A Comparison of Two Methods of Evaluation of Teat Skin Pathology

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ABSTRACT

A split-plot design with repeated measures was used to test the relationship between visual teat skin condition score, the degree of transepidermal water loss from the skin, and the colonization by Staphylococcus aureus on experimentally chapped and inoculated teats of 20 lactating Holstein cows. Visual teat skin chapping score and the number of S. aureus colonies obtained from a teat skin swab were correlated (r = 0.53). Transepidermal water loss and S. aureus count were not correlated (r = 0.02). Results indicated that visual teat skin evaluation is superior to measurements of transepidermal water loss in the prediction of the susceptibility of teat skin to colonization by S. aureus.

(Key words: teat chapping, skin colonization, Staphylococcus aureus)

Abbreviation key: TEWL = transepidermal water loss.

INTRODUCTION

The maintenance of healthy teat skin of cows is a concern for many caretakers, especially during cold, wet, and windy conditions that can predispose the skin to chapping (17). Severity of teat chapping has been positively correlated with increased colonization by Staphylococcus aureus (7) and an increased risk of IMI (16). A teat skin evaluation method that can identify when teats are at a serious risk for increased colonization would be a useful management tool to help producers further refine monitoring and intervention strategies to prevent IMI caused by S. aureus.

Logically, if a clinical grading system of teat skin condition is to be used to gauge the risk of IMI by a pathogen, its correlation with teat skin colonization by that pathogen must be evaluated. Some teat skin evaluation methods have already been correlated with S. aureus colonization. The visual scores used by Fox et al. (7), which estimated the severity of chapping and the portion of the teat affected by chapping, correlated well with S. aureus colonization, but that system was designed to interpret the rate of healing after a severe experimental chapping treatment and, as such, might not have good practical application on the farm.

A cutimeter has been tested as an objective measure of teat skin chapping (6). The cutimeter quantifies the degree that skin thickens and loses pliability as a result of chapping and drying of the stratum corneum (1, 2, 14). In a controlled study in which teats were experimentally chapped (6), cutimeter readings were significantly, but not highly, correlated with S. aureus colonization.

New and potential teat skin evaluation methods exist that require further testing. Goldberg et al. (9) at the University of Vermont have published a visual teat scoring system to monitor changes in skin condition caused by teat disinfectant treatments. The system is applicable to on-farm use but has not been correlated with S. aureus colonization.

Transepidermal water loss (TEWL) is an objective skin evaluation method that is widely used in human dermatology and that has been shown to be a sensitive biophysical measure of the water barrier function of the epidermis (11, 15, 18). If the epidermis is injured, TEWL increases (13, 20, 23), and chapped skin exhibits more TEWL than does healthy skin of humans (21). The apparatus for measuring TEWL has been adapted for use with cows, and methods to obtain reliable TEWL measures from teat skin have been developed (3). The TEWL of chapped teat skin of bovine and how it is correlated with S. aureus colonization has yet to be investigated.

The objectives of this experiment were to determine the pathologic implications of a visual teat skin evaluation system (9) and of TEWL (3) as objective measures of teat skin pathology.
MATERIALS AND METHODS

Cows

Holstein cows (n = 20) predominantly in late lactation (309 ± 142 DIM) with four functional quarters that were determined to be free from IMI caused by *S. aureus* were split into five groups for experimentation during the period from February 1996 to August 1996. A quarter was deemed to be free of IMI caused by *S. aureus* if that pathogen was not present in the blood agar cultures of two milk samples collected aseptically on different days. As cows were obtained, they were randomly assigned to one of five treatment blocks until each block was used. This procedure was repeated until 4 cows were in each of the treatment blocks.

Chapping Treatments and Arrangement

Chapping treatments were arranged in a balanced incomplete block design such that the five degrees of teat chapping defined in the visual teat evaluation system (9) were equally represented at each of the four teat positions on the mammary gland. Briefly, the degrees of teat skin chapping were 1 = teat skin is smooth and free from scales, cracks, or chapping; 2 = teat skin shows some evidence of scaling; 3 = teat skin is chapped, and some small warts may be present; 4 = teat skin is chapped and cracked, and redness is present; numerous warts may be present; 5 = teat skin is severely damaged and ulcerative with scabs or open lesions, and large or numerous warts are present.

Methods of Chapping

To create the different degrees of chapping, teats were immersed in the following compounds at 12-h intervals (after milking) until the designated chapping score was achieved: score 1, glycerin; score 2, acetone for 3 min; score 3, ether for 3 min; score 4, 0.5N NaOH; and score 5, 1N NaOH. Premilking and postmilking teat dipping ceased when chapping was initiated and did not resume until the end of the experimental period.

Challenge with *S. aureus*

When a teat reached its designated degree of chapping, it was dipped in a skim milk broth culture containing $5 \times 10^6$ cfu/ml of *S. aureus* ATCC 29740 for two consecutive milkings. To prepare the skim milk broth cultures, four 18-h cultures were grown in brain-heart infusion broth at 37°C. From each culture, 0.1 ml was transferred to new culture tubes containing 3 ml of brain-heart infusion broth for a 4-h culture. *Staphylococcus aureus* was recovered by centrifuging the 4-h cultures at 5°C (1200 × g), decanting the broth, and resuspending the *S. aureus* pellet in sterile 0.01 M PBS solution (pH 7.4). Resuspended *S. aureus* was washed twice in sterile PBS solution, and the centrifugation resuspension procedures were repeated. The final *S. aureus* resuspension was diluted to an optical density of 0.2 at 540 nm, equivalent to $100 \times 10^6$ cfu/ml. The addition of 0.2 ml of this suspension to 3.8 ml of sterile skim milk broth produced the $5 \times 10^6$ cfu/ml of culture for teat immersion.

Measurements and Samples Taken

Visual teat skin score, teat skin temperature, TEWL, and swab samples of lateral teat skin surface were obtained from teats prior to initiation of chapping and on the following days relative to the day of *S. aureus* challenge, d 1, 3, 6, and 10. All measurements and samples were taken by one experimenter to ensure that the technique used was consistent. Skin temperature of the teat barrel was obtained using a digital thermistor thermometer equipped with a skin surface probe (Cole-Parmer® Instrument Co., Vernon Hills, IL). A TEWL measurement was obtained from a teat on designated sampling days using the apparatus and methods described by Burmeister et al. (3). The TEWL measurement was made on a nonexudative section of skin located near the center of the barrel of the teat. Skin swab specimens were obtained from the teat barrel by gently rolling a sterile cotton swab moistened in sterile 0.2% thiosulfate solution up and down the length of the barrel two times. The swab was returned to the thiosulfate solution up and down the length of the barrel two times. The swab was returned to the thiosulfate solution, and solutions were stored at −5°C for subsequent bacterial culture. Ambient temperature and relative humidity in the examination room (milking parlor) were also recorded using a digital thermometer (Cole-Parmer®) and a wet bulb-dry bulb psychrometer (Belfort Instrument Co., Baltimore, MD), respectively.

Analysis of Samples

Teat swab tubes were vigorously vortexed before 50-μl portions were spread on standard sheep blood agar, selective *S. aureus* agar, and modified Baird Parker agar (4). Blood agar plates were incubated for
Statistical Analysis

Data analysis was performed on colony counts from the teat skin swab samples collected after challenge with *S. aureus*. Colony counts of *S. aureus* on the blood agar and modified Baird Parker agar were transformed by natural log and analyzed using PROC GLM (19).

The model used to test the relationship between *S. aureus* colonization of teat skin and chapping treatment, TEWL, or visual chapping score (19) was \( Y_{ijkl} = B_i + C_{j(i)} + T_k + (C \times T)_{ijk} + E_{(ijkl)} \), where \( Y \) = *S. aureus* colonization; \( B \) = block of chapping treatments; \( C \) = cow within block of chapping treatments; \( T \) = chapping treatment, TEWL, or visual chapping score; \( C \times T \) = interaction of cow within block and chapping treatment, TEWL, or visual chapping score; and \( E \) = effect of the measurement time after *S. aureus* challenge.

The interaction of chapping treatment and cow within block was the error term used to test the effect of chapping treatment. Similarly, the interaction of TEWL and cow within block and the interaction of visual chapping score and cow within block were used to test the effects of TEWL and visual chapping score, respectively. Type III sums of squares were used to test statistically the effects associated with independent factors.

Mean log e colonization, TEWL, and visual chapping score of each chapping treatment was computed and contrasted within measurement days using Duncan’s multiple range test (19).

Teat skin temperature, relative humidity, and the interaction of ambient temperature and relative humidity were tested for their effects on teat skin TEWL using a GLM ANOVA (19). Simple correlations among visual chapping score, TEWL, and *S. aureus* count were also computed (19).

RESULTS

The log e *S. aureus* colonization according to chapping treatments and day relative to teat inoculation are shown in Figure 1. Treatments that received chapping treatments 2, 3, 4, and 5 supported more *S. aureus* colonization (\( P < 0.05 \)) than did nonchapped teats (treatment 1) on day 1 and 3 postchallenge. Additionally, *S. aureus* colonization of teats that received treatments 4 and 5 increased from day 1 to 3. Teats that received chapping treatment 5 supported significantly more *S. aureus* colonization for a longer period than did teats that received the other treatments. Teat skin TEWL did not differ among the chapping treatments or days postchallenge (Figure 2). Figure 3 illustrates the changes in visual score that were associated with each chapping treatment over the test period. The three most severe chapping treatments all showed a trend toward healing during the experiment, although the visual skin condition of teats that received treatment 5 remained markedly worse than the other teats on day 10. The skin condition of the unchapped teats grew slightly worse over the test period.

Visual score was positively correlated with *S. aureus* count (\( r = 0.53; P < 0.01 \)), and TEWL was not correlated with *S. aureus* count (Table 1). Analyses of variance were used to test the effects of chapping treatment, TEWL, and visual chapping score on *S. aureus* colonization, which are reported in Table 2. The association of visual score and *S. aureus* colonization was significant (\( P < 0.01 \); Table 2). Transepidermal water loss was not associated with teat skin colonization (Table 2). In all models, the effect of day on teat skin colonization by *S. aureus* was significant (\( P < 0.01 \); Table 2), indicating the significance of the decline in *S. aureus* colonization over time. The significant effect of block and day for model 3 (Table 2)
Figure 2. Teat barrel transepidermal water loss (TEWL), (parts per million) over time by treatment. Treatments caused the following degrees of teat skin chapping: treatment 1 (◊), no chapping; treatment 2 (●), dry skin; treatment 3 (○), chapped (rough) skin; and treatment 4 (▲), cracked skin. Measurements from treatment 5, which caused severely damaged skin, could not be obtained reliably and are not reported. The standard error of the mean for TEWL was 16.3 ppm.

Figure 3. Mean teat chapping scores over time by treatment. Treatments caused the following degrees of teat skin chapping: treatment 1 (◊), no chapping; treatment 2 (■), dry skin; treatment 3 (○), chapped (rough) skin; treatment 4 (▲), cracked skin; and treatment 5 (▲), severely damaged skin. Briefly, teat condition scores were defined as follows: 1 = teat skin is smooth and free from scales, cracks, or chapping; 2 = teat skin shows some evidence of scaling; 3 = teat skin is chapped, and some small warts may be present; 4 = teat skin is chapped and cracked, and redness is present; numerous warts may be present; and 5 = teat skin is severely damaged and ulcerative with scabs or open lesions, and large or numerous warts are present.

Table 1. Correlation between the relationship between visual teat skin condition score and transepidermal water loss (TEWL) and Staphylococcus aureus colonization of teat barrel skin.1

<table>
<thead>
<tr>
<th>Method of evaluation</th>
<th>no.</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual scores</td>
<td>320</td>
<td>0.53</td>
<td>0.0001</td>
</tr>
<tr>
<td>TEWL</td>
<td>256</td>
<td>0.02</td>
<td>0.70</td>
</tr>
</tbody>
</table>

1Loge colony-forming units per milliliter of swabbing solution.  
2Number of observations. Reliable TEWL measurements could not be obtained for teats receiving treatment 5 (immersion in 1N NaOH, which caused severely damaged and ulcerative skin with scabs or open lesions; large or numerous warts were also present. Therefore, these measurements were not included in the analysis.

**DISCUSSION**

The objectives of this experiment were to determine the degree of *S. aureus* colonization that was associated with each score on a visual system and to explore the relationships among TEWL, chapping, and *S. aureus* colonization. Changes in condition scores for teats that received chapping treatment 1 were deemed to be within the range of normal variation.

The association of visual chapping scores and *S. aureus* colonization in this experiment (Figure 1; Tables 1 and 2) agrees with the results of previously published studies that investigated the colonization of chapped teats. Those studies (5, 6, 7, 8) also showed a significant positive correlation between the severity of chapping and the degree of *S. aureus* colonization.

Any degree of teat chapping significantly increased the ability of *S. aureus* to colonize the teat surface. The greatest degrees of teat skin colonization and persistence of colonization were observed when chapping severity created a crack in the stratum corneum (treatments 4 and 5), which exposed the underlying epidermal tissues.

The use of TEWL did not appear to be a good measure of pathologic changes in the skin as measured by *S. aureus* colonization after chapping. Several reasons may explain the lack of correlation between
colonization and TEWL. First, it was often difficult to find an area suitable to place a probe on teats that were severely chapped. Cracks, scaling skin, and scabs make it difficult for the probe to seal against the skin to obtain an accurate reading. Perhaps areas to probe that were chosen on severely chapped skin, those that could provide an adequate seal, were not representative of the general ulcerative condition of the skin.

The absence of differences in TEWL among the lesser degrees of chapping, all of which should have been reliably measured (3), could have been a result of changes or differences in skin physiology. The epidermis rapidly forms a very effective temporary water barrier composed of parakeratotic cells after harsh compounds contact the skin of humans (22). Alternatively, species differences may account for the lack of sensitivity in TEWL of cows compared with TEWL of humans. Although the structure of the epidermis does not vary greatly among mammals, the water barrier properties of the epidermis do differ among species and among body regions within a species (12).

Finally, the epidermal water barrier is very resistant to many types of irritation to the skin (e.g., scarification, solvent substances, or chemical irritants), which might affect the stratum corneum differently (22). Thus, the lesser chapping challenges (treatments 1, 2, and 3) apparently did not influence water barrier function despite the visual differences

### Table 2. Analyses of variance used to test the effect of chapping treatment, transepidermal water loss (TEWL), and visual teat condition score on *Staphylococcus aureus* colonization recovered from teat skin.

<table>
<thead>
<tr>
<th>Model</th>
<th>Independent variable</th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Block</td>
<td>4</td>
<td>8.6</td>
<td>0.6</td>
<td>0.6892</td>
</tr>
<tr>
<td></td>
<td>Cow (block)</td>
<td>15</td>
<td>362.8</td>
<td>6.3</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>3</td>
<td>379.5</td>
<td>33.1</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Chapping treatment</td>
<td>4</td>
<td>482.9</td>
<td>23.5</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Treatment × cow</td>
<td>56</td>
<td>288.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(block)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>237</td>
<td>907.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Block</td>
<td>4</td>
<td>3.6</td>
<td>0.2</td>
<td>0.9256</td>
</tr>
<tr>
<td></td>
<td>Cow (block)</td>
<td>15</td>
<td>74.4</td>
<td>1.2</td>
<td>0.2597</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>3</td>
<td>206.5</td>
<td>16.9</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>TEWL</td>
<td>1</td>
<td>1.8</td>
<td>0.78</td>
<td>0.3896</td>
</tr>
<tr>
<td></td>
<td>TEWL × cow</td>
<td>19</td>
<td>45.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(block)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>211</td>
<td>858.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Block</td>
<td>4</td>
<td>41.7</td>
<td>2.7</td>
<td>0.0337</td>
</tr>
<tr>
<td></td>
<td>Cow (block)</td>
<td>15</td>
<td>133.3</td>
<td>2.3</td>
<td>0.0052</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>3</td>
<td>236.1</td>
<td>20.0</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Score</td>
<td>1</td>
<td>368.0</td>
<td>57.1</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Score × cow</td>
<td>19</td>
<td>122.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(block)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td>277</td>
<td>1090.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Log$_e$ colony-forming units per milliliter of swabbing solution.
2. Five chapping treatments were arranged in five incomplete blocks so that each chapping treatment was applied to each mammary quarter in a balanced incomplete block design.
3. Chapping treatments were applied to teats such that the five degrees of skin chapping (9) were achieved. These degrees ranged from normal, unchapped skin to severely damaged skin with open, ulcerative lesions.
4. The interaction of treatment and cow within block was the error term used to test treatment.
5. The residual was used as the error term to test the effects of treatment block, cow, and day.
6. Reliable TEWL measurements could not be obtained for teats receiving treatment 5 (immersion in 1 N NaOH, which caused severely damaged and ulcerative skin with scabs or open lesions; large or numerous warts were also present). Therefore, these measurements were not included in the analysis.
7. The interaction of TEWL and cow within block was the error term used to test TEWL.
8. The interaction of score and cow within block was the error term used to test visual teat condition score.
in chapping that were apparent on the outermost layer of the teat skin.
In previous teat chapping studies (5, 8), postmilking teat disinfectant solutions were determined to be helpful in speeding the healing of chapped teat skin compared with teats that received no postmilking therapy.

CONCLUSIONS

The significant association between the visual system of assessing teat skin chapping and skin colonization by Staphylococcus aureus indicated that this assessment system could be used to predict susceptibility of the teat skin to pathogen (S. aureus) colonization. Teat TEWL measurement was not a viable method to predict teat chapping and pathogen colonization. Thus, these results indicate that the visual assessment of the teat skin condition is useful in the prediction of predisposition to teat skin colonization and, therefore, mammary gland infection by S. aureus.

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