Effect of Teat Hyperkeratosis on Somatic Cell Counts of Dairy Cows

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ABSTRACT
In one study, milk samples from individual mammary quarters (n = 4,148) in seven spring-calving dairy herds in Ireland were collected on two occasions during lactation to measure somatic cell counts (SCCs). All quarters were scored (0 to 4) for teat-end hyperkeratosis to establish a correlation between this score and SCC. In a second study, a herd of 56 autumn-calving Holstein/Friesian dairy cows were milked throughout a complete lactation, and teats on the right side of the body were disinfected after milking by submersion in chlorhexidine solution, whereas teats on the left side of the body were not disinfected. Milk samples were collected from individual quarters to measure SCCs, and the degree of hyperkeratosis was scored at intervals using a scale of 0 to 4 during the lactation. In Study 1, 46% of the teats were given a score of 1 (slight smooth ring of keratin) and 39% were scored 2 (moderately raised, smooth ring). Less than 0.5% of the teats were given a score of 4 (severe broken ring of keratin). There were no differences in SCCs among any hyperkeratosis scores. In Study 2, the mean SCC was significantly (P < .01) lower and the mean hyperkeratosis score was significantly (P < .001) higher for disinfected teats than for those that were not treated.

There was a significant (P < .01) correlation between hyperkeratosis scores 2 and 3 and SCCs in nontreated teats. The number of clinical mastitis infections was significantly (P < .001) higher for teats that were not disinfected. There were significantly (P < .01) fewer nonhemolytic staphylococci and *Staphylococcus aureus* pathogens in disinfected quarters than in those that were not disinfected.

INTRODUCTION
The teat end or orifice is an important first line of defense in protecting an udder from the invasion of mastitis pathogens. Two previous studies have suggested that changes in teat tissue due to milking may reduce the effectiveness of the teat canal barrier against infection.1-2 Others have concluded that these changes may favor penetration of bacteria into the udder.3 Mein and coworkers4 suggested the status of teat ends can change as a result of milking management and machine factors, such as slow or over milking. Prevailing weather conditions also have influenced changes in the teat end.4 A detailed classification method for assessing the condition of teat ends, referred to as teat callosity, distinguishes between smooth and rough teat ends and is useful for research.
A simplified scoring method is commonly used for routine evaluation of teat conditions such as teat hyperkeratosis. Hyperkeratosis is a term used to describe a thickened smooth keratin ring or extending fronds of keratin around the teat orifice. The condition has been observed in hand-milked and beef cows, and it is commonly observed in dairy cows. Some studies have suggested that the degree of hyperkeratosis increases as milk yield increases or with longer times on milking machines. Mein and coworkers concluded that the major factors affecting hyperkeratosis are long, pointed teats, slow milking, high-producing cows, stage of lactation, parity, weather conditions, chemical irritation, and cluster removal time.

Little or no correlation has been shown between teat orifice hyperkeratosis and intramammary infection. Most studies have calculated correlations using scores from all teat ends and do not allow for individual teat classification. When consideration was given to individual classification of teat ends for the present studies, it was concluded that teats classified with mild or moderate levels of hyperkeratosis did not appear to increase the risk of intramammary infection in lactating dairy cows. However, a greater degree of hyperkeratosis and roughness did increase the probability of new infection. Damage to the teat end allows colonization by pathogenic organisms and reduces the defense mechanism of the teat canal. Biopsies revealed that 50% of hyperkeratotic teat ends contained gram-positive bacterial colonization in the stratum corneum. Colonization of the teat end with bacteria may be greater in the absence of post-milking disinfectant application. Increased colonization in association with a high hyperkeratosis score may increase the risk of mastitis.

The present studies were conducted in milking dairy herds in Ireland to examine the effects of hyperkeratosis on somatic cell counts (SCCs) as well as to determine the effects of disinfecting teats on the presence of pathogens.

**MATERIALS AND METHODS**

**Study 1**

Seven spring-calving herds of Holstein/Friesian dairy cows in Ireland were recruited for the study. Milk samples (4,148) were collected from individual quarters before morning milking on two occasions approximately 130 and 160 days into the lactation period to measure SCCs. Milk samples were analyzed for SCCs using a Somacount 300 (Bentley Instruments). One operator, using a headlamp to illuminate the teat ends, scored all teats for hyperkeratosis immediately after cluster removal. A second operator recorded the cow number, teat position, and hyperkeratosis score using a hand-held electronic data logger. Hyperkeratosis was scored using the following scale: 0 = normal teat-end orifice, 1 = slight smooth ring of keratin, 1.5 = slight broken ring, 2 = moderate raised, smooth ring, 2.5 = moderate raised, broken ring, 3 = large raised smooth ring, 3.5 = large broken ring, 4 = severe broken ring of keratin. Scores were subsequently combined (1 and 1.5; 2 and 2.5; 3 and 3.5) and were ultimately recorded as 1, 2, 3, and 4. Results of individual SCC measurements were subsequently matched with hyperkeratosis score.

**Study 2**

Fifty-six autumn-calving Holstein/Friesian dairy cows, including 46 in their first lactation, were milked in a 14-unit, 80-degree, side-by-side milking parlor using long milk tubes with an inside diameter of 13.5 mm, a single milk line with an inside diameter of 72 mm, and a milk lift of 1.5 m above the cow. Cows were fed indoors on a grass/maize silage diet for 180 days after calving. For the remainder of the lactation, cows were kept outdoors exclusively on grass. Cows were milked with a cluster weighing 3.2 kg and a claw volume of 150 ml, wide-bore tapered liners (31.6–21.0 mm), and a simultaneous pulsation pattern. Cows were milked at intervals of 17 hours (overnight) and 7 hours (daytime). When milk flow rate dropped to 0.2 km/min, clusters were automatically removed using an electronic milk meter.
(Weighall Milk Meter, Dairymaster) linked to a software program. The pulsation rate was 60 cycles/min and the pulsation phases were \(a = 17.0\), \(b = 51.4\), \(c = 11.8\), and \(d = 19.8\) pulsations/min. The system vacuum level was 49 kPa (7.11 lb/in²) at the vacuum regulator and the effective reserve of the milking plant was 1,150 L/min.

Preparation before milking consisted of washing teats with warm running water and drying with individual paper towels. Throughout the entire lactation, teats on the right side of the body were disinfected after milking by submersion in a chlorhexidine solution containing 4,250 ppm chlorhexidine gluconate as well as a fly repellent and emollients. Teats on the left side of the cow’s body did not receive any disinfectant treatment.

One operator, using a headlamp to illuminate the teat-ends, classified teats for hyperkeratosis monthly, immediately after cluster removal at the morning milking. Teats were scored using the same scale as in Study 1. Additionally, individual quarter milk samples were collected on consecutive days on 13 occasions during the lactation and were analyzed for SCCs using the Somacount 300 instrument. Data from all months were pooled for analysis to establish a possible correlation between hyperkeratosis and SCCs.

**Microbiological Analysis**

Individual quarter milk samples were collected every 2 weeks to measure SCCs. On nine occasions, samples were analyzed to establish pathogens present in individual quarters. Milk samples were collected in an aseptic manner and were examined using International Dairy Federation guidelines for microbiological analysis. Bacterial pathogens were rated as 0 = no pathogens present, 1 = *Staphylococcus aureus*, 2 = non-hemolytic staphylococci, 3 = *Streptococcus dysgalactiae*, 4 = *Streptococcus uberis*.

A critical value of 300 \(\times\) 10⁶/ml was set for SCCs. Samples with counts greater than the critical value and quarters treated for clinical mastitis at calving were excluded from the analyses. When the SCC for an individual quarter was greater than the critical value at three consecutive samplings and pathogens were isolated, the infection was considered subclinical. If the SCC was greater than the critical value on three consecutive samplings with no pathogens present, the infection was considered nonspecific and subclinical. Subsequent subclinical infections in that quarter were not used for analyses, but clinical infections were included in the analyses. A subclinical infection was considered transient when the SCC was exceeded the critical value at one sampling date, with pathogens present. When the SCC in a sample exceeded the critical value on one sampling date, but no pathogens were present, the infection was considered transient and nonspecific. Quarters were considered clinically affected if the milk was visibly abnormal or if there were obvious signs of inflammation. Recurrent clinical cases in the same quarter were not included for analysis. Right-sided and left-sided teats were compared for hyperkeratosis, the number of new clinical and subclinical infections, and pathogen type in quarter milk samples.

Midlactation (Day 145), one operator unfamiliar with the milking treatments classified teat barrels for texture by manual palpation after cluster removal, recorded scores as 1 (normal or soft) or 2 (firm, swollen, or hard), and evaluated for color by visual assessment.

**Statistical Analyses**

Data from all farms in Study 1 were pooled for analysis to determine whether there was a correlation between hyperkeratosis and SCCs. Results from the seven farms were collated for statistical analysis. Data were compared by chi-square test to determine the significance of relationships between hyperkeratosis score and SCCs.

In Study 2, mean SCC for each cow was averaged for right-side quarters and for left-side quarters at each evaluation for analyses. Log-transformed SCCs were compared by
analysis of variance using Genstat 5, Release 3.2 (VSN International). Spearman rank correlation test was used to establish the relationship between hyperkeratosis and SCC. Chi-square analysis was used to measure differences between disinfected versus non-treated quarters for pathogens at each sampling date and over the entire lactation. Wilcoxon signed rank test was used to compare texture scores between treatments.

**RESULTS**

**Study 1**

Among the seven herds evaluated, 46% of the teats received a score of 1 and 39% were scored 2; fewer than 0.5% of teats were classified as 3 or 4. There were no differences between mean SCC and hyperkeratosis classification scores (Table 1). Hyperkeratosis scores were similar among all seven herds.

**Study 2**

Quarters that did not receive disinfectant treatments after milking had significantly ($P < .05$) higher SCCs than did disinfected quarters on seven sampling dates during the lactation (Table 2). Over the full lactation, the mean SCC (153,000) for disinfected quarters was significantly ($P < .01$) lower than the mean for quarters that were not disinfected (261,000).
The mean hyperkeratosis score was significantly \((P < .001)\) higher for disinfected quarters than for nontreated quarters at six individual measurement times. Additionally, the mean score was significantly \((P < .001)\) higher for disinfected quarters (1.69) than for nontreated quarters (1.57) over the complete lactation (Table 2). A significant correlation \((P < .01)\) was shown between hyperkeratosis score and SCC for nontreated quarters; however, no such correlation was demonstrated for disinfected quarters (Table 3). Hyperkeratosis scores of 0 and 1 had no effect on SCC; however, an intermediate score of 2 or high scores of 3 and 4 had significantly \((P < .01)\) higher SCCs when a disinfectant was not used.

The number of clinical mastitis cases was significantly \((P < .001)\) higher for nontreated quarters than for those that were disinfected. There were no differences between the two groups for subclinical, subclinical/nonspecific, transient/subclinical, or transient/subclinical/nonspecific infections (Table 4). The effect of disinfecting after milking on the number of pathogen-free quarters versus the number with pathogens is presented in Table 5. Significantly \((P < .05)\) more nontreated quarters had non-hemolytic staphylococci on Day 230 of lactation than did disinfected quarters. Nontreated quarters also tended to have more \(S. aureus\) present on Day 100 than did disinfected quarters; however, the difference was not significant. Pooled data from all sampling dates indicated that significantly \((P < .01)\) more nontreated quarters had non-hemolytic staphylococci and \(S. aureus\) than did quarters that were disinfected. There were no significant differences between treated and nontreated quarters for the number of infections with \(S. dysgalactiae\) or \(S. uberis\) (Table 5). Overall, significantly \((P < .001)\) fewer disinfected quarters harbored pathogens than did nontreated ones throughout the lactation in these cattle.

### Table 3. Effect of Teat Disinfectant after Milking on the Relationship between Hyperkeratosis Score and Somatic Cell Count in Dairy Cattle in Ireland (Study 2)

<table>
<thead>
<tr>
<th>Hyperkeratosis Score *</th>
<th>Treatment</th>
<th>No. of Quarters</th>
<th>Average SCC (\times 10^3)</th>
<th>Significance †</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (0–1)</td>
<td>Disinfected</td>
<td>537</td>
<td>126</td>
<td>NS</td>
</tr>
<tr>
<td>Low (0–1)</td>
<td>Nontreated</td>
<td>605</td>
<td>178</td>
<td>NS</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>Disinfected</td>
<td>518</td>
<td>142</td>
<td>NS</td>
</tr>
<tr>
<td>Medium (2)</td>
<td>Nontreated</td>
<td>485</td>
<td>306</td>
<td>((P &lt; .01))</td>
</tr>
<tr>
<td>High (3–4)</td>
<td>Disinfected</td>
<td>174</td>
<td>157</td>
<td>NS</td>
</tr>
<tr>
<td>High (3–4)</td>
<td>Nontreated</td>
<td>138</td>
<td>412</td>
<td>((P &lt; .01))</td>
</tr>
</tbody>
</table>

\*Hyperkeratosis scores: 0 = normal teat-end orifice, 1 = slight smooth ring of keratin, 2= moderate raised, smooth ring, 3 = large raised smooth ring, 4= severe broken ring of keratin.

\†Significance tested by Spearman rank correlation test.

NS = not significant.

### Table 4. Effect of Teat Disinfectant on the Occurrence of Clinical and Subclinical Mastitis Infection in Dairy Cows in Ireland

<table>
<thead>
<tr>
<th>Type of Infection</th>
<th>Disinfected ((n = 27))</th>
<th>Not Treated ((n = 27))</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>6</td>
<td>22</td>
<td>(P &lt; .001)</td>
</tr>
<tr>
<td>Subclinical</td>
<td>5</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td>Subclinical/nonspecific</td>
<td>2</td>
<td>3</td>
<td>NS</td>
</tr>
<tr>
<td>Transient/subclinical</td>
<td>5</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Transient/subclinical/nonspecific</td>
<td>11</td>
<td>13</td>
<td>NS</td>
</tr>
</tbody>
</table>

\*Significance tested by chi-square analysis.

NS = not significant.
Nontreated quarters had a significantly \((P < .001)\) higher texture score (0.93), indicating fewer were classified as smooth, than did quarters that were disinfected (0.85).

**DISCUSSION**

In Study 1, differences were not detected in the mean SCC among any of the hyperkeratosis scores identified. This may be because the study was conducted midlactation when SCCs would be expected to be minimal. Scores were similar among the seven herds, most likely because all seven dairies disinfected quarters after milking and practiced a high standard of milking machine maintenance. In addition, the breed of cow was similar in all herds.

In Study 2, omitting the use of disinfectant after milking resulted in higher SCCs, more pathogens, and a greater incidence of clinical mastitis. This finding is in agreement with earlier work that demonstrated the benefits of using a disinfectant after milking in the reduction of mastitis. In other published studies, concentrations of *S. aureus* recovered from skin swabs taken from quarters that were dipped in a disinfectant solution were lower than those for nondisinfected quarters. Teat skin colonized by *S. aureus* is more than three times as likely to result in mastitis. The chlorhexidine disinfectant used in this study was effective in reducing the number of nonhemolytic staphylococci, a result similar to that shown by Hogan et al. The chlorhexidine disinfectant also tended to be effective against *S. aureus*, a finding that was previously observed under an experimental challenge.

Fox and Norell concluded that teats without a disinfectant treatment after milking had significantly better skin condition than teats treated with an iodine solution.
when teats were exposed to cold and windy conditions. In the present study, teats disinfected with the chlorhexidine solution tended to have a higher hyperkeratosis score and had better texture than teats that were not disinfected. This higher hyperkeratosis score maybe due to the colder weather conditions during the winter period. Blot drying has been recommended after application of disinfectant in colder weather conditions to prevent damage to teat ends.17

Previous studies have shown that a mild degree of hyperkeratosis is not associated with an increased prevalence of clinical infection.2 Severe hyperkeratosis was associated with more cases of subclinical and clinical mastitis.11,22 Subclinical infection will also result in an increase in SCCs in the infected quarter.23 In the present study, there was no correlation between teat hyperkeratosis score and SCCs when teats were disinfected after milking; however, a correlation was evident with hyperkeratosis scores greater than 1 in the absence of teat disinfec ting. This study provides additional information regarding the relationship of teat hyperkeratosis with the increased risk of intramammary infection.

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REFERENCES

