Relationship between teat end hyperkeratosis with intra mammary infection and somatic cell counts in lactating dairy cattle

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SUMMARY

The aims of the present study were to relate between teat end hyperkeratosis with intra mammary infection and somatic cell counts in lactating dairy cattle. In total, 144 teat pairs (72 pairs of front teats, 72 pairs of rear teats, herd A: 42 teat pairs, herd B: 54 teat pairs and herd C: 48 teat pairs) were evaluated according to the presence of IMI and SCC. Scores were applied to teats according to the TEC. Teats ends with a score 1 had the largest number of IMI when compared to the other three categories. The most frequent isolated pathogens were Corynebacterium bovis, Streptococcus dysgalactiae and Staphylococcus spp. There is no evidence of association between TEC and major pathogens. Also the teat-end lesions did have a significantly effect on quarter SCC. It can be concluded that animals with very rough teat end rings may not have a greater predisposition to IMI.

Keywords: Intra mammary infection, Teat end hyperkeratosis, Somatic cell counts, Dairy cattle

RÉSUMÉ

Les buts de la présente étude étaient de relier l’hyperkératose de l’extrémité du trayon avec l’infection intra-mammaire et le comptage de cellules somatiques chez les vaches laitières. Au total, 144 paires de trayons (72 paires de trayons avant, 72 paires de trayons arrière, troupeau A: 42 paires de trayons, troupeau B: 54 paires de trayons et de troupeau C: 48 paires de trayons) ont été évalués en fonction de la présence de l’IIM et CCS. Les scores ont été appliqués à trayons selon le TEC. Les trayons avec un score 1 ont eu le plus grand nombre de l’IIM par rapport aux trois autres catégories. Les agents pathogènes isolés les plus fréquents étaient Corynebacterium bovis, Streptococcus dysgalactiae et Staphylococcus spp. Il n’y a aucune preuve d’association entre le TEC et les principaux agents pathogènes. De plus, les lésions du bout des trayons ont eu un effet significatif sur le quart (un trimestre) de CCS. On peut conclure que les animaux avec des anneaux de la fin de trayons très rudes (rugueux) n’ont pas une plus grande prédisposition à l’IIM.

Mots-clés: Infection intra-mammaire, hyperkératose de l’apex du trayon, comptage de cellules somatiques, bovins laitiers

Introduction

Mastitis continues to be one of the economically most important diseases in dairy farming. The losses are calculated from reduced milk production, discarded milk, increased cull rate, pharmacologic costs, and increased labor. Bacteria that cause clinical mastitis usually enter the udder through the teat canal. There are several important non-specific defense mechanisms of the bovine teat canal. These mechanisms include the following: the adsorption of pathogens onto keratinized teat canal epithelium (stratum corneum), the continuous removing of cell material as a result of shear force the teat canal epithelium, immunological reactions in the region of Furstenberg’s rosette, antimicrobial fatty acids and proteins and the enzyme xanthine oxidase localized in the teat canal epithelium [14, 29, 15, 7, 4, 23].

Hyperkeratosis or teat-end callosity (TEC) 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, [27, 17] and it is commonly observed in dairy cows. Cow factors including teat-end shape, teat position, teat length, milk yield, stage of lactation, and parity are associated with the degree of TEC [2, 19]. The classification of the teat end integrity into scores can serve as an important tool to control bovine mastitis because it allows for the classification of the different types of physical injuries. These irregularities can be related to problems in the management and production system, leading to a greater predisposition toward mastitis in the herd [16].

A few studies have suggested that some teat-end shapes act as risk factors for mastitis, and, as they have high daughter-dam heritability, can be eliminated by selective breeding [25, 5, 27]. The presence of teat end lesions is also regarded as important risk factors for mastitis [6, 25] and studies have investigated their relationship to somatic cell counts (SCCs) [10, 25]. The most important cause of increased SCC is bacterial infection of the mammary gland [22, 26, 28]. However at the farm level, no relationship between SCC and
degree of TEC was found [9]. The aims of the present study were to relate intra mammary infection (IMI) occurrence and SCC with teat end hyperkeratosis.

**Material and methods**

**ANIMALS**

The study was carried out in four dairy farms located in Tabriz, Iran. The Holstein cows were in herds of different size, and in different stages of lactation and lactation number (first to fifth lactation). The average milk production was 27.8 kg/cow per day (range 25.2–30.4 kg/d). Cows on each herd were milked twice daily in a milking parlor. Post milking teat disinfection and dry cow therapy were practiced in all herds. Cows were housed in open shed with concrete floor and dried manure solids were used as bedding. Teat end assessment was performed between the 30th and the 150th lactation days.

**SCC MEASUREMENT**

In total, 144 teat pairs (72 pairs of front teats, 72 pairs of rear teats, herd A: 42 teat pairs, herd B: 54 teat pairs and herd C: 48 teat pairs) were evaluated according to the presence of IMI and SCC. 4 with missing teats were excluded due to contamination. From each milk sample, 15 mL were sent to a laboratory for SCC using a Somacount 300 (Bentley Instruments). A critical value of 400 ×10³ cell/ml was set to novobiocin characterized by an inhibition halo of up to 16 mm. Readings of the tests were obtained after 24, 48, and 72 h of incubation at 37°C in an air incubator [24]. The Gram-positive, catalase-negative cocci were identified as belonging to the Streptococcaceae family and subjected to CAMP test and esculin hydrolysis. The Gram-positive, pleomorphic and catalase-positive coccobacilli were classified within the Corynebacterium genus. Blood agar plates containing three or more different colonies were considered contaminated. Major mastitis pathogens included S.aureus, Streptococcus spp., Arcanobacterium pyogenes, or coliform species. Minor pathogens were defined as CNS or Corynebacterium spp., as described by other authors [8]. A quarter was considered infected with a minor pathogen when at least three colonies were observed on the blood agar plate, and one or more colonies for a major pathogen. Blood agar plates containing three or more different colonies were considered contaminated. IMI was considered positive at the animal level when isolation was positive for at least one mammary quarter of the 72 animals. If one of the duplicate samples was contaminated, the result from the uncontaminated duplicate alone was used to diagnose infection. Milk samples were considered contaminated if three or more bacterial species were isolated. When a quarter had both a major and a minor pathogen isolated at the same time, the quarter was defined as infected with the major pathogen [12].

**TEAT-END’S SCORING**

Scores were applied at the teat level before the pre-milking routine by the same trained technician according to TEC: 1 = no ring, smooth teat end with a small even orifice; 2 = smooth ring with a raised ring around the orifice (the raised area may be smooth or slightly rough); 3 = rough ring with a raised, roughened ring with fronds of keratin extending 1–3 mm from the orifice; and 4 = very rough ring with keratin extending more than 4 mm from the orifice. Teat-end condition scores were chosen according to Mein et al., (2001) [16].

**STATISTICAL ANALYSIS**

Prior to statistical analyses, observations were checked for unlikely values and excluded from the analysis. Descriptive statistics (median, mean, standard deviation, minimum, maximum) were calculated (Table I). Teats with IMI caused by contagious and environmental pathogens were compared in terms of TEC. Chi-square test was employed to verify differences among TEC frequencies according to the type of pathogen.

The logistic regression model was applied at the teat level to assess the effect of changes in TEC and IMI status. Logistic regression model was applied and odds ratio were estimated with respective 95% confidence intervals. All analyses were made with SAS ver 9.1 (SAS, Cary, NC, USA).
Results

In this study, the subject was the teat pair and the fixed effect was the hyperkeratosis score (1, 2, 3, 4). In total, 140 teat pairs (70 pairs of front teats, 70 pairs of rear teats, herd A: 40 teat pairs, herd B: 52 teat pairs and herd C: 48 teat pairs) were evaluated according to the presence of IMI and SCC (4 with missing teats were excluded due to contamination). The teat end hyperkeratosis was scored as “1” in 117 teats (41.8%), as “2” in 80 teats (28.6%), as “3” in 56 teats (20%) and as 4 in 27 teats (9.6%). The distribution of SCC, teat end lesion and IMI within herd is detailed in Table I and II. The most frequent isolated pathogens were C. bovis (8.11%), Streptococcus dysgalactiae (10.81%) and Staphylococcus spp. (45.94%). Table II summarizes the results of microbiological tests for the 70 animals. The isolated pathogens were grouped into two categories; contagious and environmental (Table III). Statistical analysis showed that there is no evidence of Pearson correlation between TEC and major pathogens (P<0.05). The teat-end lesions did have a significant correlation with on quarter SCC (Table IV). However on bacterial culture no association was identified between TEC and IMI (Table V). The linear regression between SCC as dependent variable and TEC, as independent variable is obtained as follows:

\[ SCC = -88.99 + 103.56 \times TEC \]

The linear regression between IMI as dependent variable and TEC of right cranial quarter, as independent variable is obtained as follows:

\[ IMI = 1.072 + 0.11 \times \text{right cranial} \]

### Table I: Absolute (n) and relative frequency (%) of teat-end condition score (TEC score), somatic cell count (SCC) and intra mammary infection status (IMI) from the animals included in experiment

<table>
<thead>
<tr>
<th>Variables</th>
<th>Maximum</th>
<th>Minimum</th>
<th>StdDev</th>
<th>Mean</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>LogSCC</td>
<td>3.951</td>
<td>1.041</td>
<td>0.708</td>
<td>2.338</td>
<td>69</td>
</tr>
<tr>
<td>TEC</td>
<td>4</td>
<td>1</td>
<td>0.85</td>
<td>1.98</td>
<td>70</td>
</tr>
</tbody>
</table>

### Table II: Absolute (n) and relative frequency (%) for results of the SCC, and most frequent values (modes) of teat-end condition score (TEC score)

<table>
<thead>
<tr>
<th>TEC</th>
<th>Contagious Mastitis Pathogens</th>
<th>%</th>
<th>Environmental Mastitis Pathogens</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>15</td>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>40</td>
<td>1</td>
<td>11.11</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>35</td>
<td>4</td>
<td>44.44</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>11.11</td>
</tr>
</tbody>
</table>

### Table III: Absolute and relative frequencies (%) for contagious or environmental pathogens according to the teat-end conditions core (TEC score)

<table>
<thead>
<tr>
<th>SCC</th>
<th>Correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.24772</td>
<td>0.0401</td>
</tr>
<tr>
<td></td>
<td>0.225</td>
<td>0.0631</td>
</tr>
<tr>
<td></td>
<td>0.22619</td>
<td>0.0616</td>
</tr>
<tr>
<td></td>
<td>0.19625</td>
<td>0.1061</td>
</tr>
<tr>
<td></td>
<td>0.21986</td>
<td>0.0695</td>
</tr>
</tbody>
</table>

### Table IV: Relationship between teat-end lesions and quarter somatic cell count (SCC).
TEAT END HYPERKERATOSIS IN LACTATING DAIRY CATTLE

Discussion

Our results indicated that 28.57% of isolated bacteria were contagious mastitis agents, and *Staphylococcus aureus* was the most significant bacterium (85% of all isolated bacteria). Some reports indicate that *S. aureus* is one of the most prevalent causes of intra mammary infection (IMI) and estimate that 7% to 40% of all cows are infected [3]. In this study 12.85% of isolated bacteria were environmental mastitis agents, and the most prevalent bacterium was *Streptococcus dysgalactiae*.

As the teat-end orifice is the first line of defense against invading bacteria, changes or damage to this part of the udder may reduce its effectiveness in preventing IMI [27, 21]. It has been reported that changes to the orifice, brought about by milking methods and changes in weather conditions, may favour bacterial invasion [13, 21]. Quarter with severe teat-end lesions and those which were traumatized or leaked milk had increased Rates of IMI [25]. There is not clear relationship between IMI and TEC. Teat ends with rough rings with keratin extending more than 3mm from the orifice (score 3) had the highest chance of developing IMI when compared to the other three categories. Neijenhuis et al. [16] speculated that a small amount of TEC does not appear to increase the risk of intra-mammary infection in lactating dairy cows and may be a beneficial physiological response of the teat to machine milking. In this study there were no differences between pathogen types when considering TEC, because for both contagious and environmental pathogens the assessed variables had similar characteristics. This study contrast with Bhutto et al. [3] reported that there is evidence of some association between infections within early all major pathogens and CNS and type of teat-end lesions. Clinical mastitis cases with yeast *Kelebsiella pneumonia* and *Entrobacter aerogenes* had higher TEC than other cow [20]. Part of the observed differences within studies could be explained by differences in stage of lactation, milk yield, parity, milking machine, number of cows and teat end shape.

SCC varied according to TEC. Teats with score 2 (smooth rings teats) had lower SCC when compared to other scores. Since IMI case tended to increase with increasing teat scores, SCC was expected to follow the same pattern, which did not occur. In the present study, there was significant correlation between teat hyperkeratosis score and SCCs when teats were disinfected after milking (P<0.05). This is in accordance with research Seykora and Daniel who reported that as teat-end shape varied from pointed toward flat and inverted, SCC increased [25]. In contrast other studies the relationship between SCC and degree of teat-end callosity has not been reported [26]. With advancing of parity number, the share of teats with teat hyperkeratosis 3 and 4 and higher milk SCC, respectively could be seen. This happens because of the more prolonged effect of milking (more lactation) and occurring teat end changes, predisposing to teat bacterial infection [18]. However, the relationship between IMI and SCC is not always evident, such as in this study [6, 11, 20]. It can be concluded that animals with very rough teat end rings may not have a greater predisposition to IMI.

<table>
<thead>
<tr>
<th></th>
<th>Cranial quarters</th>
<th>Right cranial quarter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td>0.19625</td>
<td>0.21986</td>
</tr>
<tr>
<td>P value</td>
<td>0.1061</td>
<td>0.0695</td>
</tr>
</tbody>
</table>

Table V: Relationship between teat-end lesions and intra mammary infection (IMI).

References

8. - COMPTON C.W., HEUER C., PARKER K., MCDougall S.: Epidemiology of mastitis in


