Staphylococcus aureus and Streptococcus dysgalactiae
in Norwegian herds after introduction of selective
dry cow therapy and teat dipping

Anne Cathrine Whist1,2*, Olav Østera˚s1,2 and Liv Sølverød2,3

1 Department of Production Animal Clinical Sciences, Norwegian School of Veterinary Science, PO Box 8146 Dep., 0033 Oslo, Norway
2 Department of Norwegian Cattle Health Services, TINE Norwegian Dairies, PO Box 58, 1431 Ås, Norway
3 Mastitis Laboratory, TINE Norwegian Dairies, Fannestrandvegen 55, 6415 Molde, Norway

Received 24 February 2006 and accepted for publication 19 May 2006

The objective was to promote a reduction in the prevalence of Staphylococcus aureus and Streptococcus dysgalactiae after 2 years of selective dry cow therapies and teat dipping/external teat sealant implementation. Three different dry cow treatments, one long-acting and two short-acting penicillin-based products were tested at herd level together with a negative control teat dipping group, an iodine teat dipping group and DryFlex™, an external teat sealant. The regimes were independently randomly allocated to 178 dairy herds. Yearly bacteriological quarter milk samples were collected from all cows at the beginning of the trial, after 1 year and 2 years. At herd level, a total of 15% of the herds showed no Staphylococcus aureus isolates after 2 years, compared with 5% at the beginning. The distribution of Streptococcus dysgalactiae infected herds remained the same after 2 years. At cow level, there were no significant differences in the reduction of Staph. aureus between the different dry cow therapies and teat dipping regimes. But there was a significant reduction of Str. dysgalactiae in the iodine teat dipping group compared with Dryflex™ and the negative control group. The proportional rate of Staph. aureus positive quarters was reduced from 65.9% to 54.9% after 2 years. As for Str. dysgalactiae, an increase was observed from 14.2% to 15.2%.

Keywords: Herd prevalence, Staph. aureus, Str. dysgalactiae, teat dipping, selective dry cow therapy.

Total dry cow therapy is one part of a total management system recommended as an important tool in the Five-Point Mastitis Control Plan (Kingwill et al. 1970) to reduce the level of intramammary infections (IMI) during the beginning of the dry period. The use of total dry cow therapy may result in an overuse of antibiotics and selective dry cow therapy programmes have been introduced where the goal has been to optimize treatment of infected cows while minimizing treatment of healthy cows. Nordic countries use selective dry cow therapy as one part of their national mastitis control programmes since it has a well-documented effect (Østera˚s et al. 1999; Berry & Hillerton, 2002). The chosen selective procedure is scientifically based on trials conducted in Norway and the Netherlands (Østera˚s et al. 1991; Sol et al. 1997; Østera˚s et al. 1999; Østera˚s & Edge, 2000). Since only 2-3% of the Staphylococcus aureus isolates, at cow level, are reported to be penicillin resistant, penicillin would be the drug of choice in Norway (Norwegian Cattle Health Service, Annual Report 2004). The overall goal is to treat cows that are expected to respond to treatment and to cull non-responders based on prediction models put forward by Østera˚s et al. (1999).

An alternative to dry cow therapy is an internal or an external teat sealant used alone or in combination with dry cow therapy. DryFlex™, an external teat seal consists of a physical barrier covering the teat canal. Closing of the teat canal could potentially reduce the amount of pathogens during the dry period and thus reduce the incidence of IMI during the next lactation if the IMI occurs at the time the teat seal is applied.

Teat dipping with a germicidal solution immediately after every milking is another recommended part of a total management system (Kingwill et al. 1970) and is regarded as the single most effective practice for the prevention and control of clinical IMI in lactating dairy cows (Sheldrake & Hoare, 1980; Pankey, 1984; Philpot & Pankey, 1978).
Table 1. Descriptive information about the 178 participating herds before entering the selective dry cow therapy and teat dipping trial

<table>
<thead>
<tr>
<th>n of herd</th>
<th>Mean n of cows per herd (± SD)</th>
<th>Mean BMSCC† per herd (± SD)</th>
<th>Median culling rate per cow per year (25/75% percentile)</th>
<th>Median incidence of CM per cow-year (25/75% percentile)</th>
<th>n of DCT§ cows in the trial</th>
<th>Previous use of teat dips, all kinds</th>
<th>Previous DCT registered</th>
</tr>
</thead>
<tbody>
<tr>
<td>No dip/Juvanesta</td>
<td>28</td>
<td>20 (±7.9)</td>
<td>118 000 (±43 000)</td>
<td>0.05 (0–0.10)</td>
<td>0.27 (0.16–0.47)</td>
<td>108</td>
<td>3 herds</td>
</tr>
<tr>
<td>Iodine/Juvanesta</td>
<td>16</td>
<td>18 (±6.1)</td>
<td>120 000 (±37 000)</td>
<td>0.07 (0.04–0.12)</td>
<td>0.35 (0.22–0.45)</td>
<td>50</td>
<td>2 herds</td>
</tr>
<tr>
<td>DryFlex™/Juvanesta</td>
<td>18</td>
<td>22 (±9.6)</td>
<td>132 000 (±38 000)</td>
<td>0.05 (0–0.11)</td>
<td>0.23 (0.16–0.53)</td>
<td>73</td>
<td>2 herds</td>
</tr>
<tr>
<td>No dip/Siccalactin</td>
<td>22</td>
<td>24 (±9.6)</td>
<td>103 000 (±37 000)</td>
<td>0.07 (0–0.13)</td>
<td>0.22 (0.14–0.37)</td>
<td>115</td>
<td>5 herds</td>
</tr>
<tr>
<td>Iodine/Siccalactin</td>
<td>15</td>
<td>23 (±7.7)</td>
<td>117 000 (±21 000)</td>
<td>0.06 (0–0.16)</td>
<td>0.27 (0.15–0.43)</td>
<td>71</td>
<td>8 herds</td>
</tr>
<tr>
<td>DryFlex™/Siccalactin</td>
<td>23</td>
<td>18 (±5.0)</td>
<td>123 000 (±46 000)</td>
<td>0.08 (0.04–0.25)</td>
<td>0.34 (0.21–0.54)</td>
<td>136</td>
<td>3 herds</td>
</tr>
<tr>
<td>No dip/Mastipen</td>
<td>21</td>
<td>24 (±9.9)</td>
<td>115 000 (±36 000)</td>
<td>0.06 (0–0.12)</td>
<td>0.35 (0.21–0.50)</td>
<td>74</td>
<td>6 herds</td>
</tr>
<tr>
<td>Iodine/Mastipen</td>
<td>21</td>
<td>20 (±6.6)</td>
<td>123 000 (±43 000)</td>
<td>0.10 (0.06–0.15)</td>
<td>0.30 (0.21–0.43)</td>
<td>73</td>
<td>7 herds</td>
</tr>
<tr>
<td>DryFlex™/Mastipen</td>
<td>14</td>
<td>20 (±5.2)</td>
<td>123 000 (±39 000)</td>
<td>0.05 (0–0.08)</td>
<td>0.21 (0.09–0.28)</td>
<td>68</td>
<td>4 herds</td>
</tr>
</tbody>
</table>

† Bulk milk somatic cell count presented with SD
‡ Clinical mastitis
§ Dry cow therapy

Research on the efficacy of iodine post-milking teat disinfectants, published since 1980, contains seven trials (National Mastitis Council, 2004) conducted by natural exposure of the bacteria to the teat end (Bray et al. 1983; Eberhart et al. 1983; Nickerson et al. 1986; Oliver et al. 1991; Goldberg et al. 1994). Of the seven natural exposure trials, three trials have a positive control group containing Bovadine 1% iodine solution, which leaves us with four trials with a negative control group. All of these trials assessed a reduction of clinical IMI, but none of them contained Bovadine 1% iodine solution, which leaves us with four trials with a negative control group. All of these trials assessed a reduction of clinical IMI, but none of them is a large field trial where commercial dairy herds have used teat dipping product during complete lactations. There are few reports available showing the prevalence of Staph. aureus and Streptococcus dysgalactiae at herd level. Several studies were conducted at randomized cow or quarter levels within different herds. The problem with such studies is that the herd status is a product of each cow’s status. The effect of one therapy group within the same herd could influence the effect of another therapy group as there could be a possible interaction effect between individuals within the same herd. Applying a field trial at herd level avoids this problem. Österås et al. (2006) conducted a randomized survey throughout Norway in which Staph. aureus was isolated from 8.2% of the quarters while Str. dysgalactiae was isolated from 1.1% of the quarters. Pitkälä et al. (2004) presented a nationwide mastitis survey in 216 herds in Finland. Among all quarters with bacterial growth, Staph. aureus was isolated from 3.4% of the quarters and Str. dysgalactiae was isolated from 0.05% of the quarters.

In the Nordic countries, regular teat dipping and dry cow therapy have usually not been recommended. Thus, the fairly high prevalence of Staph. aureus and Str. dysgalactiae in Norway (Österås et al. 2006) could be explained by low implementation of dry cow therapy and teat dipping. The objective of the present study was to evaluate the effect of selective dry cow therapy and teat dipping/external teat sealant implemented at herd level measured as a decrease in the prevalence of Staph. aureus and Str. dysgalactiae.

Materials and Methods

Selection and randomization of the herds

We designed a longitudinal study where the population under study was stratified in six geographical areas in Norway. Our study population originally included 215 commercial Norwegian dairy herds. The first herds were enrolled in October 2002 and the last in May 2003. The descriptive data of the enrolled herds are presented in Table 1. The expected number of cows with Staph. aureus was estimated from the herd size together with the percentage of cows with a composite milk somatic cell count (CMSCC) >200 000 cells/ml (cell200) according to an equation generated from regression analyses of routinely mastitis samples in Norway during 1998:

\[
(37 \cdot 555 – 0.7985 \cdot \text{size} + 0.600853 \cdot \text{size} + 0.29688 \cdot \text{cell200} \cdot \text{size}) \quad \text{(Equation 1)}
\]

Randomization of selective dry cow therapy and teat dipping regime were conducted as a computerized systematic random assignment. All herds within each geographical area were sorted according to expected number of Staph. aureus positive cows (Equation 1). The first herd drew a starting position of A, B or C and was given the dry cow therapy regime A, B, or C accordingly. The next herd was given the next alphabetically ordered regime. The same procedure was followed for randomizing the different teat dipping strategies, but the herds were then sorted according to their size.
The inclusion criteria, at herd level, were: (1) herds had to be members of the Norwegian Dairy Herd Recording System (NDHRS), (2) the farmer was willing to use the selected dry cow therapy and teat dipping regime he/she used in his/her herd, (3) the farmer had to deliver milk to TINE BA (The Norwegian Dairies) during the study period, (4) the farmer must take CMSCC test days monthly during the study period, (5) the farmer had to implement the Nordic recommendations concerning milking routine described by Alfnes & Østera˚s (1992).

Exclusion criteria were: (1) the farmer withdrew from the study because he/she did not follow the protocol, (2) the farmer withdrew from the study if the local veterinarian suspected that the protocol was not being followed, or if 50% or more of the quarter milk samples were forgotten, (3) herds were withdrawn if co-ownerships were dissolved during the study period.

Selective dry cow therapy and treatment protocols

The protocol and sampling strategy were:

Cows with a geometric mean of the three last CMSCC test days $>$100,000 cells/ml prior to drying-off, and cows with a clinical mastitis history during the last lactation were sampled for bacteriological examination before drying-off.

Cows with positive Staph. aureus or Str. dysgalactiae isolates were treated at drying-off according to the herd’s given randomized regimen.

Cows with positive Staph. aureus or Str. dysgalactiae isolates prior to drying-off and a geometric mean of the three last CMSCC test days $>$600,000–700,000 cells/ml were assessed for culling after calving, no dry cow treatment were recommended.

The different dry cow therapies were:

A: Juvanesta® Comp. vet. A short acting antibiotic with 300 mg penethamate hydroiodide BAN (300 000 i.u. benzylpenicillin) and 300 mg dihydrostreptomycin sulphate (Boehringer Ingelheim Vetmedica AS). If one or two quarters were infected with Staph. aureus or Str. dysgalactiae, only these will be treated, if three or four quarters had infection, all four will be treated. The treatment was repeated four times with a 24-h interval.

Bacteriological examination of quarter milk samples

Annual quarter milk samples were taken for bacteriological examination at the beginning of the study period, and again after 1 year and 2 years. The two last annual samples were taken as close to 365 d as possible to avoid any seasonal effect as described by Østera˚s et al. (2006). The first annual samples were collected by the local veterinarians when they taught the farmers the aseptic milk sampling technique. The two last annual samples were then collected by the farmers. The samples were cooled in a refrigerator as soon as possible, at least within a few hours, after sampling and kept cooled until sending by mail as fast as possible to the mastitis laboratory. Samples arriving at the laboratory on Saturday were cooled at the laboratory until processing on Monday, thus avoiding an uncontrolled cooling chain for more than 24 h. Samples were analysed for bacterial growth on blood agar plate (Blood Agar Base, Oxoid Ltd, Basingstoke, UK) mixed with 5% washed bovine erythrocytes. Examination of bacterial growth and diagnostics followed the official Norwegian
procedure (National Veterinary Institute, 1993) and was in agreement with the recommendations of the International Dairy Federation (International Dairy Federation, 1981). Plates were divided into quarters with β-haemolytic Staph. aureus streak. Quarter foremilk (0.01 ml) was streaked out on each quarter before incubation at 37 °C±1 °C for 18–24 h. Typical colonies for Staph. spp. producing a typical β-haemotoxic zone was classified as Staph. aureus. Combined growth of Staph. aureus and Str. dysgalactiae was classified separately. Other colonies of Staph. spp. were classified as coagulase positive or negative using Prolex™ Staph Latex Kit (PRO-LAB Diagnostics, Toronto, Canada). Coagulase positive isolates were diagnosed as Staph. aureus. Str. dysgalactiae were classified based on colony morphology, haemolytic properties, CAMP reaction and fermentation of aesculine. They were grouped according to Lancefield’s grouping system.

Data analysis

Herd level. From the annual samples, the herd prevalence of Staph. aureus or Str. dysgalactiae was calculated as the number of cows with these isolates in one or more quarters divided by the total number of cows sampled in the herd (Dohoo et al. 2003). Data were compiled using SAS software (version 8e). Multivariable models were constructed using PROC MIXED procedures with herd as the repeated observation. In herd model 1, the herd prevalence of Staph. aureus or Str. dysgalactiae each year was applied as dependent variable. A compound symmetry matrix was chosen according to the –2 log likelihood assessment of model fit after having explored the results of the autoregressive matrix, the compound unstructured matrix and the Toeplitz matrix. The independent fixed effects in the model were dry cow therapy (A, B or C), teat dipping regime (A, B or C), the annual sampling in the trial (0, 1, 2), interaction between dry cow therapy regime and annual sampling and the interaction between teat dipping and annual sampling. The interaction variables were always included in the model as long as dry cow therapy or teat dipping was included. These variables made sure that we analysed for the associations during the trial compared with the starting point. In herd model 2, separate models were made where the prevalence of Staph. aureus or Str. dysgalactiae after 1 year and 2 years were used as dependent variable. In these separate models, the prevalence of Staph. aureus or Str. dysgalactiae at the beginning of the study were used as independent fixed effects together with the interactions between this prevalence and both the dry cow therapy and the teat dipping regime. For herd models 1 and 2, the independent variables in the full models were excluded one by one with backward elimination procedure until all remaining variables were assessed as being significant or $P<0.10$.

In herd model 3, we checked for the differences in the random HERDID effect within each teat dipping group. We used separate PROC MIXED models for each teat dipping group where annual sampling 1 and 2 were used as independent variables and HERDID as random effect.

Cow level. Dry cows and cows with missing samples were not included in the analysis. A positive Staph. aureus or Str. dysgalactiae cow was assessed as having an isolate of Staph. aureus or Str. dysgalactiae (1) if the bacteria were isolated from one or more quarters. Cows were classified as negative (0) if none of the quarters had any such isolate. The same procedure was used for Str. dysgalactiae. Multivariable models were fitted separately with Staph. aureus or Str. dysgalactiae positive cows or not as dependent variables. PROC GENMOD with binomial distribution and logit link function with Walds’ statistics for Type 3 contrasts was used to assess significant contribution for each independent fixed effect.

The full model consisted of these independent fixed variables: parity (class 1, 2, 3 and >3), sine and cosine of calving day to evaluate any seasonal effect as described by Østérås et al. (2006), the natural log of days in milk (lnDIM), dry cow therapy group (A, B or C), teat dipping group (A, B or C), yearly sampling number (0, 1 or 2), days from sampling to receipt at the mastitis laboratory (0, 1, 2, 3 or >3), geographical area (A, B, C, D, E or F) and finally the interaction between the teat dipping groups and the annual sampling. DIM was also tested in the model, instead of lnDIM, but lnDIM gave a better fit of the model. Cow identity (COWID) was included as a random effect nested within the herd identity (HERDID). The random effect was expressed as the log (OR) by using the LOGOR statement in the model as alternative logistic regression (Carey et al. 1993). Goodness of fit was assessed with deviance or Δ deviance in the different models. Variables were assessed as significant when $P<0.05$. The independent fixed effects in the full models were excluded one by one from the model with backward elimination procedure until all remaining variables were assessed as being significant or $P<0.010$.

Results

Of the 215 herds initially enrolled, 178 herds remained in the study after 2 years. Twelve farmers withdrew from the study because they did not manage the protocol or they suffered some accidents or force majeure outside this project. Another 23 farmers withdrew because their local veterinarian suspected that the protocol was not being followed or 50% or more of the milk samples were forgotten. Two farmers withdrew because their co-ownerships were dissolved during the study period. The distribution of the dry cow therapies and teat dips, together with herd descriptions are given in Table 1.

Results of the bacteriological findings, at quarter level, are summarized in Table 2. At quarter level, the proportional ratio of the different pathogens changed, with
less *Staph. aureus*, the same level of *Str. dysgalactiae* and an increased level of *Str. uberis* and coagulase negative staphylococci. There were no differences between the different dry cow therapy and teat dipping groups.

**Herd level**

The distribution of herd prevalence of *Staph. aureus* infected cows changed during the study period (Fig. 1). The prevalence of *Staph. aureus* at herd level, adjusted for the herd effect was 0.18 (±0.009) at the beginning, 0.14 (±0.009) after 1 year and 0.13 (±0.009) after 2 years. Altogether 19% of the variation in the herd level prevalence of *Staph. aureus* could be attributed to the herd. In the 178 herds, 15% of the herds showed no *Staph. aureus* positive cows after two years compared with 5% at the beginning. The distribution of herd prevalence of *Str. dysgalactiae* isolates at the beginning (year 0), after 1 year and after 2 years in the trial. The PROC MIXED herd model 1, with repeated measurements at herd level, revealed that there were no significant effects of either the different dry cow therapies, teat dipping regimes or any of the interactions between these two independent fixed effects and the annual sampling in the study. There were no significant associations between any of the independent fixed effects and the *Str. dysgalactiae* herd prevalence. In this model, 20% of the variation in the *Str. dysgalactiae* herd prevalence could be attributed to the herd.

In herd model 2, where *Staph. aureus* prevalence after 1 year and 2 years were used as the dependent variable and the *Staph. aureus* prevalence at the beginning of the study was used as independent variable, the only significant effect was the *Staph. aureus* prevalence at the beginning of the trial (b=0.14±0.05). There was no significant effect of the different dry cow therapies or teat dipping regimes. However, in this model, 24% of the variation in the *Staph. aureus* herd prevalence could be attributed to the herd. Where the prevalence of *Str. dysgalactiae* after 1 and 2 years was used as a dependent
variable, the only significant independent fixed effect identified was the prevalence of \textit{Str. dysgalactiae} at the beginning of the study (\(b=0.27\pm0.07\)).

Herd model 3, the separate PROC MIXED models for each of the teat dipping group, revealed a herd effect of 38\% for group A (control), 28\% for group B (iodine) and 11\% for group C (DryFlexTM) for \textit{Staph. aureus} and 12\% for group A, 0\% in group B and 22\% in group C for \textit{Str. dysgalactiae}.

Cow level
At cow level, there were no significant differences in the reduction of \textit{Staph. aureus} between the different teat dipping regimes (Fig. 2). In the PROC GENMOD models, the classification ‘\textit{Staph. aureus} cow’ or not, from the annual sampling 1 and 2, was used as dependent variable. The only significant independent fixed effect was lnDIM (Table 3). In the same model, but with ‘\textit{Str. dysgalactiae} cow’ or not as dependent variable, there was a significant effect of parity, lnDIM, teat dipping (B vs. A or C) and some geographical areas. Results are presented in Table 3.

Discussion
We observed a reduction of \textit{Staph. aureus} after 2 years in the trial. This is due to the implementation of the selective dry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Estimate</th>
<th>se</th>
<th>OR† (95% CI)</th>
<th>P-value</th>
<th>Estimate</th>
<th>se</th>
<th>OR† (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>6776</td>
<td>-1.07</td>
<td>0.15</td>
<td>0.15</td>
<td>&lt;0.001</td>
<td>-4.24</td>
<td>0.37</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2861</td>
<td>-1.16</td>
<td>0.31</td>
<td>0.31 (0.21–0.45)</td>
<td>&lt;0.001</td>
<td>-0.09</td>
<td>0.91</td>
<td>(0.62–1.35)</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>1846</td>
<td>-0.34</td>
<td>0.71</td>
<td>0.71 (0.53–0.96)</td>
<td>&lt;0.05</td>
<td>-0.09</td>
<td>0.20</td>
<td>(0.62–1.35)</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>1123</td>
<td>-0.09</td>
<td>0.91</td>
<td>0.91 (0.62–1.35)</td>
<td>NS</td>
<td>-0.09</td>
<td>0.20</td>
<td>(0.62–1.35)</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;3</td>
<td>946</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>NS</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>lnDIM‡</td>
<td>6776</td>
<td>-0.17</td>
<td>0.03</td>
<td>0.84 (0.80–0.89)</td>
<td>&lt;0.001</td>
<td>-0.15</td>
<td>0.06</td>
<td>0.86 (0.77–0.97)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Fixed

<table>
<thead>
<tr>
<th>Teat dipping</th>
<th>n</th>
<th>Estimate</th>
<th>se</th>
<th>OR† (95% CI)</th>
<th>P-value</th>
<th>Estimate</th>
<th>se</th>
<th>OR† (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Negative)</td>
<td>2913</td>
<td>0.43</td>
<td>0.18</td>
<td>1.54 (1.08–2.19)</td>
<td>&lt;0.05</td>
<td>0.43</td>
<td>0.18</td>
<td>1.54 (1.08–2.19)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B (iodine)</td>
<td>1842</td>
<td>0.59</td>
<td>0.20</td>
<td>1.80 (1.22–2.67)</td>
<td>&lt;0.01</td>
<td>0.59</td>
<td>0.20</td>
<td>1.80 (1.22–2.67)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C (DryFlex™)</td>
<td>2021</td>
<td>0.90</td>
<td>0.29</td>
<td>2.46 (1.39–4.34)</td>
<td>&lt;0.01</td>
<td>0.90</td>
<td>0.29</td>
<td>2.46 (1.39–4.34)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Geographical areas

| A                  | 1367| 1.20     | 0.27| 3.32 (1.96–5.64) | <0.001  | 1.20     | 0.27| 3.32 (1.96–5.64) | <0.001  |
| B                  | 1445| 1.13     | 0.28| 3.10 (1.79–5.36) | <0.001  | 1.13     | 0.28| 3.10 (1.79–5.36) | <0.001  |
| C                  | 1343| 0.44     | 0.34| 1.55 (0.80–3.02) | NS      | 0.44     | 0.34| 1.55 (0.80–3.02) | NS      |
| D                  | 739 | -0.87    | 0.47| 0.42 (0.17–1.05) | NS      | -0.87    | 0.47| 0.42 (0.17–1.05) | NS      |
| E                  | 656 | 0       | 0    | 1            | NS      | 0       | 0    | —            |         |
| F                  | 1226| 0.92     | 0.18| 2.51 (1.76–3.57) | <0.001  | 0.92     | 0.18| 2.51 (1.76–3.57) | <0.001  |

Random§

| Within cow         |     | 0.18   | 2.51 (1.76–3.57) | <0.001  | 0.18   | 2.51 (1.76–3.57) | <0.001  |
| Within herd        | Alpha 1 | 0.21  | 0.04 | 1.23 (1.14–1.33) | <0.001  | 0.21  | 0.04 | 1.23 (1.14–1.33) | <0.001  |

† Odds ratio with 95\% confidence intervals
‡ natural log of days in milk, mean days in milk 130 d ±92
§ Number of clusters =178, minimum cluster size 9; maximum cluster size 94

![Fig. 2. Crude cow level proportion with confidence interval, \textit{Staphylococcus aureus} or \textit{Streptococcus dysgalactiae} positive cows divided by total number of sampled cows within each teat dipping regimes at the beginning (year 0), after 1 year and after 2 years in the trial.](image-url)
cow therapy protocol and the selective culling strategy of cows with a long history of high CMSCC. This is in accordance with the results of Østeraås et al. (1991) who conducted a similar study where a negative control group was included. They found a significant decrease in subclinical Staph. aureus mastitis in cows using dry cow therapy compared with a negative control group. They used the same dry cow therapy formula as Juvenasta (300 000 i.u. benzylpenicillin and 300 mg dihydrostreptomycin) which served as a positive control in our trial, and justifies the lack of a negative control (Dohoo et al. 2003).

The reason why we did not observe a reduction in Str. dysgalactiae after 2 years is unknown and was unexpec-
ted. We should expect a therapeutic effect of the three different selective dry cow therapies close to 100%, which would be in accordance with Østeraås et al. (1991). Str. dysgalactiae mastitis is an emerging problem in Norway, causing more clinical and subclinical mastitis than in previous years (Norwegian Cattle Health Service, Annual Report 2004). Little research has been conducted on Str. dysgalactiae, a microbe which may be contagious in some dairy herds and environmental in other herds (International Dairy Federation, 1999).

Our present results demonstrate that neither iodine dip nor DryFlex™ had any effect on reducing the prevalence of Staph. aureus or Str. dysgalactiae at herd level. At cow level, we found a significantly higher odds ratio (OR) of Str. dysgalactiae positive cows when comparing the DryFlex™ group with the iodine group (OR=1·5, CI 95% 1·1 to 2·2) and the negative control with the iodine group (OR=1·8, CI 95% 1·2 to 2·7). The lack of improvement in prevalence and in occurrence of Staph. aureus at cow level are contradictory to other iodine teat dipping trials, at cow or quarter level, where udders have been either experimentally or naturally exposed to Staph. aureus. One explanation for these results could be that the present trial was conducted at herd level in 178 commercial dairy farms where other herd effects may play an important role in reducing the prevalence of Staph. aureus. A DryFlex™ trial is not approved by the NMC protocol, and as far as we are concerned, DryFlex™ has never been tested in large field trials where the included herds have a high proportion of Staph. aureus and Str. dysgalactiae isolates and the reason for including DryFlex™ was to see whether it had an impact on Staph. aureus and Str. dysgalactiae infected herds. There have been many efficacy studies conducted on DryFlex™ in research herds, but very few with published data and only one trial mentions Staph. aureus, which was a natural exposure trial conducted in Argentina. The results showed that DryFlex™ was especially effective against environmental streptococci and coliforms, but no differences were observed between the two groups in reducing Staphylococcus aureus infections (Corbellini et al. 2002).

The significant variables in the PROC GENMOD models (at cow level) were lnDIM (Staph. aureus model) and lnDIM and some geographical areas (Str. dysgalactiae). The choice of lnDIM, instead of DIM, was based on better fit of the model using lnDIM, which tells us that both Staph. aureus and Str. dysgalactiae positive isolates are associated with samples taken close to calving compared with later in the lactation. These findings are partly in accord with Østeraås et al. (2006) who found more Staph. aureus positive samples after calving compared with late lactation, but Str. dysgalactiae positive isolates increased in late lactation. Our results also tell us that there are differences between the different geographical areas with regard to the prevalence. The farmers and the veterinarians’ effort and expertise in taking samples, treating and culling correct cows are one factor influencing these differences.

The effect of teat dipping over time was carefully looked into by including the interaction between teat dipping and the two annual samplings in herd model 1. It is important to look at the interaction between the prevalence and the teat dipping over time since this interaction will illustrate any increase or decrease related to teat dipping as the trial progresses. The effect of dry cow therapy over time was not significant and was excluded from the model after dry cow therapy was excluded. Since the prevalence of Staph. aureus and Str. dysgalactiae differed between the three teat dipping groups at the beginning of the trial (Fig. 2), the first annual sampling (year 0) was included as an independent variable in separate models to adjust for different starting positions.

In herd model 3, the random herd effect of Staph. aureus seems to be higher for the control group and lower for the DryFlex™ group. Higher random herd effects demonstrate a higher correlation between the herd prevalence in year 1 and year 2 in the trial. For Str. dysgalactiae, this clustering turned out to be zero in the iodine group. This means there is no correlation between the prevalence in the herds in year 1 and in year 2, which indicates that it would be difficult to control Str. dysgalactiae in these herds with current knowledge as the mean prevalence stays constant.

In the present study, the herd effect was taken care of by randomly allocating the herds independently into the different dry cow therapies or teat dipping regimes. However, herd level studies could be biased by any herd effect. In statistical analysis at cow level, the herd effect may overestimate the significance of the responsible variable as the variation usually will be underestimated (McDermott et al. 1994). It is therefore important to include this effect as a random term.

The present results give no indication that the prevalence of Staph. aureus in Norwegian herds could be significantly decreased by a general recommendation for teat dipping with iodine or application of DryFlex™ as done in this study. However, they do indicate that iodine teat dipping could be beneficial in herds with a high prevalence of Str. dysgalactiae positive cows as demonstrated by a significant effect of iodine teat dip at cow level. The zero cluster effect at herd level for iodine teat dip also indicates
that there are some additional new infection mechanisms that have to be controlled, despite using iodine teat dip.

We thank Boehringer-Ingelheim, VetPharma and DeLaval for their contribution with support of free intramammarys and teat dips. The access to the data was given by the Norwegian DHRS and the Norwegian Cattle Health Services (for health data) in agreement number 4/1998. The study was financially supported by grants from the Research Council of Norway.

References

Alfnes T & Østera˚s O 1992 Milking and milking management. Landbruksforlaget, Oslo


Kingwill RG, Neave FK, Dodd FH, Griffin TK & Westgarth DR 1970 The effect of a mastitis control system on levels of subclinical and clinical mastitis in two years. Veterinary Record 87 94–100

McDermott JJ, Schukken YH & Shoukri MM 1994 Study design and analytic methods for data collected from clusters of animals. Preventive Veterinary Medicine 18 175–191


National Veterinary Institute 1993 Laboratory routines for mastitis diagnostics at the State Veterinary Laboratories. Oslo


Østera˚s O, Solderod L & Relsen O 2006 Milk culture results in a large Norwegian survey – effect of season, parity, days in milk, resistance and clustering. Journal of Dairy Science 89 1010–1023

Østera˚s O & Edge VL 2000 Factors prior to dry period associated with high and low levels of cow milk somatic cell counts in next lactation. Acta Veterinaria Scandinavica 41 63–77

Østera˚s O, Edge VL & Martin SW 1999 Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. Journal of Dairy Science 82 1221–1231


