P = 0.03).

Model 2

The following independent variables were retained in the model with SU in early lactation as the outcome of interest when cows that had SU at pre-calving or calving check were excluded: Farm, parity, BCS at early lactation check, DIM at early lactation check, and incidence of CM in the first 30 DIM. Results are presented in Table 3.

Of the cows that had CM in the first 30 DIM and no previous SU recorded at pre-calving or calving check 13.04% developed SU in early lactation, whilst only 4.52% of cows that did not have CM in the first 30 DIM and no previous SU developed SU in early lactation (P = 0.02). After correcting for farm, parity, BCS at early lactation check and DIM at the early lactation check, the odds of developing SU in early lactation were not statistically significantly greater in cows that had CM in the first 30 DIM compared to cows that did not have CM in the first 30 DIM (OR 2.25; CI 0.81-5.35, P = 0.09). The attributable fraction of the risk of presence of SU in early lactation to CM in the first 30 DIM was 3.12% (CI -1.54-7.78%) for cows with no SU recorded at pre-calving or calving check.

Model 3

The following independent variables were retained in the model with SU or severe SH at early lactation as the outcome of interest: Farm, parity, BCS at early lactation check, presence of SU or severe SH at pre-calving check or calving check. Incidence of CM in the first 30 DIM was forced into the final model. Results are presented in Table 4.

After adjusting for farm, parity, BCS at early lactation check, presence of SU or severe SH pre-calving or at fresh check, presence of SU or severe SH by BCS at early lactation check and farm by parity, the odds of developing SU or severe SH in early lactation were not statistically significantly greater in cows that had CM in the first 30

DIM compared to cows that did not have CM in the first 30 DIM (OR 1.19, CI 0.61-2.27, P = 0.59). The attributable fraction of the risk of presence of SU or severe SH in early lactation to CM in the first 30 DIM was 0.2% (95% CI -0.81-1.36%).

The odds of developing SU or severe SH in early lactation were 6.36 times greater if cows had SU or severe SH pre-calving or when freshly calved compared to cows that did not have SU or severe SH pre-calving or when freshly calved (CI 2.79-16.06, P < 0.001).

Model 4

The following independent variables were retained in the model with presence of SU in early lactation as the outcome of interest: farm, parity, BCS at early lactation check, DIM at early lactation check, presence of SU at pre-calving or calving check and high SCC (>200,000 cells/ml).

When high SCC in the first 30 days was entered into the model, there was no association with presence of SU in early lactation (OR 1.19, CI 0.61-2.27, P = 0.59). The attributable fraction of the risk of presence of SU in early lactation to high SCC in the first 30 DIM was 3.04% (CI -2.70 to 8.79%).

However, when SCC in the first 100 DIM was considered, the odds of SU in the early lactation check were 1.70 times greater in cows that had high SCC compared to cows that did not have high SCC in the first 100 DIM (CI 1.13 - 2.55, P = 0.01) after correcting for farm, parity, BCS at early lactation check, DIM at early lactation check and presence of SU at pre-calving check or calving check. The attributable fraction of the risk of presence of SU in early lactation to high SCC in the first 100 DIM was 13.45% (95% CI 2.73 to 24.18%). Results for high SCC in the first 100 DIM are presented in Table 5.

DISCUSSION

We show here that there is an association between CM in the first 30 DIM and presence of SU in early lactation (after the first 30 DIM), which is in agreement with our previous study (7). We also investigated whether CM in the first 30 DIM precedes the development of a new SU by excluding cows with a pre-existing SU. The association was no longer statistically significant but the same numeric trend still existed. It does appear that severity of foot lesion is important as the association is only statistically significant for SU and not for milder lesions (SH). High SCC in the first 100 DIM was also associated with presence of SU in early lactation.

Studies of the risk factors for SU have tended to look at large datasets from national foot trimming records (28,36,37) or from foot trimming records made by farm staff (14). These lesion records may over represent lame animals or under report mild lesions. Not many studies have explored links between CM and other aspects of claw health and very few have directly explored the association between incidence of CM and SU. Peeler et al. (1994) (10) found that lameness and CM in the period before service were significantly associated in a study of 3,603 lactation records from 10 dairy herds in the UK. A genetic association has also been found between CM and SU in Swedish Red Cows, meaning that some cows may be genetically more susceptible to both conditions (38).

Although our results indicate there could be an association between clinical mastitis in the first 30 DIM and presence of SU in early lactation this is unlikely to be a causal relationship as the attributable fraction of SU risk was only 3%, suggesting that the incidence of SU would only be marginally reduced if CM in the first 30 DIM was controlled. A possible explanation for the observed association is supported by a study that found that cows with experimentally-induced CM spend more time standing,

presumably because their udder is painful (39,40). Cows who spend more time standing around the time of calving are then more likely to develop SU later in lactation (41). An alternative hypothesis is that cows developing claw horn lesions spend more time lying down (42); this could predispose them to mastitis through udder contact with dirty bedding. It is likely that any association is multifactorial and may change with farm management. For example, stocking density both affects lying behaviour of cows with mastitis (43) and affects risk for later development of sole lesions (44).

Excessive or impaired inflammatory response may also play a role in linking these two diseases. Essentially, all cows experience some increased systemic inflammation after parturition (31,45), and the risk for various periparturient diseases has been linked to the degree of this inflammation (46–48). The suspensory apparatus of P3 is weakened around the time of calving (contributing to SU development) and inflammatory mediators associated with CM may exacerbate this phenomenon. A measurable acute phase response (a marker for systemic inflammation (49)) has recently been associated with SU in dairy cattle (13). Rumen acidosis and coliform mastitis have been suggested to be common predisposing metabolic conditions for the development of SU, acting via endotoxin-induced inflammatory mediators (15,50). There are many examples of the severity of inflammatory response being linked to genetic factors in many species, including cattle (51) and potentially acting independently of the nature of the inflammatory stimuli. Several cattle lameness issues have been shown, in multiple studies, to have a genetic component. For example, SU cases have been linked to SNPs in the BTA8 chromosome by genome wide association studies (GWAS) (52). Key inflammatory genes are present on BTA8, including genes for inflammatory cytokines produced by the bovine mammary epithelial cells. Mammary production of one of these cytokines, interleukin-6, is

strongly stimulated by both E. coli and S. aureus, key pathogens in CM (53). Furthermore, genetic influences on SCC in Holsteins have been demonstrated with specific gene variants identifiable in multiple genes relating to inflammation and related processes (54). Hence, it is possible that some cattle are just more susceptible to inflammatory outcomes, whatever the trigger and this may explain the link between CM, SU and SCC.

Many previous studies have found that cows are more likely to have an SU if they had SU in the previous lactation (27,28). We had no previous lactation history here but our results showed that cows were at 7.56 greater odds of having SU present in early lactation if they had SU present at the pre-calving or calving check. The same lesion may have been seen at successive checks. Recent work shows that changes occur both in the bone structure of P3 (55) and sole soft tissue thickness (56) of cows with SU that may predispose them to future episodes.

Cows had reduced odds of developing SU in early lactation if they were in their second parity compared to their first or later parities. Previous studies have found that risk for SU increases with parity (14) particularly for cows in parity 4 or greater (23) but not that cows in their second parity have reduced odds for developing SU. The explanation for this is not clear.

Increased BCS seemed to have a protective effect against development of SU and this reflects findings from other studies. Cows with BCS greater than 2.5 had reduced odds of having SU present at early lactation, and the effect was more significant for cows with BCS greater than 3.0. Low BCS and loss of BCS through lactation is known to be a risk factor for formation of claw horn disruption lesions and there is evidence that this is related to the thickness of the digital cushion (7,25). A recent post-mortem study of digital cushion tissue found that cows with BCS > 3.0 at slaughter had

significantly greater adipose cell size than cows with BCS < 2.5 (57). Another possible explanation for our BCS findings is that cows that develop SU in early lactation are likely to mobilise more fat due to the pain they experience and the associated reduced dry matter intake.

Farm also appeared to be a significant factor in the development of SU, with cows on farm 4 having reduced odds of having SU present although specific reasons for this are not clear from this study. It is known that large variation exists in lesion-specific lameness between farms (58), and association between lameness and other factors may vary (59).

We found a significant association between SU and CM but when SH lesions were included along with SU lesions, this association was no longer statistically significant. SH are regarded by some as a precursor for SU (19), but it would appear a visible SU lesion in early lactation was important for an association with CM in the first 30 DIM in this study.

Further evidence for the relationship between udder inflammation and SU is provided by our finding that elevated SCC at any milk recording in the first 100 DIM was also found to be significantly associated with presence of SU in early lactation. The attributable fraction of risk for presence of SU in early lactation to high SCC in the first 100 DIM (13.45%) was higher than that for CM in the first 30 DIM (2.95%). Interpreting this finding is difficult because of the overlap in the timing of data collection, but this suggests the presence of SU is linked to subclinical mastitis in the first 100 DIM. More work would be needed to elucidate this relationship.

Strengths of this study include the relatively large number of cattle enrolled and the consistent and regular recording of foot lesions by a qualified veterinary surgeon expressly for the purposes of the study. One weakness of this study is the reliance on

farm records for incidence of CM cases. It is known that there can be significant variation in diagnosis and recording of CM between and within farms (60). The incidence of cows enrolled in this study that had CM recorded in the first 30 DIM was 2.2%, when the NMR 500 median in 2019 was 5% (61). The farms involved in the study all proactively managed CM but it is likely there has been some under recording of cases. Misclassification bias may actually mean that some cows with SU in early lactation had CM in the first 30 DIM that was not recorded and that the associations described here could be even stronger (we cannot however prove this with our current dataset). A number of cows were excluded from our analyses which may lead to a level of bias if cows were culled due to lameness or mastitis problems.

Farms chosen for enrolment were a convenience sample of proactive farms that employ large numbers of staff and were interested in veterinary involvement. There is a possibility the results do not represent the true picture among all the UK dairy herds. In our study, we attempted to use treatment as an indicator of severity but did not include these results in the final models due to inconsistencies in recording among farms. CM can be graded on severity depending on the degree of tissue involvement. Cows affected with different severities of CM are likely to suffer different short and long term health consequences and receive different treatments (62). Future studies would be improved by training farm staff to detect and record CM cases using a consistently defined scale before data collection started.

CONCLUSION

We have found that there is an association between clinical mastitis in the first 30 DIM and the presence of SU in early lactation. The association was no longer statistically significant if cows with SU at pre-calving or calving check were excluded from the

dataset, but the same numeric trend still existed. There was also a significant association between presence of SU in early lactation and high SCC at any time in the first 100 DIM. Elucidating the mechanisms behind these relationships will improve our understanding of the diseases' aetiopathogenesis and could lead to new preventive strategies.

DECLARATIONS

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Ethics approval

The study was conducted following ethical approval from the University of Liverpool Veterinary Research Ethics Committee.

Consent for publication

Consent was obtained from participating farms prior to the start of the study.

Availability of data and material

The datasets generated and analysed during the current study are available at reasonable request.

Competing interests

All authors declare that they have no competing interests.

Contributorship statement

CW performed statistical analyses and wrote the first draft of the manuscript, MB collected field data and assisted with data analysis, BG, AA, CB collected field data, HH advised on study design and statistical analysis, SC advised on study design, AP

advised on study design, GB advised on study design, GO (corresponding author) designed and supervised the study and assisted statistical analysis. All authors critically evaluated the manuscript and approved the submitted version.

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Table 1. Datasets used for final models. 2,353 Holstein-Friesian cows and heifers were initially enrolled

Model	1	2	3	4
Total cows in dataset	2131	2036	2131	2101
Farm 1	124	110	124	102
Farm 2	212	208	212	212
Farm 3	1397	1343	1397	1390
Farm 4	398	375	398	397
CM recorded (first 30 DIM)	49	46	49	47
CM recorded (first 100DIM)	139	130	139	136
SCC>200 (first 30 DIM)	214	175	184	184
SCC>200 (first 100DIM)	529	498	521	513
SU at pre-calving check	72	0	72	72
SU at calving check	46	0	46	45
SU at early lactation check	131	96	131	129
SH 2 or 3 at pre-calving check	156	124	156	152
SH 2 or 3 at calving check	131	110	131	127
SH 2 or 3 at early lactation check	519	467	519	511

Table 2. Results from Model 1 which had presence of SU at the early lactation check as the outcome of interest. Presented Odds Ratios (OR) are for each level against the reference category for the odds of developing the stated outcome; P-values and 95% confidence intervals (CI) are Wald based estimates. Model fit was assessed using Hosmer-Lemeshow test (P = 0.66) and McFadden's Pseudo R2 (0.19).

Independent variable	Level	Odds ratio	95% CI	P value
Intercept		0.041	0.007-0.26	<0.001
Farm	1	Reference		
	2	0.49	0.20-1.22	0.13
	3	0.62	0.31-1.34	0.2
	4	0.27	0.11-0.67	0.004
Parity category	1st	0.78	0.40-1.47	0.46
	2nd	0.12	0.06-0.25	<0.001
	>= 3rd	Reference		
BCS category at early lactation check	<2.5	Reference		
	2.5-3.0	0.45	0.27-0.75	0.002
	>3.0	0.32	0.18-0.57	<0.001
DIM at early lactation check	Continuous	1.02	1.0-1.04	0.025
SU at pre-calving or calving	Yes	7.56	4.46-12.76	<0.001
	No	Reference		
Mastitis in first 30 DIM	Yes	2.44	0.97-5.54	0.042
	No	Reference		

Table 3. Results from Model 2 where cows that had pre-existing SU present at drying off or calving were excluded from the dataset. Presented Odds Ratios (OR) are for each level against the reference category for the odds of developing the stated outcome; P-values and 95% confidence intervals (CI) are Wald based estimate. Model fit was assessed using Hosmer-Lemeshow test (P = 0.74) and McFadden's Pseudo R2 (0.12).

Independent variable	Level	Odds ratio	95% CI	p value
Intercept		0.04	0.006-0.31	0.0016
Farm	1	Reference		
	2	0.48	0.18-1.26	0.13
	3	0.55	0.26-1.30	0.15
	4	0.19	0.06-0.57	0.004
Parity category	1st	0.76	0.37-1.48	0.43
	2nd	0.12	0.05-0.25	<0.001
	>= 3rd	Reference		
BCS category at early lactation check	<2.5	Reference		
	2.5-3.0	0.49	0.28-0.90	0.018
	>3.0	0.35	0.18-0.65	<0.001
DIM at early lactation check	Continuous	1.025	1.00-1.05	0.02
Mastitis in first 30 DIM	Yes	2.25	0.81-5.35	0.09
	No	Reference		

Table 4. Results from Model 3 with presence of both SU or SH>2 or 3 as the outcome of interest. Presented Odds Ratios (OR) are for each level against the reference category for the odds of developing the stated outcome; P-values and 95% confidence intervals (CI) are Wald based estimates. Model fit was assessed using Hosmer-Lemeshow test (P = 0.86) and McFadden's Pseudo R2 (0.13).

Independent variable	Level	Odds ratio	95% CI	p value
Intercept		1.04	0.53-2.02	0.9
Farm	1	Reference		
	2	0.84	0.41-1.71	0.49
	3	0.61	0.33-1.11	0.09
	4	0.48	0.25-0.96	0.03
Parity category	1st	3.99	1.55-10.77	0.01
	2nd	0.33	0.12-0.88	0.02
	>= 3rd	Reference		
BCS category at early lactation check	<2.5	Reference		
	2.5-3.0	0.54	0.36-0.82	0.005
	>3.0	0.4	0.26-0.61	<0.001
SU/SH 2 or 3 at pre-calving or calving	Yes	6.36	2.79-16.06	<0.001
	No	Reference		
Mastitis in first 30 DIM	Yes	1.19	0.61-2.27	0.59
	No	Reference		

Table 5. Results from Model 4 with high SCC in the first 100DIM as an independent variable and SU at the early lactation check as the outcome of interest. Presented Odds Ratios (OR) are for each level against the reference category for the odds of developing the stated outcome; P-values and 95% confidence intervals (CI) are Wald based estimates. Model fit was assessed using Hosmer-Lemeshow test (P = 0.42) and McFadden's Pseudo R2 (0.19).

Independent variable	Level	Odds ratio	95% CI	p value
Intercept		0.035	0.006-0.23	<0.001
Farm	1	Reference		
	2	0.56	0.22-1.42	0.22
	3	0.63	0.30-1.42	0.24
	4	0.31	0.12-0.80	0.015
Parity category	1st	0.87	0.44-1.60	0.67
	2nd	0.14	0.06-0.27	<0.001
	>= 3rd	Reference		
BCS category at early lactation check	<2.5	Reference		
	2.5-3.0	0.45	0.27-0.76	0.003
	>3.0	0.33	0.19-0.56	<0.001
DIM at early lactation check	Continuous	1.03	1.00-1.05	0.015
SU at pre-calving or calving	Yes	7.98	4.69-13.55	<0.001
	No	Reference		
SCC>200 first 100DIM	Yes	1.7	1.13-2.55	0.01
	No	Reference		

Supplementary material: Descriptive summary of herds

Farm	Enrolled cows	Mean parity (sd)	Average 305-d yield, L/cow	Mean bulk milk SCC, cells/ml
1	132	2.6 (1.5)	11,100	160,000
2	239	2.9 (1.6)	8,900	170,000
3	1550	2.6 (1.6)	11,400	220,000
4	432	2.8 (1.4)	11,100	94,000

Supplementary material: Criteria used to grade lSU and SH lesions in study. Based on ICAR atlas (Egger-Danner 2015)

Lesion	Description
SH Grade 1	Light pink lesion <2cm
SH Grade 2	Light pink lesion >2cm or dark red lesion <2cm
SH Grade 3	Dark red lesion >2cm or any size blue lesion
SU Grade 1	Ulceration <2cm wide
SU Grade 2	Ulceration >2cm or granulation tissue protruding <1.5cm
SU Grade 3	Granulation tissue protruding >1.5cm or secondary infection present