CNS mastitis: Nothing to worry about?


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ABSTRACT

In this paper, we analyzed a very large field data set on intramammary infections (IMI) and the associated somatic cell count (SCC) in dairy cows. The objective of the study was to analyze the impact of coagulase-negative staphylococci (CNS) IMI on cow SCC, both mean and variability, and on the potential of these infections to have a major impact on the bulk milk SCC (BMSCC). Data and milk samples for bacterial culture were collected by Quality Milk Production Services (QMPS) between 1992 and March of 2007. The QMPS program services dairy farms in New York State and other states in the Northeastern USA and operates in conjunction with Cornell University. Only records from cows where SCC and milk production data were available, and where only one organism was isolated from bacterial cultures of milk samples (or where culture was negative) were used for this analysis. A total of 352,614 records from 4200 whole herd mastitis screening sampling qualified for this study.

Within herds an average of 15% (S.D. 12%) of cows sampled were infected with CNS, ranging between 0 and 100%. Average within herd prevalence of cows with a CNS IMI and an SCC over 200,000 cells/ml was 2% (S.D. 4%) with a minimum of 0% and a maximum of 50%. Results of linear mixed models showed three distinct populations of IMI statuses: negative cultures with the lowest SCC; CNS and Corynebacterium bovis with a moderate increase in SCC, and Streptococcus agalactiae, Streptococcus spp. and Staphylococcus aureus showing an important increase in SCC. Surprisingly, milk production was slightly but significantly higher in CNS infected cows compared to culture-negative cows, whereas it was strongly reduced in cows with a major pathogen IMI. The percentage contribution of CNS infections to the BMSCC was 17.9% in herds with a BMSCC less than 200,000 cells/ml. This value decreased to 11.9 and 7.9% in herds with bulk milk SCC between 200,000 and 400,000 and over 400,000 cells/ml, respectively. We concluded that very few herds with milk quality problems would have an important increase in BMSCC that could be mostly attributed to CNS infections. On the other hand, in herds with low BMSCC, CNS infections may be an important contributor to the total number of somatic cells in the bulk milk.

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1. Introduction

Coagulase-negative staphylococci (CNS) intramammary infections (IMI) have been associated with an increase in somatic cell counts (SCC) of affected cows (Jarp, 1991). However, the importance of these IMI is debated. Classically, CNS were classified as minor pathogens and their importance as an independent cause of subclinical or clinical mastitis was judged to be limited.
These CNS infections are typically associated with only a moderate increase in cow SCC (Lam et al., 1997). Some studies would indicate that CNS infections are preventive for IMI by other major pathogens (White et al., 2001), although this is much debated and other observational studies did not find this protective effect (Zadoks et al., 2001). However, more recent studies propose that infections with CNS may cause a more serious harm than thought before (Taponen et al., 2006). CNS infections have been studied in pre-partum treatment trials in heifers and bacteriological cure was associated with a decrease in SCC (Borm et al., 2006). Most studies lacked a sufficient sample size to evaluate both the mean effect of CNS and the variability of the effect. A large variability would imply that in some cows and herds, CNS infections might play a major role in udder health and milk quality.

Even though individual CNS infections may only have a moderate impact on SCC (Lam et al., 1997), many cows infected with this family of organisms, in herds where the producer goal is to achieve a relatively low bulk milk SCC (BMSCC), would potentially lead to herd level SCC problems. No field studies on a large number of dairy farms exist to quantify this particular situation and to estimate its relative importance relative to other causal mastitis organisms.

In this manuscript, we analyzed a very large field data set on IMI and the associated SCC in dairy cows. The objective was to study the impact of CNS IMI on cow SCC, both mean and variability, and on the potential of these infections to have a major impact on the BMSCC.

2. Materials and methods

2.1. Data

Data and milk samples for bacterial culture were collected by Quality Milk Production Services (QMPS) between January 1992 and March of 2007. The QMPS program services dairy farms in New York State and other states in the Northeastern USA and operates in conjunction with Cornell University. Data included bacterial culture results from individual cow milk samples, individual cow data retrieved from Dairy Herd Improvement Association (DHIA) records, and herd management information obtained by questionnaires completed at the time of sampling. Herds with high BMSCC (>750,000 cells/ml) were required by New York state law to participate in the program; the same program was voluntary for other herds. Milk sample collection and isolate identification were performed by QMPS as described previously (Wilson et al., 1997). Composite milk samples were collected by QMPS personnel and cultured in one of the four regional QMPS laboratories. Collection of mammary secretion was done aseptically according to National Mastitis Council guidelines (Hogan et al., 1999). In brief, teat ends were scrubbed with cotton pads soaked in 70% isopropyl alcohol, and the first few streams of foremilk were discarded. Approximately equal amounts of milk were collected from each quarter into a composite milk sample. Milk samples were stored at 4–8 °C until cultured. An approximate 20-µl aliquot from each milk sample was spread onto blood agar plates containing 0.01% esculin using individual sterile cotton swabs and incubated for 48 h at 37 °C. After incubation, plates were observed and organisms identified presumptively as Staphylococcus aureus, CNS, Streptococcus spp., and coliform bacteria based on colony morphology on blood and MacConkey agar, CAMP reaction, Gram stain and catalase and coagulase test reactions (Hogan et al., 1999). Streptococcus agalactiae was identified using colony morphology and a positive CAMP reaction. A sample was considered contaminated when three or more dissimilar colony types were observed. SCC and milk production data were obtained from DHIA records and were matched to the culture results by closest test day relative to the day of milk sampling for bacteriology. Milk SCC was transformed to linear score (LS) using the formula: 

$$\text{LS} = \left(\log_{e}(\text{SCC})/0.6931\right) - 3.6439,$$

where SCC is the somatic cell count in thousands per ml.

Records were only included in the analysis from cows where SCC and milk production data were available and where only one organism was isolated in bacterial culture of the milk sample (or culture-negative). A total of 352,614 records from 4200 full herd samplings qualified for this study. Average herd size in these herds was 69 cows (S.D. 93) that average approximately 9330 kg milk (S.D. 3147) per 305 day standardized lactation. Throughout, a cow will be defined infected when a pathogen was detected in the composite milk sample. It is recognized that this definition of IMI is not uniformly accepted given our sampling method.

2.2. Statistical analyses

Descriptive analyses were performed. Means and standard deviations were calculated for each micro-organism. Analyses were performed separately for heifers and cows.

Herds of greater than or equal to 10 cows were used for further analysis on the impact of individual micro-organisms on estimated BMSCC. For each observation in these herds the percent contribution of the cow to the herd’s estimated BMSCC was calculated. For each pathogen, total contribution to BMSCC during a whole herd mastitis screening sampling was calculated as the sum of contributions of all cows infected with this pathogen. The contribution of an individual cow was calculated as

$$\frac{\text{cow SCC} \times \text{cow milk (kg)}}{\text{sum over all cows of (cow SCC} \times \text{cow milk (kg))}} \times 100\%$$

Once the individual cow contribution to the bulk tank was estimated, contributions per organism were calculated by adding the individual contributions of all cows infected with each organism within a herd sampling. This provided the proportion of cells in the bulk tank attributed to a particular organism at a given sampling day.

A mixed model linear regression of LS and milk production was performed. Herd was treated as a random effect and forced into all models; days in milk (categorized by month in lactation), lactation number (heifers versus cows), pathogen code and all possible interactions were
included as fixed effects. Statistical significance was set at $P < 0.05$. Backward elimination of independent variables was employed. All analyses were performed in the Statistical Analysis System, SAS (version 9.1, SAS Institute, Cary, NC). Least squared means were calculated for the interaction of pathogen with month in lactation and lactation number to obtain milk production and LS lactation curves per pathogen and per parity group (cows versus heifers).

3. Results

The infection status of within herd prevalence of CNS in all sampled cows per herd is shown in Fig. 1. Approximately 9% of all cows were infected with CNS. At herd level, an average of 15% (S.D. 12%) of cows sampled were infected with CNS, ranging between 0 and 100%. Average within herd prevalence of cows with a CNS IMI and SCC over 200,000 cells/ml was 2% (S.D. 4%) and a range of 0–50%. Descriptive statistics of sample numbers, LS and SCC with their standard deviation for the most prevalent pathogens are shown in Table 1. The results showed three distinct populations of infection status in cows: negative cultures with the lowest SCC, CNS and *Corynebacterium bovis* with a moderate increase in SCC and *S. agalactiae*, *Streptococcus* spp. and *S. aureus* with an important increase in SCC.

The mixed model analysis showed a statistically significant and important contribution of all pathogens to the total LS variability ($P < 0.05$). Least square means of the most prevalent pathogens by month in milk are shown in Fig. 2. Infections with CNS showed a larger increase in LS in heifers compared to cows. This was mostly because culture-negative heifers had a lower LS compared with older culture-negative cows. The resulting impact of CNS IMI was then larger in heifers relative to cows, even though the absolute LS for CNS infected cows was not much different compared to that of heifers (Fig. 2).

The linear mixed model with milk production per day as outcome variable showed that CNS infected cows had a slightly higher milk production (0.45 kg/day, S.D. 0.12, $P < 0.001$) when compared to culture-negative cows. In contrast, all major pathogens showed a significant decrease in milk production per day. The loss per day was 3.6 kg/day (S.D. 0.12, $P < 0.001$) for *S. agalactiae*,

![Fig. 1. Prevalence of coagulase-negative staphylococci intramammary infection within herd. Number of herds is shown in classes with a width of 5 percentage points.](image)

![Fig. 2. Least square means linear score of somatic cell count by pathogen for heifers (top) and cows (bottom). Least square means were calculated from a mixed model linear regression of LS. Herd was treated as a random effect; days in milk (categorized by month in lactation), lactation number (heifers versus cows), pathogen code were included as fixed effects.](image)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Heifers</th>
<th>Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>LS</td>
</tr>
<tr>
<td>Negative</td>
<td>54,947</td>
<td>2.29</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>667</td>
<td>4.85</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>2,623</td>
<td>4.64</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2,756</td>
<td>4.63</td>
</tr>
<tr>
<td><em>Corynebacterium bovis</em></td>
<td>2,803</td>
<td>3.01</td>
</tr>
<tr>
<td>Coagulase-negative <em>Staphylococci</em></td>
<td>13,926</td>
<td>3.06</td>
</tr>
</tbody>
</table>
1.6 kg/day (S.D. 0.18, \(P < 0.001\)) for *Streptococcus* spp., and 1.8 kg/day (S.D. 0.18, \(P < 0.001\)) for *S. aureus* infected cows. Predicted least square mean lactation curves for each pathogen are shown in Fig. 3.

Table 2

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>BMSCC &lt; 200,000</th>
<th>BMSCC &lt; 400,000</th>
<th>BMSCC &gt; 400,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean S.D.</td>
<td>Minimum Maximum</td>
<td>Mean S.D.</td>
</tr>
<tr>
<td>Negative</td>
<td>41.4 20.0 0.45</td>
<td>95.6</td>
<td>26.6 19.6 0.04</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>8.3 10.2 0.02</td>
<td>38.8</td>
<td>18.2 17.4 0.0</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>10.8 11.1 0.11</td>
<td>54.9</td>
<td>14.2 13.2 0.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12.8 12.4 0.03</td>
<td>65.7</td>
<td>15.2 17.0 0.0</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>17.9 13.4 0.14</td>
<td>72.2</td>
<td>7.9 9.1 0.0</td>
</tr>
<tr>
<td><em>Corynebacterium bovis</em></td>
<td>7.1 9.7 0.02</td>
<td>41.7</td>
<td>8.6 10.7 0.01</td>
</tr>
</tbody>
</table>

In Table 2, descriptive statistics of percent contribution to the herd BMSCC per pathogen are presented, divided into three BMSCC classes: less than 200,000, between 200,000 and 400,000 and herds with BMSCC greater than 400,000 cells/ml. The percentage contribution of CNS infections to BMSCC was 17.9% in herds with a BMSCC less than 200,000 cells/ml. This contribution of CNS IMI decreased to 11.9 and 7.9% in herds with BMSCC between 200,000 and 400,000 and greater than 400,000 cells/ml, respectively. Noticeably, standard deviations of these values were low in herds over 200,000 cells/ml compared to the contributions due to major pathogens. This would indicate that rarely, CNS IMI are responsible for a very large proportion of BMSCC in herds with higher cell counts; while this would be more common with major pathogens.

Bulk milk SCC was plotted against the total percentage contribution of CNS infected cows in Fig. 4. The shaded area identifies the herds where CNS IMI contribute at least 10% of all cells in the bulk tank and would make the difference between a BMSCC over 400,000 cells/ml and a count below that cut-off point. This cut-off point was chosen because of European milk quality regulations. Approximately 2% of herds (86 of 4200) potentially have had high SCC problems (>400,000 cells/ml in bulk milk) that may be contributed in part to CNS IMI. These herds are represented in the

Fig. 3. Estimated least square milk production curve of cows that are infected with one of four individual pathogens or cows that were culture-negative. Least square means were calculated from a mixed model linear regression of milk-production. Herd was treated as a random effect; days in milk (categorized by month in lactation) and pathogen code were included as fixed effects.

Fig. 4. Bulk milk somatic cell count (BMSCC; *1000 cells/ml*) vs. contribution of somatic cell count to bulk milk of cows with coagulase-negative staphylococci intramammary infection. Each data point in the graph reflects a herd. The shaded area identifies the herds where CNS infections contributed at least 10% of cells and were considered responsible for bulk milk SCC going over 400,000 cells/ml. The dotted lines indicate that a bulk milk SCC of 800,000 must have at least 50% contribution due to CNS to consider CNS infections responsible for going over 400,000 cells/ml. There are a total of 86 herds out of 4200 (2.1%) in the shaded area.
shaded area in Fig. 4. Even though CNS IMI played a contributing role in the increased BMSCC, all herds with these high BMSCC values still showed considerable percentages of cows infected with other (major) pathogens (results not shown).

4. Discussion

Our data indicated that CNS IMI in dairy cows were an important part of infection prevalence in dairy herds. Approximately 9% of cows included in our study were infected with CNS. Average within herd prevalence was approximately 15%. The difference between mean animal prevalence and mean herd prevalence indicates that small herds typically had higher prevalence. The impact of CNS IMI on cow level SCC was intermediate when compared to culture-negative animals and cows infected with major pathogens. Our data indicated that depending on stage of lactation and parity, cow level SCC increases between 0.5 and 1 LS point in CNS infected cows relative to culture-negative cows. These findings are in line with previous observations where CNS infected cows also showed a moderate increase in SCC (Lam et al., 1997).

Cows with CNS IMI have a slightly higher daily milk production when compared to culture-negative cows, and much higher daily milk production when compared to cows infected with major pathogens. These results are somewhat surprising, given the previously shown increase in LS and the general negative correlation between LS and milk production (Green et al., 2006). However, similar findings have been reported by Piepers et al. (2008) who observed that heifers infected with CNS in early lactation showed slightly higher milk production than culture-negative heifers. This Belgian study investigated a much smaller sample of cows, but had much more precise longitudinal data per animal. This increase in milk production is somewhat unexpected and certainly would need further understanding of potential biological phenomena. A potential mechanism might be in the protective effect of CNS on clinical mastitis occurrence (Lam et al., 1997). Although previous studies have not been conclusive on this protective effect, a decrease in clinical mastitis incidence would likely be associated with a reduced milk loss (Wilson et al., 2004) (observed as a moderate production increase compared to non-CNS infected cows). In this study and in many others, no species identification beyond the generic classification as CNS was attempted. This may results in a loss of precision with regard to virulence of individual species. It has been shown that some species have greater virulence characteristics such as invasion of epithelial cells compared to others (Almeida and Oliver, 2001). Also the presence or absence of virulence genes such as genes coding for antimicrobial resistance, biofilm formation, or cytotoxicity may determine the clinical presentation in the mammary gland. Further studies would be valuable to identify the potential differences between CNS species as it relates to mammary pathogenicity.

Because of the nature of our data, where a large number of herds was sampled completely, there was a unique opportunity to study the impact of CNS IMI on BMSCC (as measured by the weighted average SCC of all included cows). The contribution of CNS IMI to BMSCC decreased with increasing BMSCC. It was approximately 12% for herds with a BMSCC higher than 200,000 and lower than 400,000 cells/ml and only approximately 8% in herds with a BMSCC higher than 400,000 cells/ml. Probably, even more important was that the standard deviations of these percentages were relatively low (approximately 6 and 9%, respectively), indicating that a very high percentage contribution due to CNS IMI was seldom present. This finding was further illustrated when we plotted the contribution to BMSCC due to CNS IMI versus BMSCC. Typically, CNS IMI had a higher contribution in low BMSCC herds. In contrast, less than 2% of herds would be able to implicate CNS IMI as an important contribution to BMSCC levels higher than 400,000 cells/ml.

On the other hand, in herds striving for a low BMSCC, cows with CNS infections were a sizeable proportion of the issue to be resolved. Herds with BMSCC below 200,000 cells/ml had approximately 18% of cells being shed in CNS infected animals. As a group of organisms, CNS had a larger contribution than any of the individual major pathogens. Along the same lines, the proportion of somatic cells shed by CNS infected cows was approximately the same as the proportion of somatic cells by any of the major pathogens in the herds with a BMSCC between 200,000 and 400,000 cells/ml (Table 2). This effect was due to a much larger number of infected cows with CNS when compared to cows infected with major pathogens. Individual animals with CNS infections have much lower SCC compared to cows infected with major pathogens. Therefore, even though all CNS infected cows and all cows infected with major pathogens shed approximately the same total number of SCC, individual animal management decisions (such as treatment, segregation or culling) will be easier and more cost-effective in cows infected with major pathogens compared to cows infected with CNS. Still, at herd level the importance of CNS herds with lower BMSCC cannot be denied.

The data we analyzed have important advantages and disadvantages. Obviously the large size of the data provided an amazing population overview of the importance of CNS IMI. A large variety of herds in all ranges of BMSCC values were observed in detail. Moreover, the large data set allowed us to select only cows with a single organism in their composite milk and discard any animal with multiple pathogens or contaminated samples. Infection status and SCC were observed in the co-mingled milk, making these values strongly associated since both were measured at cow-level. An important disadvantage of these data lies in the very same issue: cow-level infection status was obtained by using composite milk samples. This provides a composite measurement of infection status of the four quarters combined, but no specific information on the individual quarter. The same is true for SCC data, and combining the two pieces of information may introduce potential ecological fallacy (Schukken et al., 1988): an increase in SCC may be due to one-quarter, while this quarter may or may not be infected with a specific pathogen. Analyzing data at cow-level would correlate these findings and may potentially lead to erroneous
causal conclusions. We believe that this error is minimized because we only included cows with one pathogen cultured in co-mingled milk. Cows with multiple quarters infected with multiple pathogens would be much more likely to such causation errors. Sensitivity of composite samples may be lower when compared to sensitivity of quarter samples. This would lead to a misclassification of IMI as culture-negative, reducing the apparent difference in SCC and/or milk production between cows with an IMI and culture-negative cows.

Some important conclusions would be justified from our data. First CNS infected cows did show an increase in SCC, but this increase is relatively small when compared to cows infected with major pathogens. Obviously, herds striving for the very best SCC may still consider these CNS infections to be associated with an unwarranted increase in their BMSCC. The second conclusion would be that CNS infected cows did not show a reduction in milk production compared to culture-negative cows, despite and increase in SCC. Thirdly, our population data allowed us to evaluate the importance of SCC infections as a contribution to BMSCC. Our data indicated a decrease in the contribution of CNS infected cows to BMSCC with increasing BMSCC. Only very rarely did we observe herds where high BMSCC levels could be contributed predominantly to cows with CNS infections. Further investigations into these unusual herds where CNS infections contributed significantly to BMSCC would be very valuable. These herds may show a specific pattern of infection or CNS species that might be considerably different when compared to the vast majority of herds. Precise molecular diagnostic techniques would be important in investigations to be undertaken in these herds (Zadoks and Schukken, 2006). These outliers may be of value to our understanding of the pathological potential of CNS infections.

5. Conclusions

Intramammary infections with CNS resulted in a moderate increase in SCC. CNS infections were relatively more important in heifers when compared to cows. The difference in SCC between CNS infected animals and culture-negative animals was larger in heifers than in cows. Daily milk production was somewhat, but statistically significant, higher in CNS infected cows compared to culture-negative cows. Very few herds with milk quality problems had an important increase in BMSCC that could be attributed mostly to CNS infections. On the other hand, in herds with low BMSCC, CNS infections may be an important contributor to the total number of somatic cells in bulk milk.

Conflict of interest

None of the authors (Y.H. Schukken, R.N. González, L.L. Tikofsky, H.F. Schulte, C.G. Santisteban, F.L. Welcome, G.J. Bennett, M.J. Zurakowski, R.N. Zadoks) has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the paper entitled “CNS mastitis: nothing to worry about?”.

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