Evaluation of test-day milk somatic cell count to predict intramammary infection in late lactation grazing dairy cows

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ABSTRACT

Use of selective dry cow antimicrobial therapy requires to precisely differentiate cows with an intramammary infection (IMI) from uninfected cows close to drying-off to enable treatment allocation. Milk somatic cell count (SCC) is an indicator of an inflammatory response in the mammary gland and is usually associated with IMI. However, SCC can also be influenced by cow-level variables such as milk yield, lactation number and stage of lactation. In recent years, predictive algorithms have been developed to differentiate cows with IMI from cows without IMI based on SCC data. The objective of this observational study was to explore the association between SCC and subclinical IMI, taking cognizance of cow-level predictors on Irish seasonal spring calving, pasture-based systems. Additionally, the optimal test-day SCC cut-point (maximized sensitivity and specificity) for IMI diagnosis was determined. A total of 2,074 cows, across 21 spring calving dairy herds with an average monthly milk weighted bulk tank SCC of ≤200,000 cells/mL were enrolled in the study. Quarter-level milk sampling was carried out on all cows in late lactation (interquartile range = 240–261 d in milk) for bacteriological culturing. Bacteriological results were used to define cows with IMI, when ≥1 quarter sample resulted in bacterial growth. Cow-level test-day SCC records were provided by the herd owners. The ability of the average, maximum and last test-day SCC to predict infection were compared using receiver operator curves. Predictive logistic regression models tested included parity (primiparous or multiparous), yield at last test-day, and a standardized count of high SCC test-days. In total, 18.7% of cows were classified as having an IMI, with first parity cows having a higher proportion of IMI (29.3%) compared with multiparous cows (16.1%).

Staphylococcus aureus accounted for the majority of these infections. The last test-day SCC was the best predictor of infection with the highest area under the curve. The inclusions of parity, yield at last test-day, and a standardized count of high SCC test-days as predictors did not significantly improve the ability of last test-day SCC to predict IMI. The cut-point for last test-day SCC which maximized sensitivity and specificity was 64,975 cells/mL. This study indicates that in Irish seasonal pasture-based dairy herds, with low bulk tank SCC control programs, the last test-day SCC (interquartile range days in milk = 221–240) is the best predictor of IMI in late lactation.

Key words: mastitis, dry cow therapy, dry-off, predictive values, somatic cell count

INTRODUCTION

Mastitis treatment and control accounts for the majority of antimicrobial use in dairy cattle (Pol and Ruegg, 2007; Saini et al., 2012; Hyde et al., 2017). Blanket dry cow therapy (DCT) to treat existing IMI and to prevent new IMI over the dry period by treating all cows in a herd with an intramammary antibiotic at dry-off, has been a key pillar of mastitis control for many decades (Dodd et al., 1969). However, public concern over the use of antibiotics, and its implications for antimicrobial resistance has led to the development of regulation 2019/6 on use of veterinary medicines by the European Union (European Parliament and the Council of the European Union, 2019). This regulation came into force on 28 January 2022, and states that the preventative use of antimicrobials in groups of animals should not be used. An alternative strategy to blanket DCT is to treat cows that demonstrably have an IMI or that are at higher risk of IMI during the dry period with antibiotics, while the remaining cows are treated with an internal teat seal alone (selective DCT). In Ireland blanket DCT with or without internal teat seal is widely used, with most recent estimates showing...
blanket DCT usage in the majority of Irish dairy herds (More et al., 2017; McAloon et al., 2021). It is important now that Irish dairy farmers implement selective DCT to reduce antimicrobial use.

Implementing selective DCT involves correctly categorizing quarters or cows as probably having IMI and infusing them with antibiotics and conversely categorizing quarters or cows as probably not having IMI and infusing them with an internal teat seal alone. The decision to use selective DCT is first a herd-level decision (i.e., whether selective DCT is an appropriate approach for a specific herd; Madouasse et al., 2022). In addition to the potential general problems with mastitis control, it has been suggested that herd prevalence of IMI can affect the efficacy of the SCC tests to discriminate IMI status (Torres et al., 2008). Then, at the cow-level, identification of IMI with a high sensitivity (Se) and specificity (Sp) is required (McDougall et al., 2021). A low Se results in more false negatives, leading to a failure to treat truly infected cows. Conversely, a low Sp is associated with false positive diagnosis of an IMI and subsequent overuse of antimicrobials. To detect IMI at drying-off, bacteriological culture of milk samples remains the gold standard (Hogan et al., 1999). However, due to financial and practical constraints, it is not often used. Alternative methods used to differentiate between cows with and without IMI are on-farm cultures and the California mastitis test (Kabera et al., 2020; McDougall et al., 2022). The most common measurement to differentiate between cows with and without an IMI is the use of SCC (Vanhoudt et al., 2018; McDougall et al., 2021; Rowe et al., 2021). Some scientific publications use a predictive algorithm based on one or more cow-level SCC measurements during lactation, and in most cases, clinical mastitis history to predict IMI status (Bradley et al., 2018; Vanhoudt et al., 2018; Lipkens et al., 2019). However, each publication uses varying number of milk test-day SCC records and different thresholds for SCC and clinical mastitis history (Bradley et al., 2018; Vanhoudt et al., 2018; Lipkens et al., 2019). The development of an accurate criteria to classify cows with IMI needs to be evaluated in different production systems and with different pathogen profiles. Ireland’s dairy production system is characterized for being pasture-based, with a seasonal spring calving pattern and where most infections are attributed to *Staphylococcus aureus* (Clabby et al., 2022). *Staphylococcus aureus* is typically characterized as a contagious pathogen, capable of establishing subclinical infections and can be difficult to detect as sometimes there is no obvious spike in the SCC of *Staph. aureus* infected quarters (Djabri et al., 2002).

It is hypothesized that an accurate prediction of IMI using test-day SCC (among other variables) may differ for Irish pasture-based seasonal calving systems given that the predominant pathogen responsible for infection is *Staph. aureus*. Therefore, the primary objective of this study was to explore the potential of cow-level factors to develop a prediction model to differentiate between cows with and without an IMI at the end of the lactation. A secondary objective was to explore the possibility of identifying a test-day SCC cut-point that maximized Se and Sp for identification of IMI in late lactation.

**MATERIALS AND METHODS**

This study was approved by the Teagasc Animal Ethics Committee (License No. 1542017), and all procedures were authorized and carried out in accordance with the Health Products Regulatory Authority (HPRA) of Ireland.

**Herds Enrollment**

A total of 21 herds located in the south of Ireland were enrolled for this observational study; 20 of which were milk suppliers of Kerry Agribusiness (www.kerryagribusiness.ie) and one research herd (Clonakilty Agriculture College, operated by Teagasc, Animal & Grassland Research and Innovation Centre) which was a milk supplier of Barryroe Co-operative (www.barryroeco-op.ie). All herds were spring calving (whereby 80% of cows calved between February 1 and March 31 and 95% of cows were dried off between November 1 and December 31) pasture-based systems of milk production. One research herd was selected by the research team and 20 commercial herds were nominated by the milk processor on the basis of: (1) up to the time of enrollment in October 2020 an average monthly milk collected weighted bulk tank SCC (BTSCC) ≤200,000 cells/mL; (2) conducted regular whole-herd test-days (minimum of 4 test-dates) throughout the lactation; (3) good ongoing cooperative relationship with milk processors, supply good routine herd records and agreeing to allow access to cow- and herd-level data; and (4) herds were located within a radius of no more than 150 km from the research center to facilitate sampling.

**Data**

Bulk tank and cow-level data were available for the 2020 lactation. The majority of bulk milk tanks were collected and analyzed every 2–3 d and bulk tank data were provided by the respective milk processors. At the cow-level, all commercial herds conducted individual cow whole-herd test-days across the lactation. Test-day information was obtained from Irish Cattle Breeding Federation (www.icbf.com), Ireland’s national cattle information was obtained from Irish Cattle Breeding Federation (www.icbf.com), Ireland’s national cattle
breeding database. Each individual cow test-day provided cow-level information on milk yield (kg), milk fat and protein content (%), and SCC (cells/mL). Quarter-level milk samples were collected by trained Teagasc research personnel in late lactation (Table 1), as described below. Late lactation was defined as >230 DIM and herds considered were planning to dry-off cows within 3 to 6 wk after quarter sampling.

### Quarter-Level Sampling

Whole-herd quarter-level milk samples were aseptically collected before milking, by trained Teagasc research personnel in late lactation (Table 1; Sampling period ranged from October 15 to November 19, 2020). Aseptic collection of quarter milk samples was achieved by disinfection of teat ends with cotton swabs soaked in methylated spirits. Front teats were disinfected first followed by hind teats. Fore strips were discarded and milk collected on a per quarter basis in the opposite direction to avoid contamination of disinfected teats; hind teats were stripped and milk was collected first, followed by the front teats (Adkins et al., 2017). Sample bottles were brought to the laboratory immediately after collection and frozen at −20°C.

### Quarter-Level Bacteriology Analysis

The quarter-level milk samples were stored at −20°C for up to 3 wk before processing. Before analysis quarter milk samples were defrosted to 4°C and were adjusted to room temperature (16–18°C). To identify the pathogens causing IMI, a nonselective medium Blood Agar Base No 2 (OXOID) was used to isolate and identify bacteria from aseptic foremilk samples. Blood agar allows good differentiation between colonies of Streptococcus spp., Staphylococcus spp. and Micrococcus spp. To improve differentiation between Streptococcus spp., 0.1% esculin was added to the medium. The plates were divided into 4 equal quadrants, one for each quarter of the same cow. Plating was carried out by streaking 10 μL of the sample using aseptic disposable loops. Plates were incubated at 37°C and examined 24h to 48h after incubation and colony morphology was assessed. Growth morphologic features (colony size, shape, color, hemolytic characteristics) as described by Adkins (et al., 2017) were used to identify and quantify bacterial colonies present. *Staphylococcus aureus* were identified as creamy, grayish-white, and occasionally golden-yellow colonies on blood agar, 3 to 5 mm in diameter with typical zones of hemolysis. Streptococcus spp. were identified by small (1–3 mm diameter) colonies that were smooth, translucent, cone shaped on blood agar. *Escherichia coli* were identified as having large gray colonies after 24 h incubation and frequently produce mucoid colonies on blood agar. Infection was defined as the isolation of at least 6 cfu of the same pathogen in the plated quarter milk sample (600 cfu/mL; McParland et al., 2019; Clabby et al., 2022). This cut-point was selected mainly to allow for sufficient S. to precisely identify infection based on the work by Dohoo et al. (2011). If we detected 2 types of colonies on the same plate, the predominant colony was considered the main cause of IMI. A sample was considered contaminated if >2 types of colonies were identified on the plate.

### Statistical Analyses

Statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc.). Cow-level test-day SCC data were natural log-transformed before analyses.

### Table 1. Descriptive statistics for the 21 Irish grazing dairy herds (2,074 cows) enrolled in the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size</td>
<td>99</td>
<td>47</td>
<td>66</td>
<td>122</td>
</tr>
<tr>
<td>Parity</td>
<td>3.3</td>
<td>0.4</td>
<td>3.0</td>
<td>3.5</td>
</tr>
<tr>
<td>DIM at quarter sampling</td>
<td>250</td>
<td>11</td>
<td>240</td>
<td>261</td>
</tr>
<tr>
<td>Number of test-days</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Interval from last test-day to quarter sampling (d)</td>
<td>17</td>
<td>11</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Milk yield 305 d (kg)</td>
<td>6,700</td>
<td>695</td>
<td>6,300</td>
<td>7,400</td>
</tr>
<tr>
<td>Fat yield 305 d (kg)</td>
<td>290</td>
<td>26</td>
<td>263</td>
<td>312</td>
</tr>
<tr>
<td>Protein yield 305 d (kg)</td>
<td>249</td>
<td>24</td>
<td>231</td>
<td>268</td>
</tr>
<tr>
<td>Jersey crossbred (%)</td>
<td>15.7</td>
<td>21.4</td>
<td>0</td>
<td>25.3</td>
</tr>
<tr>
<td>Yield per day at last test-day (kg)</td>
<td>17.4</td>
<td>3.2</td>
<td>14.8</td>
<td>20.1</td>
</tr>
<tr>
<td>Cows within herd with ≥1 test-day &gt;200,000 cell/mL (%)</td>
<td>15.1</td>
<td>4.7</td>
<td>12.1</td>
<td>19.6</td>
</tr>
<tr>
<td>BTSCC full lactation (cells/mL) (%)</td>
<td>127,500</td>
<td>36,900</td>
<td>99,000</td>
<td>143,500</td>
</tr>
<tr>
<td>Cows within herd with IMI (%)</td>
<td>18.7</td>
<td>4.8</td>
<td>15.2</td>
<td>22.2</td>
</tr>
</tbody>
</table>

1Jersey crossbreed defined as at least 25% Jersey.
2BTSCC = average monthly milk collected weighted bulk tank SCC.
Data checks were carried out and cows with <3 test-day milk records, or >65 d between last test-day and quarter-level milk sampling were removed from the data set (n = 2).

Bacteriological results were used to define cows as having a pathogen IMI. If bacteria were identified after the milk culture described above in at least one-quarter, the cows were deemed infected (INF), otherwise cows were deemed uninfected (no INF). Bacterial isolation is commonly used as the gold standard for IMI (Hogan et al., 1999). The herd-level of infection in late lactation was calculated as the proportion of cows infected divided by the total number of cows that were quarter milk sampled in each herd. The FREQ procedure was used to describe study population characteristics (categorical variables) and the prevalence of the different microorganisms identified after milk culture. The MEANS procedure was used to describe study population characteristics for continuous variables.

**Infection Predictive Equation**

A logistic regression model was used to estimate the ability of different variables at predicting IMI in late lactation. Bacterial infection (INF) was the outcome of interest. Cow-level test-day SCC was used to define the base model for prediction of INF (Taponen et al., 2017; Lipkens et al., 2019). Different cow-level test-day SCC measures were considered as potential predictors for INF in the base model: the average SCC for the lactation (MeanSCC), the maximum SCC of the lactation (MaxSCC), and the last SCC record before bacterial culture (LastSCC).

Parity (initially first, second, third, fourth or ≥fifth) was summarized into a binary variable (primiparous and multiparous; first or ≥ second) based on results from its univariable association with INF using a logistic regression model (GENMOD procedure with Bonferroni adjustment). Statistically significant differences for the probability of INF were only observed when primiparous were compared with all other parity groups. Additional cow-level predictors evaluated for inclusion in the model were: milk yield per day at last test-day before bacterial culture (Yield; continuous variable) and a standardized count of high SCC test-days (HiSCC; continuous variable calculated as weighing factor (WF) × number of test-days with SCC >200,000 cells/mL, where WF was calculated as the total number of test-days per cow over the median number of test-days conducted by the study herds).

The potential herd effect was evaluated by 2 methods before data analyses: a null logistic regression model with herd as the only predictor to examine the association between herd and INF, were built (McDougall et al., 2021). Herd was not unconditionally associated with the outcome of interest (INF; \( P = 0.14 \)) and the intraclass correlation was low (0.004) and not statistically significant (\( P = 0.32 \)). Thus, herd and herd-level effects were not considered in the predictive equation. This decision was confirmed after data analysis by visually assessing the model residuals plotted by herd; no clustering by herd was observed (data not shown).

To develop the predictive equation, data were randomly split into training and testing subsets, containing 80 and 20% of the data, respectively, using the SURVEYSELECT procedure. Using the training data set, the different cow-level test-day SCC measures (MeanSCC, MaxSCC, and LastSCC) were evaluated by logistic regression with the GENMOD procedure. The associated areas under the curve (AUC) from the receiver operating characteristics (ROC) curve analysis were computed for each using the LOGISTIC procedure. Using the training data set, parity, yield, and HiSCC were evaluated individually and in combination for their contribution to INF prediction by logistic regression. Multicollinearity was assessed using variance inflation factor by the REG procedure before further multivariable modeling; multicollinearity was not detected.

Each model was compared with the base model using nonparametric ROC analyses. Receiver operating characteristic curve analyses were built for the base model, and those including the additional cow-level predictors using the LOGISTIC procedure with a logit link function. Associated AUC were compared by nonparametric ROC analyses using the ROC contrast statement using the base model as the referent. The effects of first-order interactions on prediction of the multivariable models were evaluated using nonparametric ROC analyses as described above; the inclusion of interactions did not improve the model prediction.

The assumption of linear relationship between the outcome logit and each continuous predictor was assessed by plotting the logit (log-odds) of INF against each continuous predictor using the GPLOT procedure. To assess the validity of the developed logistic regression model, the optimal performing model was then fitted to the testing data set and model performance on the training and testing data sets compared as described above.

**Infection Predictive SCC Cut-point**

The optimal SCC cut-point for diagnosis of INF was determined by ROC curve analyses and maximized
AUC, weighting false positive and false-negative results equally using MedCalc (Version 20.110; MedCalc Software Ltd.). The whole data set was used for the SCC cut-point identification.

**RESULTS**

**Descriptive**

A total of 2,074 cows across 21 herds were used for the study. The average herd size was 99 cows [SD = 47; interquartile range (IQR) = 66–122] and herds carried out an average of 7 test-days (SD = 6; IQR = 5–7) across the lactation (Table 1). The average number of days in milk at quarter-level milk sampling was 250 (SD = 11; IQR = 240–261). The average number of days from last test-day to quarter sampling was 17 (SD = 11; IQR = 8–23). The average monthly milk collected weighted herd BTSCC (rounded to the nearest hundred) for the 2020 lactation was 127,500 (SD = 36,900; IQR = 99,000–143,500 cells/mL). The average herd-level infection rate was 18.7% (SD = 4.8; IQR = 15.2–22.2; Table 1). Additional descriptive data at herd-level are provided in Table 1. Figure 1 shows the herd percentage of IMI and the average monthly milk collected weighted BTSCC for each herd.

*Staphylococcus aureus* was the predominant pathogen causing IMI in all 21 herds. Of the cows identified with IMI (n = 393/2,074), 84.0% (n = 330/393) were infected only with *Staph. aureus* and 4.1% (n = 16/393) only with *Streptococcus uberis* (Table 2). *Staphylococcus aureus* was the predominant pathogen in primiparous and multiparous cows, accounting for 88.2 and 81.7% of IMI, respectively. *Streptococcus uberis* did not cause IMI in primiparous cows and accounted for 6.2% of IMI in multiparous cows. *Streptococcus dysgalactiae* accounted for 2.2 and 2.7% and nonaureus staphylococci accounted for 6.6 and 3.1% of primi- and multiparous IMI, respectively. A total of 92 cows had a missing sample from 1 quarter (either contaminated or missing). Table 3 shows the average untransformed SCC for LastSCC by parity for INF and no INF cows. Second, third, and fourth parity cows had numerically lower average untransformed LastSCC compared with primiparous cows, 88,300 (SD = 104,500) cells/mL (Table 3). In the overall multiparous cows group (parity ≥2), 16% of cows were infected and their average untransformed LastSCC was 81,400 (SD = 127,800) cells/mL. Nineteen percent and 14% of primi- and multiparous cows, respectively had at least one test-day record >200,000 cells/mL across the lactation.

The average untransformed MeanSCC (SD), LastSCC, and MaxSCC for all cows was 103,000 (225,000) cells/mL, 82,900 (122,900) cells/mL, and 190,900 (623,400) cells/mL, respectively. The percentages of cows with a LastSCC ≤50,000, 51 to 100,000, 101 to 200,000, 201 to 400,000, and ≥400,000 cells/mL were 52.5, 28.5, 12.1, 4.8, and 2.1%, respectively. Figure 2 shows the distribution of LastSCC for each herd. Distribution of natural log-transformed LastSCC, MeanSCC, and MaxSCC for cows with and without IMI is presented in Figure 3.

**Infection Predictive Equation.** Among the evaluated cow-level test-day SCC measures, LastSCC showed the best prediction ability as represented by the AUC with maximized Se and Sp (0.79; Table 4; Figure 4). The base logistic regression model for developing the infection predictive equation was then defined with

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**Table 2.** Cow-level pathogen profile (≥1 quarter sample with bacterial growth) of the cows (n = 393/2,074) enrolled in the study across 21 Irish grazing dairy herds

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>330</td>
<td>84.0</td>
</tr>
<tr>
<td><em>Streptococcus uberis</em></td>
<td>16</td>
<td>4.1</td>
</tr>
<tr>
<td><em>Streptococcus dysgalactiae</em></td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>Nonaureus staphylococci</td>
<td>17</td>
<td>4.3</td>
</tr>
<tr>
<td>Nonhemolytic <em>Escherichia coli</em></td>
<td>4</td>
<td>1.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> and other*</td>
<td>16</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*Cows with >1 infected quarter. Infected with *Staph. aureus* and with one additional pathogen (*S. uberis, S. dysgalactiae*, or nonaureus staphylococci).
LastSCC. The considered additional predictors (Parity, HiSCC, or Yield) alone or in combination did not result in a better prediction value compared with the model including only LastSCC. Table 5 shows model fit, AUC, and ROC contrast P-values for the comparison of logistic regression models including potential additional predictors against the base model (LastSCC only) for predicting INF.

Using the testing data set (20% of the data), the predictive equation performed as well as with the training data set (80% of the data). Model performance on training and testing data sets is summarized in Table 6 and Figure 5.

Infection Predictive SCC Cut-Point. Considering the results of the predictive equation, the SCC cut-point was estimated using LastSCC. The cut-point for LastSCC to detect IMI with maximized Se and Sp was 64,975 cells/mL. At this cut-point, Se and Sp were 72.5% (95% CI = 67.8–76.9) and 75.1% (95% CI = 72.9–77.1), respectively. The positive predictive value was 40.5% (95% CI = 38.0–43.0%) and the negative predictive value was 92.1% (95% CI = 90.8–93.2%). Figure 6 shows the trade-off between Se and Sp at different SCC cut-points.

DISCUSSION

The 21 herds recruited for this study are considered a representative sample of Irish dairy herds that have low BTSCC. In 2020, the national average monthly milk collected weighted BTSCC of Irish dairy herds was 155,000 cells/mL (the first, second, third, and fourth quartiles were 91,000, 131,000, 169,000, and 243,000 cells/mL, respectively; Agricultural Economics and Farm Surveys Department, 2021). This indicates that the dairy herds on the current study represented a cohort of best 50% in terms of BTSCC.

The average herd-level IMI in the current study herds was 18.7%. This is higher than that reported by McDougall et al. (2021) with 7.2% of cows with a major pathogen IMI in late lactation on 36 herds across 4 regions in New Zealand. The levels of IMI in the current study were also greater than that reported by Lipkens et al. (2019) with 15.6% of cows across 15 commercial dairy herds in Belgium with a major pathogen IMI at dry-off. In the current study the predominant pathogen causing IMI was Staph. aureus (84%). This compares to only 8.1 and 7.7% of IMI caused by Staph. aureus in the McDougall et al. (2021) and Lipkens et al. (2019) studies, respectively. Primiparous cows are generally thought to be predominantly infected by NAS (De Vliegher et al., 2012). In our study, primiparous cows had a higher proportion of NAS IMI compared with multiparous cows (6.6 vs. 3.1% of IMI, respectively), however, the majority of infections in both groups were caused by Staph. aureus alone (88.2 and 81.7% of IMI, respectively). This highlights that the scope of the current study might apply to a different population.
than that of McDougall et al. (2021) and Lipkens et al. (2019). *Staphylococcus aureus* produces a low SCC response (Djabri et al., 2002) which can result in difficulties in timely detection and treatment. *Staphylococcus aureus* is mostly a contagious pathogen and requires effective implementation of mastitis control measures to contain IMI levels and prevent rapid emergence in herds (Bradley and Green, 2004).

**Figure 3.** Distribution of (a) natural log SCC of last test-day (LastSCC), (b) average of natural log SCC over the lactation (MeanSCC), and (c) natural log of maximum SCC (MaxSCC), for cows deemed as having intramammary infection (INF; where ≥1 late lactation quarter sample resulted in bacterial growth; green diagonally lined bar) or cows deemed as not having intramammary infection (No INF; where late lactation quarter samples resulted in no bacterial growth; blue solid bar) from 2,074 cows from 21 Irish grazing dairy herds.

**Table 4.** Area under the receiver operating curve comparison for the logistic regression model using SCC at last test-day, mean SCC from all test-days or maximum SCC from all test-days

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>SE</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>LastSCC</td>
<td>0.79</td>
<td>0.014</td>
<td>0.76</td>
<td>0.82</td>
</tr>
<tr>
<td>MeanSCC</td>
<td>0.77</td>
<td>0.015</td>
<td>0.74</td>
<td>0.80</td>
</tr>
<tr>
<td>MaxSCC</td>
<td>0.75</td>
<td>0.015</td>
<td>0.72</td>
<td>0.78</td>
</tr>
</tbody>
</table>

1Data are from the training data set comprising 1,659 cows from 21 Irish grazing dairy herds.
2AUC = area under curve.
3LastSCC = natural log of last test-day SCC; blue triangle.
4MeanSCC = mean natural log test-day SCC; dark green square.
5MaxSCC = maximum test-day SCC; light green circle.
6LastSCC = natural log test-day SCC before quarter sampling.
7MeanSCC = natural log of SCC averaged over all test-days.
8MaxSCC = natural log of maximum SCC determined from all test-days.

**Figure 4.** Receiver operating characteristic curve to predict intramammary infection (INF) based on LastSCC (defined as natural log of last test-day SCC; blue triangle), MeanSCC (defined as mean natural log test-day SCC; dark green square), and MaxSCC (defined as maximum test-day SCC; light green circle) from 2,074 cows from 21 Irish grazing dairy herds. Dotted line represents the line of no discrimination.
The higher level of IMI (29.3%) in primiparous cows is worrisome and unexpected considering these herds are low SCC herds, based on their BTSCC records. In New Zealand, McDougall et al. (2021) reported that IMI was higher in multiparous cows (9.4%) compared with primiparous cows (2.1%). Archer et al. (2014) reported that in Irish herds with a herd-level SCC of ≥120,000 cells/mL, 33% of test-day milk records of primiparous cows were >200,000 cells/mL across the lactation. It is possible that there is a higher infection level in primiparous cows in Irish herds compared with the international literature. In fact, Archer et al. (2014) highlighted that the majority of Irish herds would increase farm profitability by improving udder health in primiparous cows in early lactation. Retrospectively, in the current study the primiparous cows identified with IMI in late lactation had an average SCC (SD) of 288,000 (680,000) cells/mL at the first milk recording of the lactation (average 36 DIM) and 166,000 (163,000) cells/mL in mid lactation (average 163 DIM). Similarly, the primiparous cows without IMI in late lactation had an average SCC (SD) of 152,000 (134,000) cells/mL across the lactation.

Table 5. Comparison between a logistic regression model evaluating the association between bacterial infection and natural log SCC at the last test-day (LastSCC; base model) and logistic regression models with additional effect modifiers

<table>
<thead>
<tr>
<th>Model</th>
<th>AIC</th>
<th>AUC</th>
<th>SE</th>
<th>Low</th>
<th>High</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LastSCC (base model)</td>
<td>1,620</td>
<td>0.789</td>
<td>0.014</td>
<td>0.762</td>
<td>0.816</td>
<td>ref</td>
</tr>
<tr>
<td>LastSCC + Parity</td>
<td>1,620</td>
<td>0.787</td>
<td>0.015</td>
<td>0.759</td>
<td>0.816</td>
<td>0.75</td>
</tr>
<tr>
<td>LastSCC + HiSCC</td>
<td>1,620</td>
<td>0.790</td>
<td>0.014</td>
<td>0.762</td>
<td>0.817</td>
<td>0.51</td>
</tr>
<tr>
<td>LastSCC + Yield</td>
<td>1,620</td>
<td>0.788</td>
<td>0.014</td>
<td>0.760</td>
<td>0.815</td>
<td>0.41</td>
</tr>
<tr>
<td>LastSCC + Parity + HiSCC</td>
<td>1,620</td>
<td>0.791</td>
<td>0.015</td>
<td>0.763</td>
<td>0.820</td>
<td>0.62</td>
</tr>
<tr>
<td>LastSCC + Parity + Yield</td>
<td>1,620</td>
<td>0.788</td>
<td>0.015</td>
<td>0.759</td>
<td>0.817</td>
<td>0.84</td>
</tr>
<tr>
<td>LastSCC + HiSCC + Yield</td>
<td>1,620</td>
<td>0.789</td>
<td>0.014</td>
<td>0.761</td>
<td>0.816</td>
<td>0.94</td>
</tr>
<tr>
<td>LastSCC + Parity + HiSCC + Yield</td>
<td>1,620</td>
<td>0.792</td>
<td>0.015</td>
<td>0.763</td>
<td>0.820</td>
<td>0.57</td>
</tr>
</tbody>
</table>

1Data are from the training data set comprising 1,659 cows from 21 Irish grazing dairy herds.
2AIC = Akaike information criteria.
3AUC = area under the curve.
4Parity = primiparous or multiparous.
5HiSCC = a standardized count of high SCC recordings (continuous variable calculated as: weighing factor (WF) × number of test-days with SCC >200,000 cells/mL, where WF was calculated as the total number of test-days per cow over the median number of test-days conducted by the study herds).
6Yield = milk yield at the last test-day.
tation had an average SCC (SD) of 113,000 (294,000) cells/mL and 62,000 (59,000) cells/mL at the first and mid-lactation milk recording, respectively. This would suggest that the IMI identified in late lactation could be associated with infections that originated in early lactation. Therefore, there is a need for Irish dairy herds to place greater emphasis on controlling IMI in primiparous cows. Additionally, it is worth highlighting that a reduction in the prevalence of Staph. aureus infected primiparous cows within Irish herds could have a significant effect in reducing the risk of new IMI, as Staph. aureus infected primiparous cows have been reported to act as significant reservoirs of infection to uninfected herdmates (Roberson et al., 1994).

Identifying cows with an IMI at dry-off is important for decision making at a crucial time in the lactation. An accurate prediction model that can be developed into an on-farm decision making tool would be an additional benefit for farmers to identify which cows are suitable for selective DCT at dry-off. Our model showed an acceptable capacity to discriminate between the 2 states of intramammary infection (INF and no INF, AUC = 0.789) and was able to replicate its accuracy in an untrained data set (testing set). The model by McDougall et al. (2021) showed an AUC of 0.85 for discriminating infection status using similar variables. In the current study, using the LastSCC as a predictor of infection in late lactation was better compared with using MeanSCC or MaxSCC, which is different to that found by McDougall et al. (2021) where there were no differences in the ability of LastSCC, MeanSCC or MaxSCC to predict infection. The prevalence of different pathogens in the different studies could also be a potential reason for this finding. Given that the studied herds had a higher prevalence of Staph. aureus and considering that Bradley and Green (2004) identified Staph. aureus as a significant cause of IMI at dry-off, it is within reason to assume that for the population of our study, the LastSCC would be more precise. The current study showed no evidence of a herd clustering effect, therefore it can be assumed that the model should perform equally well regardless of their specific BTSCC, provided they are low BTSCC herds. Whether the prediction accuracy of the model would be different in high BTSCC herds is outside of the scope of this paper but is an interesting topic for future research.

The use of the last test-day as the basis for the prediction of infection in late lactation is of important practical use for dairy farmers. In Ireland, it is estimated that approximately 50% of the herds conduct regular whole-herd test-day milk recording (Balaine et al., 2020). Efforts need to be made to increase the uptake of this practice to justify the use of antibiotics at dry-off in the context of the implementation of EU regulation of veterinary medicinal products use. Potentially, one test-day in late lactation could be a feasible transition for farmers not currently milk recording. This would help in the efforts of guiding decision making at dry-off. However, this practice should be eventually extended to the entire lactation, as it has been shown that conducting milk recording during the lactation is a very important tool for mastitis control, improved udder health, and to avoid IMI close to drying-off (Hennessy et al., 2013; Madouasse et al., 2022).

Decision making at dry-off is more commonly based on using a specific SCC cut-point (Bradley et al., 2010; Vanhoudt et al., 2018). The cut-points, however, applied for identification of cows with IMI varies substantially in different countries (Rowe et al., 2021). The current study showed that the cut-point that maximized Se and Sp was almost 65,000 cells/mL. We did not separately estimate a cut-point for primiparous and multiparous cows as our prediction model showed that parity was not a good predictor for IMI. McDougall et al. (2021) also reported a single optimal cut-point for SCC before dry-off, 108,000 cells/mL, with an overall Se and Sp of 67.1% and 59.5%, respectively. Lipkens et al. (2019) observed that the highest Youden’s index was for the geometric mean of the last 3 test-days and corresponded with a cut-point of 100,000 cells/mL, with an overall Se and Sp threshold of 86% and 71%, respectively. The results of the current study lie in between the previously 2 mentioned papers with a Se of 72.5%, and a Sp of 75.1%, which could be determined by the population studied and the consequent ROC curves obtained.

The selection of an optimal SCC cut-point for decision making at herd level needs to consider several practical aspects. To provide an example, if the level of IMI in late lactation for a herd is near 20% and we were to classify all the cows in the herd as having no IMI, then our Se would be 80%. This means that one criterion for choosing an SCC cut-point is that it detects the true positive at least as good as classifying all cows as without IMI, given a set level of IMI. The challenge is to assess the true IMI level in a herd, which

<table>
<thead>
<tr>
<th>Data set</th>
<th>No. cows</th>
<th>AUC</th>
<th>SE</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training</td>
<td>1,659</td>
<td>0.789</td>
<td>0.014</td>
<td>0.762</td>
<td>0.816</td>
</tr>
<tr>
<td>Testing</td>
<td>415</td>
<td>0.792</td>
<td>0.030</td>
<td>0.733</td>
<td>0.851</td>
</tr>
</tbody>
</table>

*The model included only LastSCC (natural log SCC determined at the last test-day preceding milk culture) of 2,074 cows from 21 Irish grazing dairy herds.*
is one of the practical applications of the predicted algorithm developed in this study. Another important point to consider is the positive predictive value, which is related to the Se. The positive predictive value found in this study for a maximized Se and Sp was 40.5%, which is higher than that reported by McDougall et al. (2021; 20%) and Lipkens et al. (2019; 29%). This means that if a farmer chose the 65,000 cells/mL cut-point for DCT decisions, up to 60% of the cows treated with antibiotics would have had no IMI, while only 8% of the untreated cows would have had an IMI (negative predictive value = 92%).

The Se of a single bacteriological culture milk sample can largely vary depending on the criteria used for classifying the quarters as having an IMI (Dohoo et al., 2011), and consequently, this can influence Se and Sp estimates for SCC measured against bacteriological culture. In the current study it was not possible to assess the Se and Sp of the bacteriological culture used, however, the Se and Sp of a single milk sample have been reported in other studies. Dohoo et al. (2011) reported a Se and Sp of 90.4 and 99.8% for Staph. aureus and 86.5 and 100.0% for Streptococcus spp. for culture results of a single milk sample. Buelow et al. (1996) observed that a single milk quarter sample had a Se of 91% to detect Staph. aureus infection versus a gold standard of 2 culture positive quarter milk samples from 6 consecutive days of sampling.

The SCC threshold in this study was determined by optimizing both the Se and Sp. However, Rowe et al. (2021) highlights it may be appropriate to optimize Se or Sp individually, depending on circumstance or desired outcomes. For example, if the goal is focused on correctly identifying infected cows then a higher Se may be desired. However if the goal is to reduce antibiotic use, then a higher Sp may be desired. Additionally, herd-level factors may affect the Se and Sp of SCC thresholds. Lipkens et al. (2019) reported that high IMI prevalence may result higher Se but lower Sp compared with low prevalence herds. Decisions on dry cow treatment should be based considering antimicrobial stewardship principles but acknowledging that antibiotic DCT provides the best opportunity to achieve a cure in a subclinically infected cow (Bradley and Green, 2004). There is potential for this prediction model or the derived SCC threshold to be used in combination with other testing methods, for example bacteriological culture, which may further improve the decision making at dry-off. An example would be, choosing a higher SCC threshold to classify cows as having an IMI and combine it with a confirmation bacteriological culture which could enhance both the Se and Sp of the decision-making criteria. Future research will explore the effect of using this prediction model for DCT decisions on SCC and IMI in the following lactation.

CONCLUSIONS

The current study found that in Irish dairy herds the predominant pathogen responsible for IMI was Staph. aureus. Primiparous cows had a higher level of IMI than multiparous cows. The last test-day SCC was the best predictor of IMI in late lactation. The inclusion of parity, milk yield, and lactation history of high SCC tests in addition to LastSCC as predictors did not significantly improve the ability to discriminate between cows with or without IMI in late lactation. In the current study, the SCC cut-point that maximized Se and Sp was 65,000 cells/mL and should be explored as a decision-making tool for dry cow treatment selection on Irish dairy herds.

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