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MASTITIS THERAPY: CAN WE IMPROVE EFFICACY?

JOHN W. HALLBERG, D.V.M., PhD., Pharmacia & Upjohn Animal Health, Kalamazoo, MI, USA

SUMMARY

Extended therapy, both 3X duration and 8X, with pirlimycin significantly increases the treatment efficacy and significantly decreases SCC following the treatment of cows with induced Staphylococcus aureus mastitis. This model study confirms field observations and results with 3X duration therapy. Daily therapy (8X) provides better efficacy than the 3X duration, as the milk concentration of pirlimycin is more constant during the entire treatment period. Extended therapy does not alter the milk discard period after the last treatment but it will lengthen the pre-slaughter withdrawal period for pirlimycin. For chronic infections, extended therapy with pirlimycin offers an alternative treatment regime that will significantly increase bacterial cure rates.

INTRODUCTION

Mastitis is a continuing problem on all dairy farms. It results in billions of dollars of lost milk production, production of lower quality milk, culled cows, and treatment costs. Mastitis is caused by a diverse number of mastitis pathogens that can present many different clinical presentations to the dairy producer and veterinarian. These presentations range from sub-clinical mastitis with normal appearing milk to severe clinical mastitis.

Treatment of mastitis has centered on the application of antibiotics to the cow via intramammary infusion (IMM) or parenteral injection (intramuscular, subcutaneous, or intravenous) infusion. At the 3rd International Mastitis Seminar in Tel Aviv in 1995, Dr Ziv noted that “very little progress was made during the last ten years towards solving some of the basic problems associated with antimicrobial treatment of mastitis, i.e. the low cure rate for clinical and sub-clinical S. aureus infection” (1). In review of the literature from the previous 10 years, Dr Ziv noted that the literature, “repeatedly dealt with the possible reasons for failure to eliminate intramammary infection (IMI), the interaction between infection and inflammation as a complicating factor in antimicrobial therapy, justification for antimicrobial use in the treatment of acute coliform mastitis, rational for treatment of sub-clinical mastitis during lactating and dry cow therapy and assessment of treatment efficacy.” So after many years of research and many new therapies, the cure for mastitis has not been identified. Several potential causes for treatment failure include: failure of the antibiotic to gain contact with the pathogen, use of the wrong antibiotic, presence of a resistant organism, or inadequate treatment dose or duration.

A logical starting place is the proper use of antibiotic including dose and duration of therapy for the treatment of mastitis. Each antibiotic family has a different mechanism of action. For example, β-lactam antibiotics (penicillins/cephalosporins) inhibit cell wall synthesis. Sufficient concentration of these antibiotics must be in contact with the bacteria for a sufficient period of time to have the
maximum effect, time above the minimum inhibitory concentration (MIC). In contrast, the aminoglycosides must have a sufficiently high peak concentration for maximum activity (2). A specific example is pirlimycin hydrochloride. Pirlimycin is a lincosamidine antibiotic that is active against Gram positive mastitis pathogens such as *S. aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. It binds to the 50S ribosomal sub-unit of mRNA and inhibits protein synthesis. Pirlimycin is a bacteriostatic antibiotic. Pirlimycin’s efficacy is dependent on the length of time pirlimycin concentration in milk remains above the minimum inhibitory concentration of the susceptible bacteria (a time above MIC antibiotic). Data from multiple clinical field efficacy studies demonstrated that 50 mg of pirlimycin administered twice at a 24 hour interval into each affected quarter is effective for the treatment of clinical mastitis caused by Gram positive pathogens in lactating dairy cattle.

Following regulatory approval in the US and Canada, the efficacy for the treatment of cows infected *S. aureus* and *Str. uberis* has been examined. Timms (3) presented data from two herds that received recommended label therapy of pirlimycin in an attempt to “blitz” treat two poorly managed herds chronically infected with *S. aureus*. The bacteriological quarter cure rates were 9 and 12% for these two herds, but the quarter cure rate decreased from 33% at day 12 post treatment to 9% at day 40 post treatment in one herd. However, this herd had a 4% new infection rate. The decrease in cure rate has two potential causes. These may have been a high rate of quarter re-infection or pirlimycin therapy was initially effective but the duration of therapy was not long enough to eliminate the infection from these chronically infected cows.

Because pirlimycin is a bacteriostatic antibiotic and its efficacy is governed by the time its concentration remains above the MIC of the pathogen being treated, lengthening of therapeutic duration should increase pirlimycin’s efficacy. This is especially true for mastitis pathogens such as *S. aureus* and *Str. uberis* that become chronic infections. Label and extended duration therapy with pirlimycin were subsequently investigated by several researchers. This work is summarized in Table 1.

In an extended therapy regime where 50 mg of pirlimycin (one plastet) was administered per infected quarter twice at a 24 hour interval followed by a 36 hour discard period (4). At 48 hours after the last treatment, a second treatment regime was administered into all affected quarters followed by a second 36 hour discard period. Again at 48 hours after the second discard period, a third treatment regime was administered. This was called 3X duration treatment. This treatment regime was an attempt to extend treatment duration and increase efficacy without using the product in an extra label manner. By repeating label therapy three times after the label milk discard period, the therapeutic period of pirlimycin was extended to 8 days. Cows chronically infected with *S. aureus* were examined in this study. Quarter milk microbiology samples were collected prior to therapy, at each milking for the first 19 milkings during treatment and after treatment, and at days 14 and 28 after the last treatment. A cured quarter bacteriologically negative in samples taken on both days 14 and 28 post last treatment was defined as cured. In these chronically infected cows, 17 of 41 quarters (42%) were cured.
Previous use of label therapy of pirlimycin in similar cows cured 1 of 24 quarters (4.2%). This study provided the first demonstration that extension of therapy duration could significantly increase bacteriologic cure rate of cows chronically infected with *S. aureus*.

**Table 1. Summary of literature extended quarter cure rates**

<table>
<thead>
<tr>
<th>Author (Date)</th>
<th>Pirlimycin Treatment</th>
<th>Pathogen</th>
<th>Study Location</th>
<th>Quarter Cure Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timms (1995)</td>
<td>Registered Dose</td>
<td><em>S. aureus</em></td>
<td>Iowa</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>Registered Dose</td>
<td><em>S. aureus</em></td>
<td>Iowa</td>
<td>12%</td>
</tr>
<tr>
<td>Owens (1995)</td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Louisiana</td>
<td>42%</td>
</tr>
<tr>
<td>Owens (1997)</td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Louisiana</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Louisiana</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Louisiana</td>
<td>41.5%</td>
</tr>
<tr>
<td>Belschner (1996)</td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Missouri</td>
<td>47%</td>
</tr>
<tr>
<td></td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>California</td>
<td>6%</td>
</tr>
<tr>
<td>Catell (1997)</td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Colorado</td>
<td>40%</td>
</tr>
<tr>
<td></td>
<td>No Treatment</td>
<td><em>S. aureus</em></td>
<td>Colorado</td>
<td>1%</td>
</tr>
<tr>
<td>Catell (1999)</td>
<td>Registered Dose</td>
<td><em>Str. iberis</em></td>
<td>Colorado</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>No Treatment</td>
<td><em>Str. iberis</em></td>
<td>Colorado</td>
<td>39%</td>
</tr>
<tr>
<td>Timms (1998)</td>
<td>Registered Dose</td>
<td><em>S. aureus</em></td>
<td>Iowa</td>
<td>21%</td>
</tr>
<tr>
<td></td>
<td>Registered Dose</td>
<td><em>Str. iberis</em></td>
<td>Iowa</td>
<td>63%</td>
</tr>
<tr>
<td></td>
<td>Registered Dose</td>
<td><em>S. aureus</em></td>
<td>Iowa</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Iowa</td>
<td>50%</td>
</tr>
<tr>
<td>Timms (1999)</td>
<td>Registered Dose</td>
<td><em>Str. ag.</em></td>
<td>Iowa</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Registered Dose</td>
<td><em>Str. dysgalactiae</em></td>
<td>Iowa</td>
<td>86%</td>
</tr>
<tr>
<td></td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Iowa</td>
<td>85%</td>
</tr>
<tr>
<td>Keefe (1999)</td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Canada (PE1)</td>
<td>67%</td>
</tr>
<tr>
<td></td>
<td>No Treatment</td>
<td><em>S. aureus</em></td>
<td>Canada (PE1)</td>
<td>17%</td>
</tr>
<tr>
<td>Sears (1999)</td>
<td>3X Duration</td>
<td><em>S. aureus</em></td>
<td>Michigan</td>
<td>58%</td>
</tr>
</tbody>
</table>

In a Missouri herd using 3X treatment, 15 chronically infected *S. aureus* quarters from 12 cows were treated (5). The treated quarters had a 47% quarter cure rate. Somatic cell counts (SCC) were also determined for these cows. The SCC for 9 of 12 cows dropped dramatically from 1.2 million to 107,000 cells/ml at 4 weeks after the last treatment. The SCC from the other three cows that were treatment failures remained greater than 1 million cells/ml. In a 700 cow California herd that maintained a “Staph” string of 120 cows, 37 cows received 3X duration therapy. As in other herds, milk samples were collected at day 28 after the last treatment for bacteriology to define cure. In these 37 cows, only 5 cows were cured. SCC data were also examined. Pre-treatment SCC for all 37 cows was >1 million cells/ml. The SCC for cured cows dropped from to an average of 106,000 cells/ml. Twenty three of 37 cows ended the study with SCC <300,000 cells/ml.
Another study was conducted in a Colorado herd of 400 lactating cows where 30% of the cows were infected with *S. aureus*. For this study, 120 cows were randomly assigned to two treatment groups, no treatment and 3X duration treatment with pirlimycin. A composite aseptic milk sample was obtained from each cow pretreatment and at days 14, 28, and 48 following the last treatment. Cure was defined as the absence of *S. aureus* in all three post treatment samples. Bacterial cure rates were 1% for the non-treated control and 40% for the cows receiving the 3X duration treatment with pirlimycin. The herd SCC dropped to 300,000 cells/ml. It was noted that 47% of the cows with SCC <1,000,000 cells/ml prior to therapy became culture negative whereas only 28% of the cows with SCC >1,000,000 cells/ml became culture negative. If a SCC of 1,000,000 cells/ml is used as a chronicity threshold in this herd, than treatment of chronically infected cows was less likely to be successful.

Pirlimycin therapy used according to the label was examined in cows with *Str. uberis* mastitis in a 1700 cow herd in Colorado (8). Infected cows were assigned randomly to a treatment group receiving 50 mg of pirlimycin per affected quarter twice at a 24 hour interval or a control group that received no therapy. Milk samples were collected for bacteriology obtained prior to treatment and 14 days after treatment. Cure was defined as no growth in samples obtained 14 days after treatment. Quarter cure rates were 70% for the treated group and 39% for the non-treated control group and these cure rates are significantly different (P<0.05). Label pirlimycin treatment was very effective for the treatment of *Str. uberis* mastitis in this herd.

Owens (8) further examined 3X duration pirlimycin therapy in four additional herds. Milk samples were obtained prior to therapy and 14 and 28 days after the last treatment for determination of microbiologic status and SCC. Quarter cure rates were 42, 86, 26, 41.5% for the four herds. In this study, seven quarters were not treated and the quarter cure rate of these was 0%. After therapy, the average SCC was 465,000 cells/ml for cured quarters and 2,534,000 cells/ml for the failed quarters.

Timms (9) presented data from two Iowa herds with elevated somatic cell counts where pirlimycin therapy was examined. In the first herd, eight quarters in 7 cows were infected with *Str. uberis* and 19 quarters in 14 cows were infected with *S. aureus*. Cows were treated with the recommended label dose of pirlimycin. The quarter cure rates were 63% for the *Str. uberis* infected quarters and 21% for the *S. aureus* infected quarters. Exclusion of one chronically infected cow raised the *S. aureus* rate to 24%. In the second herd, 61 of 86 cows and 46% of the quarters in these cows were infected with *S. aureus*. Of these cows 46 cows and 123 quarters were treated. From this group, 63 quarters from 27 cows whose SCC had been >300,000 cells/ml for less than 60 days received pirlimycin at the label dose. The quarter cure rate was 3%. Sixty quarters of 19 cows received extended therapy. Of the cows receiving extended therapy, those cows with a SCC >300,000 cells/ml for fewer than 60 days had a 50% quarter cure rate. Chronically infected cows (SCC >300,000 cells/ml for <60 days) had a 6% quarter cure rate. This study demonstrates that the extension of therapy duration does significantly increase quarter cure rates for *S. aureus*. It also indicated that treatment of chronically infected cows (SCC>300,000 cell/ml for >60 days) should be questioned and culling maybe the best option for these cows. This study also demonstrated that label pirlimycin treatment is effective for the treatment of *Str. uberis*. 
Data was obtained for 3X duration therapy in *S. aureus* herds on Prince Edward Island, Canada (10). In this study, milk samples were taken prior to treatment and on days 14, 21 and 28 after the last treatment. A quarter was defined as cured if it was culture negative at all three post treatment time points. Some 44 of 65 (67%) of the treated quarters cured but only 5 of 29 (17%) non-treated quarters were cured.

In a 45 cow Iowa herd with an elevated SCC (>1 million cells/ml) 46% quarters were uninfected, 5% were infected with *Strep. agalactiae*, 23% were infected with *Strep. dysgalactiae*, 24% with *S. aureus*, and 3% with coagulase negative Staphylococci (11). The quarters infected with the streptococci received the recommended label treatment of pirlimycin, the quarters infected with *S. aureus* received the 3X duration treatment of pirlimycin. Milk samples were obtained for bacteriology 31 days post last treatment to assess cure. The quarter cure rates were 100% for *Strep. agalactiae*, 86% for *Strep. dysgalactiae*, and 85% for *S. aureus*. Herd SCC dropped to 256,000 cells/ml within one month post treatment and remained there for the following 12 months. This study demonstrates efficacy of the label dose of pirlimycin for quarters infected with *Strep. agalactiae* and *Strep. dysgalactiae* and the efficacy of the 3X duration treatment with pirlimycin for quarters infected with *S. aureus*.

Sears (12) examined a program for the elimination of *S. aureus*. In these herds, he insured adequate immune system status with the injection of 5 ml of a vitamin E/Selenium and an autogenous *S. aureus* bacterin. He treated all infected quarters with a 3X duration treatment of pirlimycin. Milk from all affected quarters was examined prior to treatment and from samples taken days 7, 14, 21, and 28 days after the last treatment. In 10 herds tested, the quarter cure rate following 3X duration pirlimycin therapy ranged from 29% to 95% with a mean herd average quarter cure rate of 58%.

Collectively, the data from these studies support the hypothesis that a longer duration of therapy with pirlimycin significantly increases the quarter cure rates and significantly decreases SCC post treatment of *S. aureus* mastitis. The data also support the conclusion that the label dose of pirlimycin is effective for the treatment of mastitis caused by *Strep. iberis, dysgalactiae* and *agalactiae*. These studies only examined one extended treatment regime (3X duration). Pharmacia & Upjohn have initiated a model mastitis study to examine further the effect of other extended therapy regimes on efficacy.

**MODEL STUDY**

**OBJECTIVE**

The objectives of this study were to determine if extension of therapy to more than two infusions of 50 mg of pirlimycin per infected quarter resulted in a significant increase in the efficacy of pirlimycin for the treatment of mastitis caused by *S. aureus* and if extension of therapy affects the post-treatment milk discard period and the pre-slaughter withdrawal period.

**MATERIALS AND METHODS**

Thirty four lactating Holstein cows provided 106 quarters that were free of clinical mastitis, bacterial infection, udder edema and had SCC <200,000 cells/ml during the entire 7 day pre-
infection period. Cows were infected with either 243 or 413 cfu *S. aureus* via intramammary infusion. During the following 14 days, cows were examined at each milking for signs of mastitis. A quarter/cow was classified as having acute clinical mastitis (ACM) when clinical signs of mastitis were observed for 2 consecutive milkings. Cows identified with ACM were treated immediately in all four quarters. The ACM quarter has severe clinical mastitis. Cows/quarters not classified as ACM but where *S. aureus* was isolated and clinical mastitis was observed at least once during the 14 day post challenge period were classified as induced persistent infection (IPI). The IPI quarters had at least one milking with abnormal milk but not severe enough to be classified as an ACM quarter. These quarters returned to normal milk but they remained infected for the remainder of the 14 day post infection observation period. The IPI quarters are sub-clinically infected quarters. All quarters of the IPI cows were treated on the 14th day after infection. In this study, 30 quarters (10 cows) were classified as ACM and 76 quarters (24 cows) were classified as IPI. Cows were blocked by parity and milk production and then were randomly assigned to four treatment groups: label therapy of 2 infusions of 50 mg of pirlimycin into all four quarters twice at a 24 hour interval (2X), 5 daily infusions of 50 mg of pirlimycin into all four quarters (5X), three label treatments of 50 mg of pirlimycin with a 36 hour interval between treatments into all four quarters (3x Duration), and 8 daily treatments of 50 mg of pirlimycin into all four quarters (8X). Two non-treated control cows were included in the study. Cows were observed daily for up to 28 days after the last treatment. Bacterial cultures were obtained weekly over the 28 day period. A quarter was bacteriologically cured if all bacterial cultures obtained at 7, 14, 21, and 28 days after the last treatment were negative for *S. aureus*.

Milk samples were obtained from all treated cows at each milking for 96 hours after the last treatment. A microbiological cylinder plate assay was used to determine milk pirlimycin concentrations. The assay has a limit of quantitation (LOQ) of 0.02 µg/ml (ppm). All cows were slaughtered either at the determination of treatment failure or following the 28 day post treatment period for determination of pirlimycin residue concentration in the liver (the target tissue) by the determinative HPLC Thermospray Mass Spec assay. This assay has a LOQ of 0.025 µg/g (ppm).

**RESULTS**

The classification of quarter infections for the study is found in Table 2. Overall bacterial quarter cure rates in the study regardless of ACM/IPI classification were 0, 54, 27, 61 and 71% for the non-treated control, 2X, 5X, 3X duration, and 8X treatments, respectively (Table 3). The 2X, 5X, 3X duration and 8X overall bacterial cure rates were all significantly better than the non-treated control. The overall bacterial cure for the 3X duration and 8X groups were numerically better than the overall bacterial cure rates for the 2X and 5X duration groups. The 8X group was significantly better than the 5X group. Quarter cure rates for ACM cows were 0, 29, 44, and 40 percent for 2X, 5X, 3X duration and 8X treatments.

**Table 2. Summary of quarter classification trial 782-7923-1-RAR-97-003**

<table>
<thead>
<tr>
<th>Sterile Pirse Treatment Group</th>
<th>Cows (N)</th>
<th>Qualified Quarters</th>
<th>Quarter Classification</th>
<th>Total Quarters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ACM</td>
<td>IPI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>---------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2X</td>
<td>8</td>
<td>26</td>
<td>4</td>
<td>22</td>
</tr>
<tr>
<td>5X</td>
<td>8</td>
<td>26</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>3X Duration</td>
<td>8</td>
<td>23</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>8X</td>
<td>8</td>
<td>24</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>106</td>
<td>30</td>
<td>76</td>
</tr>
</tbody>
</table>

1A quarter classified as ILI is not included in data sets for statistical analysis.

Table 3. Analysis of treatments for overall proportion of quarters cured

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cure Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/7=0.00</td>
</tr>
<tr>
<td>2x</td>
<td>14/26=0.54&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5x</td>
<td>7/26=0.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3X Duration</td>
<td>14/23=0.61&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>8x</td>
<td>17/24=0.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1Analysis used jackknife methodology. Each nonzero treatment group had eight cows. Since the ratio of the maximum to minimum estimates’ variance was 1.36, the variances were pooled; the pooled variance was 0.0239 associated with 28 degrees of freedom. Note that the individual φs ranged from 0.46 to 0.89 with an average of 0.66.

2Nonzero Treatments with different superscripts are significantly different at the (two-sided) α=0.10 level
Table 4. Analysis of treatments differences for proportion of quarters cured within IPI and ACM categories

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cow Category</th>
<th>IPI[^1]</th>
<th>ACM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0/7=0.00</td>
<td>--</td>
</tr>
<tr>
<td>2x</td>
<td></td>
<td>14/22=0.64[^a]</td>
<td>0/4=0</td>
</tr>
<tr>
<td>5x</td>
<td></td>
<td>5/19=0.26[^a]</td>
<td>2/7=0.29</td>
</tr>
<tr>
<td>3X Duration</td>
<td></td>
<td>10/12=0.83[^b]</td>
<td>4/11=0.44</td>
</tr>
<tr>
<td>8x</td>
<td></td>
<td>15/16=0.94[^b]</td>
<td>2/8=0.40</td>
</tr>
</tbody>
</table>

[^1] Analysis of the IPI cows used jackknife methodology. The 2x, 5x, 3p, and 8x treatment groups had 7, 4, 6, and 5 cows, respectively; therefore the cure rate’s variances were not pooled. Pair wise tests used the square root of the sum of the individual variances as standard error and used as degrees of freedom the smaller of the two degrees of freedom associated with standard error.

[^2] The two categories had significantly different cure rates: p<.005.

[^3] Nonzero treatments with different superscripts are significantly different at the (two-sided) α=.10 level.

respectively (Table 4). There were no ACM control cows. The cure rates show a numerical trend to improvement of cure rates following extended therapy duration. Quarter cure rates for IPI cows were 0, 64, 26, 83 and 94 percent for the control, 2X, 5X, 3X duration and 8X treatments, respectively (Table 4). The IPI cure rates for all four treatment groups were all significantly better than the non-treated control. The IPI cure rates for the 8X and 3X duration treatment groups were significantly better than the 2X and 5X IPI cure rates. A breakdown of treatment cure rate by post treatment sampling time is found in Table 5. For the 2X treatment group, the largest drop in cure rate percentage occurred between the 7 and 14 day post-treatment samples. For the 5X and 3X duration groups, this cure rate decrease occurred between the day 14 and 21 day post-treatment samples. This cure rate decrease did not occur in the 8X treatment group. Cured quarters from all groups had significant reductions in SCC (Table 6).
Table 5. Overall post-treatment quarter cure\(^1\) rate

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Qualified Quarters</th>
<th>Days Post-Treatment</th>
<th>Overall Quarters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2X</td>
<td>26</td>
<td>(19) 73.1</td>
<td>(14) 53.8</td>
</tr>
<tr>
<td>5X</td>
<td>26</td>
<td>(10) 38.5</td>
<td>(9) 34.6</td>
</tr>
<tr>
<td>3X-Duration</td>
<td>23</td>
<td>(18) 78.3</td>
<td>(16) 69.6</td>
</tr>
<tr>
<td>8X</td>
<td>24</td>
<td>(19) 79.2</td>
<td>(18) 75.0</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
<td>(66) 48.5</td>
<td>(57) 41.9</td>
</tr>
</tbody>
</table>

\(^1\) (N) = number of cured quarters. Quarters culture positive for *S. aureus* at any post-treatment time are included as failures for each subsequent sample day irrespective of subsequent culture results.

Examination of the milk samples revealed that extension of treatment duration did not affect the milk decline profiles of pirlimycin between the treatment groups. For extended therapy with pirlimycin, the milk discard time remains the same after the last treatment as for label use (Table 7). The liver residue data is found in Table 8. Examination of these data demonstrate that extension of therapy duration will lengthen the pre-slaughter withdrawal period when compared to the application of shortest duration therapy (one plaset per quarter twice at a 24 h interval). P&U is generating further tissue residue decline data.

**STUDY CONCLUSION AND SUMMARY**

Extended therapy, both 3X duration and 8X, with pirlimycin significantly increases the efficacy and significantly decreases SCC following the treatment of cows with induced *S. aureus* mastitis. This model study confirms field observations and results with 3X duration therapy. Daily therapy (8X) provides better efficacy than the 3X duration, as the milk concentration of pirlimycin is more constant during the entire treatment period. Extended therapy does not alter the milk discard period after the last treatment but it will lengthen the pre-slaughter withdrawal period for pirlimycin. For chronic infections, extended therapy with pirlimycin offers an alternative treatment regime that will significantly increase bacterial cure rates.

In the past, the goal for lactational therapy has centered on returning cows to normal milk as quickly as possible with a minimum of amount of discarded milk and not bacterial cure. The complete bacterial cure has not been the goal. The use of antibiotics for the treatment of
Table 6. Geometric mean somatic cell counts (x1000; SCC) for quarter milk samples for untreated control cows and cows treated with Pirlimycin

<table>
<thead>
<tr>
<th>Pirlimycin Treatment</th>
<th>Quarter Treatment Response (No. of Quarters)</th>
<th>Pre-Infection Day 1-7</th>
<th>Pre-Treatment&lt;sup&gt;1&lt;/sup&gt; Study Days –14 to 0</th>
<th>Post-Treatment Study Days 1-28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14-8</td>
<td>7-1</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>Cure (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Failure (7)</td>
<td>10</td>
<td>954</td>
<td>1371</td>
</tr>
<tr>
<td>2x</td>
<td>Cure (14)</td>
<td>7</td>
<td>392</td>
<td>377</td>
</tr>
<tr>
<td></td>
<td>Failure (12)</td>
<td>6</td>
<td>301</td>
<td>383</td>
</tr>
<tr>
<td>5x</td>
<td>Cure (7)</td>
<td>12</td>
<td>849</td>
<td>1073</td>
</tr>
<tr>
<td></td>
<td>Failure (19)</td>
<td>7</td>
<td>691</td>
<td>978</td>
</tr>
<tr>
<td>3X-Duration</td>
<td>Cure (14)</td>
<td>5</td>
<td>289</td>
<td>342</td>
</tr>
<tr>
<td></td>
<td>Failure (9)</td>
<td>9</td>
<td>1314</td>
<td>1301</td>
</tr>
<tr>
<td>8x</td>
<td>Cure (17)</td>
<td>7</td>
<td>511</td>
<td>379</td>
</tr>
<tr>
<td></td>
<td>Failure (7)</td>
<td>11</td>
<td>2201</td>
<td>1757</td>
</tr>
</tbody>
</table>

<sup>1</sup>The SCC value for pre-treatment day 14 is from the morning milking immediately before challenge inoculation with <i>S. aureus</i> and extends through to pre-treatment day 1 which is the morning milking immediately before the first treatment.
Table 7.  Milk Pirlimycin residue decline following extended therapy

<table>
<thead>
<tr>
<th>Time Post Last Treatment</th>
<th>2X</th>
<th>5X</th>
<th>3x - Duration</th>
<th>8X</th>
<th>FOI 2X</th>
<th>Sterile 2X</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>6.61</td>
<td>7.74</td>
<td>5.84</td>
<td>6.30</td>
<td>8.45</td>
<td>10.4</td>
</tr>
<tr>
<td>24 h</td>
<td>0.42</td>
<td>0.99</td>
<td>0.45</td>
<td>0.65</td>
<td>0.88</td>
<td>0.82</td>
</tr>
<tr>
<td>36 h</td>
<td>0.20</td>
<td>0.29</td>
<td>0.22</td>
<td>0.26</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>48 h</td>
<td>0.10</td>
<td>0.12</td>
<td>0.10</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>60 h</td>
<td>0.08</td>
<td>0.09</td>
<td>0.08</td>
<td>0.09</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>72 h</td>
<td>0.06</td>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>84 h</td>
<td>0.05</td>
<td>0.07</td>
<td>0.05</td>
<td>0.07</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>96 h</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 8.  Liver Pirlimycin residue decline data following extended therapy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Day 15</th>
<th>Day 16</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 29</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.36 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.07±0.09 (7)</td>
</tr>
<tr>
<td>5X</td>
<td>-</td>
<td>-</td>
<td>1.00±0.23 (4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.07±0.02 (4)</td>
</tr>
<tr>
<td>3x - Duration</td>
<td>-</td>
<td>-</td>
<td>1.88 (1)</td>
<td>-</td>
<td>-</td>
<td>0.75 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.09±0.05 (6)</td>
</tr>
<tr>
<td>8X</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.05 (1)</td>
<td>-</td>
<td>0.08±0.08 (7)</td>
</tr>
<tr>
<td>FOI 2X</td>
<td>-</td>
<td>0.49±0.15 (5)</td>
<td>-</td>
<td>-</td>
<td>0.07±0.03 (5)</td>
<td>-</td>
<td>-</td>
<td>0.04±0.01 (5)</td>
<td>-</td>
<td>0.06±0.03 (8)</td>
</tr>
<tr>
<td>Sterile 2X</td>
<td>1.42±0.22 (4)</td>
<td>0.24±0.04 (4)</td>
<td>&lt;0.025 (4)</td>
<td>-</td>
<td>-</td>
<td>&lt;0.025 (4)</td>
<td>-</td>
<td>-</td>
<td>&lt;0.025 (4)</td>
<td></td>
</tr>
</tbody>
</table>
clinical mastitis is one part of a mastitis treatment program. The veterinarian and the dairyman must define a successful outcome and assess each cow prior to therapy. Creation of a treatment decision tree and the proper use of antibiotic therapy will increase the number of successful outcomes. To improve efficacy, the veterinarian and dairyman must evaluate several areas prior to therapy. These areas include: the patient, the mastitis pathogen, and potential approved treatments.

The chances of successful therapy decrease with age and the number of clinical mastitis cases. A first lactation heifer with her first case of mastitis has the greatest chance for success whereas the 5th lactation cow with chronic S. aureus has the least chance for success. The veterinarian must utilize bacteriological culture and antibiotic sensitivity testing for the mastitis pathogens on each farm so only potentially effective antibiotics are utilized. The veterinarian and the dairy producer should carefully assess treatment of older chronically infected cows as they serve as a source of new infections. The best duration of therapy is difficult to determine in that each cow and each mastitis pathogen will provide a different opportunity. The farm veterinarian is best qualified to make this determination and the availability of flexible dose/treatment duration labels with supporting milk and tissue residue decline information will make these treatment options viable.

REFERENCES

MONITORING ANTIBIOTICS IN MILK - THE CHANGING WORLD OF TEST METHODS

PAUL NEAVES, Williams & Neaves, The Food Microbiologists, "Moleview", 28, Randalls Road, Leatherhead, Surrey, KT22 7TQ

SUMMARY

For many years, the UK operated a national approach to the detection of antibiotics in milk, the Delvotest P (Gist-brocades BV, The Netherlands) being, initially, the only test listed in the Joint Committee Code of Practice for the Assessment of Milk Quality (the 'Blue Book'). Thus, the Delvotest P was accepted nationally for the examination of tanker milks and became known as the 'UK Industry Standard' test. In 1992, the LacTek β-lactam test (Idexx Laboratories, USA) became the first 'rapid' test to be incorporated into the Blue Book but, since deregulation in 1994, several new rapid tests have appeared that differ significantly in sensitivity as well as in the range of substances detected. Although the newer tests are not yet listed in the Blue Book, their presence within the dairy industry, raises the question, "Are we now spoiled for choice?" and there is considerable debate over the advantages and limitations of different proprietary tests. To enable objective comparisons to be made, the International Dairy Federation has produced two guideline documents for the evaluation of tests that detect antibiotics in milk, the first of which has been adopted by ISO as an International Standard (1). In England & Wales, the Milk Quality Forum and the Dairy Industry Federation commissioned the Hannah Research Institute to assess independently the test manufacturers' claims. This work has formed the basis for revising the Blue Book list of tests and the final report of the Hannah work (2) has recently been issued (28th July). At the time of writing, however, the revised list of Blue Book 'acceptable' tests remains eagerly awaited.

PRINCIPLES AND PRACTICE OF ROUTINE TESTING FOR ANTIBIOTICS

The 'traditional' tests for antibiotics in milk, known as 'microbial inhibitor' tests, involve incubating a susceptible organism in the presence of the milk sample. In the absence of an antibiotic, the organism grows and can be detected visually either by opacity of the agar growth medium or by a colour change resulting from acid production. In the presence of an antibiotic, or any other inhibitor, the organism fails to grow and a zone of inhibition or lack of a colour change is observed. Such tests are exceptionally sensitive to β-lactam antibiotics, though they can also be made to detect sulphonamides and other antimicrobials. They are generally reliable and cost-effective but require incubation for several hours before the result can be visualised.

The desire for a more rapid result has promoted the development of tests that employ the 'immune receptor' test principle, which is a variation of the well-established enzyme-linked immunosorbent assay (ELISA). Essentially, a specific target antibiotic is captured by immobilised antibodies, or by a broader-spectrum receptor such as a bacterial cell. Most tests involve a competitive principle in which antibiotic in the sample competes with an internal antibiotic standard for the immune receptor. The antibody-antibiotic complex is then usually linked to an enzyme that catalyses a colour or fluorescence reaction and a comparison of the intensity of the 'test' reaction with that of a 'control' determines whether the sample is positive
or negative. Because of their competitive principle, a low intensity usually means 'positive' whilst a high intensity is regarded as 'negative'. Immune receptor tests can be made quantitative but are generally used to provide a 'pass/fail' result. They are generally more expensive than microbial inhibitor tests but only detect substances that react immunologically with the immobilised receptor and they provide a result in less than 10 min.

The commercially available immune receptor tests employ several variations of capture mechanism and colour reaction but most possess the common features of an immunological reaction coupled with a change in colour (or fluorescence). There are, however, two exceptions. The Penzym test (UCB Bioproducts, Belgium) employs the inhibition of an enzyme reaction (DD-carboxypeptidase activity), instead of an immune reaction, to detect the presence of a β-lactam and it visualises this by a colour change. Conversely, the Charm II assay (Charm Sciences Inc., USA) employs an immune reaction to bind the antibiotic to a microbial receptor but detects this complex using a low-level ³H or ¹⁴C radio-label, instead of an enzyme reaction.

The commercial availability of microbial inhibitor and immune receptor tests has created a significant dilemma for the dairy industry. Although β-lactam antibiotics are the most commonly used antimicrobials in veterinary medicine, especially for intramammary administration, preparations containing sulphonamides, tetracyclines or other antimicrobials are also available. Microbial inhibitor tests have a broad spectrum and can therefore detect substances other than β-lactams; however, they are not specific for antibiotics and there are occasional reports of positive reactions associated with other inhibitors such as lactoferrin, lysozyme or sanitisers. Conversely, immune receptor tests are specific for a particular antibiotic group or even a specific substance. Most of these tests are capable of detecting the β-lactam group although at least 2 commercially available immune receptor tests are specific for penicillins and would fail to detect the presence of a cephalosporin. Although immune receptor tests for sulphonamides and tetracyclines are also commercially available, these tests need to be undertaken separately, with obvious adverse implications for cost and convenience.

The UK dairy industry currently employs essentially two (but, increasingly, three) levels of testing for antibiotics. 1) A farmer quality payment scheme was first introduced in England & Wales by the Milk Marketing Board in 1982. Bulk tank samples were tested weekly and the test results were therefore retrospective. Thus, there was (and still is) no commercial need for a rapid result, the main requirements of a test being low cost, broad spectrum and reliability. 2) Tanker milks are also usually tested on arrival at the dairy where there is a desire to accept or reject the consignment before off-loading. Similarly, for silo samples, it is desirable to obtain the test result before the milk is released into production. In this situation, a rapid result is essential and consequently many dairies have chosen to sacrifice a broad spectrum and, to some extent, cost for the benefit of speed. 3) Since deregulation, tests for antibiotics have begun to be applied at the individual cow level, notably for freshly calved animals that have received dry cow therapy. The ideal test for this situation would possess a broad spectrum, speed and low cost; however, no test currently achieves all three attributes and the farmer is thus faced with a choice between speed and broad spectrum. This approach to the control of antibiotics in the milk supply has received international approval and is embodied in the IDF Integrated Detection System for Antimicrobials (3).
The diversity of tests now available and the fragmentation of the UK dairy industry since
deregulation have created a significant dilemma. Firstly, different tests may be applied by
purchaser and supplier, since there is no longer a standardised, national approach and there is
an increased danger that conflicting results might be obtained between two tests undertaken
on the same consignment of milk. The supplier-customer relationship often defines which
tests are to be used, to ensure that consistent results are obtained by both parties, an aspect
that is becoming increasingly complex as the movement of milk across national boundaries
expands. Thus, tests may be employed simply because they are used by an important
customer. Secondly, dairies must decide whether they should screen milk supplies for the
widest possible range of substances using a test where the result is obtained retrospectively or
whether they should use a rapid test to detect only those antimicrobials most commonly
encountered. It seems that some UK dairies have taken the former approach whilst others
have adopted the latter. However, most dairies employ the Delvotest as the 'definitive test'
and may confirm rapid test results by re-testing a positive sample with the microbial inhibitor
test.

Three additional factors further complicate the situation. 1) The EU Maximum Residue
Limits (MRL) for veterinary medicinal residues in milk apply to an ever-expanding list of
substances. Milk processors have a responsibility to ensure that their milk supplies do not
contain any of these substances at levels that exceed the MRLs, yet test methods cannot keep
pace with the rate at which the list is expanding. 2) The UK Food Safety Act defines the
concept of 'Due Diligence' under which, in the event of legal proceedings, a milk processor
must demonstrate that all reasonable precautions had been taken. Since some antibiotic
groups are much more commonly found than others, is it necessary to screen milk for all
antimicrobial groups? 3) Both the dairy industry and the test manufacturers have precious
little specific information on the market breakdown for veterinary medicinal preparations.
The information that does exist appears largely to be anecdotal making it difficult for test
manufacturers to design appropriate tests and difficult for dairies to choose the test that best
fits their needs.

COMMERCIALY-AVAILABLE MICROBIAL INHIBITOR TESTS

The Delvotest (Gist-brocades BV, The Netherlands) is the best known microbial inhibitor test
but it is less widely recognised that several versions of this test exist. The first version to be
developed, in the 1970s, was the Delvotest P, designed to detect β-lactams. The target
organism, *Bacillus stearothermophilus*, is encapsulated in an agar medium containing a pH
indicator, a nutrient tablet and the milk sample both being dispensed onto the agar surface.
The 'ampoule version' is designed for individual tests or small-scale testing whilst a micro-
titre plate version is designed for mass testing where 96 tests can be undertaken
simultaneously. A negative result is indicated by a colour change from purple to yellow, due
to acid development during incubation at 64°C for 2½ hours. The Delvotest P has been used
throughout the world and has a sensitivity to penicillin G of 0.005 IU/ml although the
Delvotest P kits distributed within the UK have historically been selected especially to meet
the UK's unique demand for a sensitivity to 0.006 IU/ml penicillin G!

A more recent development, the Delvotest SP, is capable of detecting a wider spectrum of
substances, notably sulphonamides, but also has increased sensitivity to tylosin, erythromycin,
neomycin, gentamicin, trimethoprim and other antimicrobials. The Delvotest SP appears
identical to the Delvotest P, the only difference being the need to incubate the Delvotest SP
for 2½ hours. The Delvetest SP is sold throughout the world and, universally, has a sensitivity to penicillin G of 0.003-0.004 IU/ml.

The Delvetest Cow Test was introduced into the UK in 1994 for testing individual animals as well as bulk tank milk and is identical to the ampoule version of the Delvetest P, differing only in its packaging. However, as the UK dairy industry is now beginning to change to the use of Delvetest SP (see below), an 'SP' version of the Delvetest Cow Test has recently become available.

Finally, a fourth version of the Delvetest, the Delvetest MCS test, is soon to be launched in the UK. This test is similar to the micro-titre plate version of the Delvetest SP but has the nutrients included in the agar which makes the addition of a nutrient tablet is unnecessary but gives the test materials a shorter shelf life. This test is aimed at the high-volume, quality testing laboratory (or 'Milk Control Station') market where low cost and simplicity are vital and reduced shelf life is not an inconvenience.

Although the Delvetest is by far the most widely used microbial inhibitor test in the UK, three similar tests, manufactured by Charm Sciences Inc. (USA), are also available. The Charm AIM-96 test is a micro-titre plate test, similar to the Delvetest and capable of detecting β-lactams, sulphonamides, tetracyclines, macrolides and aminoglycosides in 96 samples simultaneously. Unlike the Delvetest, however, it employs a liquid medium instead of agar. The inoculated micro-titre plate is incubated on a heating block, programmed to provide a time-temperature profile suited to the batch of Bacillus stearothermophilus spores being used; the incubation period is typically 3-4 hours, at the end of which a blue-yellow colour change indicates that a sample is negative. The Charm Farm test is a 'test-tube' version of the AIM-96 test, designed for on-farm use and employs the same microbial inhibitor principle with a colour change. There are two versions of this test: the Charm Farm Test-'Vial' and the Charm Farm Test-'Mini Vial' that both employ larger quantities of test medium than the AIM-96 test but are designed for fewer samples.

In addition, there are several other microbial inhibitor tests, produced by several companies. These include the Brilliant Black Reduction Test, the Valio T101 test, the Copan microbial inhibitor test, the Lumac rapid antibiotic test, the Arla micro test and the Biosys bioluminescence method. However, these tests are either not available in the UK or have not yet captured the interest of the UK dairy industry.

COMMERCIALY-AVAILABLE IMMUNE RECEPTOR TESTS

Probably the most widely used immune receptor test in the UK is the LacTek test for β-lactams (Idexx Laboratories, USA). The test has a test tube format that is suited to laboratory use and the test takes 7 minutes to complete. The milk sample and a competitive enzyme tracer are added to an antibody-coated test tube and bind to the tube surface whilst the tube is shaken at room temperature, any antibiotic in the milk competing with the tracer for the surface receptor. The tube is washed and a colour developer is added to visualise the surface-bound complex. The colour intensity is measured in a spectrophotometer and compared with that of a penicillin 'standard', an intense colour indicating that the sample is negative, a pale colour indicating a positive result. The LacTek β-lactam test is highly specific for penicillins and does not detect cephalosporins. Several lesser-known versions of
the LacTek test are also available that separately detect ceftiofur, tetracyclines, sulphamethazine, gentamicin and the banned substance, chloramphenicol.

The Delvo X-Press β-lactam II test (Gist-brocades BV, The Netherlands) employs the same principle as the LacTek test and also has a test tube format, suited to laboratory use. The test takes 7 minutes to complete, all incubations and colour measurements being undertaken in an 'Incubator-Shaker-Reader-Printer' instrument. Both penicillins and cephalosporins are detected.

The SNAP β-L test (Idexx Laboratories, USA) was the first of a growing number of tests to employ capillarity to draw the milk sample and test reagents over an immobilised antibody. It consists of a test tube and a disposable plastic unit or 'SNAP' device (rather like an electrical rocker switch) that contains the test reagents. The test is therefore essentially 'dry', making it suitable for laboratory or field use and it has also found some applications for use on tankers. The milk sample is first incubated in a test tube, placed in a heating block, then poured into one end of the SNAP device where it flows along a filter paper strip. After 30s, the device is 'snapped' to allow the colour developing reagents (contained at the opposite end) to flow in the opposite direction. At the end of the test (<10 min.) two colour spots ('control' and 'test') appear in the middle of the device and the intensity of these is compared either visually or in a colour reader. The SNAP test detects both penicillins and cephalosporins and there are separate SNAP tests for tetracyclines, sulphonamides and gentamicin.

The Beta Screen test (Advanced Instruments, USA) is an 'add-on' to the Fluorophos test for phosphatase used in many dairy laboratories and employs a fluorescent end-point. The milk sample and enzyme conjugate are first incubated in an antibody-coated test tube, placed in a heating block. After washing the tube, fluorescence is developed by the addition of reagents and further incubation; finally, the fluorescence intensity is measured in the Fluorophos fluorimeter and compared with that of a penicillin 'standard'. The Beta Screen test takes 10 min.; it is highly specific for penicillins and does not detect cephalosporins.

The Charm II assay (Charm Sciences Inc., USA) is not a single test but a family of separate tests for specific groups of antibiotics, notably β-lactams, sulphonamides, tetracyclines, novobiocin, aminoglycosides and macrolides, as well as various other substances such as chloramphenicol. There are several versions of the test that can detect different substances within an antibiotic group such as the aminoglycosides or macrolides. The Charm II assay is an immune receptor test but is suitable for large laboratories only, requiring a range of laboratory equipment, including a centrifuge and sample mixers to prepare samples as well as a scintillation counter to detect the radio-label. Calibration curves need to be prepared for each analyte and a 'negative control' sample must be tested each day, constraints that may require the laboratory to obtain a source of antibiotic-free raw milk powder.

Several tests for antibiotics have been launched in the UK within the last 18 months. The Beta STAR test (UCB Bioproducts, Belgium) involves a specific β-lactam receptor linked to gold particles. It is a 'dipstick' test that detects penicillins and cephalosporins within 5 min., though extending the incubation to 8 min. makes the test more sensitive. The milk sample is added to a vial containing the test reagents (25 test kit only: for the 100 test kit these are added separately) and incubated, the dipstick is added and incubation is continued. A red 'control' band appears on the dipstick together with a red 'test' band of variable intensity and
the latter is compared visually with the former. If the 'test' band is weaker than the 'control' band the result is positive; if the 'test' band gives a stronger reaction, the result is negative.

The Charm MRL test (Charm Sciences, USA) is very similar to the Beta STAR test and detects penicillins and cephalosporins in 8 min. The test strip is placed in a heating block, the milk sample is added to an absorbent pad at one end and the test is incubated. Two lines appear on the dipstick, a sample being considered positive if the 'test' line is lighter than the 'control' line. The results can be read visually or using an image reader.

The Penzym test (UCB Bioproducts, Belgium) is newly launched in the UK, though it has been used in Northern Ireland and in mainland Europe for many years. Two versions are available (Penzym and Penzym S) that have different incubation periods and different levels of sensitivity. The test detects β-lactams and is especially sensitive to some cephalosporins. The test is based on the principle that β-lactam antibiotics prevent bacterial multiplication by inhibiting the activity of the enzyme DD-carboxypeptidase. During the test, DD-carboxypeptidase activity liberates D-alanine from an enzyme substrate which is visualised by a colour change; in the presence of antibiotic, no D-alanine can be liberated and no colour change occurs. The Penzym test produces a pink colour when a sample contains no antibiotic whilst a yellow colour is interpreted as 'positive'. In the case of Penzym S, a peach-orange colour is considered to be a negative result whilst a colour with a 'yellow tendency' indicates that the sample contains a β-lactam.

Finally, another proprietary test is currently being developed by Idexx Laboratories (USA) though the UK launch date is not yet clear. The Parallux test is designed to detect a range of antimicrobials as well as other substances of interest to the dairy industry, though the exact range of compounds has yet to be announced. Parallux is a laboratory instrument that automates the pipetting, mixing, incubating and reading stages of an immune receptor test by means of a series of pumps, a centrifuge and a fluorescence detector all built into one unit. Each test is presented to the instrument in the form of a disposable cartridge that contains 4 glass capillary tubes, each coated with a different range of antibodies. For antibiotics, two cartridge types are being considered: one with 4 tubes each containing the same range of antibodies (the 'cillins' multi-cartridge) so that 4 different samples can be screened simultaneously and another with 4 tubes containing different antibodies (the 'individual' cartridge) so that a positive sample can be further identified. The instrument has two parts: a 'prep' station and a 'read' station. A cartridge is fitted to the 'prep' station, the milk sample(s) is (are) pipetted into its tray and the instrument is set to run. The milk is mixed with pre-dispensed, dried reagents, any antibiotic competitively binds to the coated tube and the tube is washed. When these operations are complete the cartridge is manually transferred to the 'read station' where it is centrifuged and the fluorescence is measured, a high level of fluorescence indicating that the sample is 'negative'. The test is described as a 'solid-phase fluorescence immunoassay' and takes 4 min. to complete.
SENSITIVITY AND SPECIFICITY OF ANTIBIOTICS TESTS

The UK dairy industry now faces the dilemma that the proprietary tests currently available have yielded a confusing array of detection spectra and test sensitivities. Some sensitivities and specificities for antibiotics tests are given in Table 1. This list is not exhaustive; not all claims made by test manufacturers are listed and most, if not all, tests also detect substances for which no claim is made. The list does, however, encompass many of the substances that may be of concern in milk processing.

Whilst all tests have similar detection limits for penicillin G, there are some wide variations for other β-lactams. The Delvotest P, for example, detects cloxacillin at 25 µg/Kg which is near the EU MRL of 30 µg/Kg whilst the LacTek test is much more sensitive (7 µg/Kg) and this difference created some discrepancies for dairies and the Milk Marketing Board when the LacTek enter the Blue Book in 1992. Conversely, it recently became apparent that the Delvotest P is sensitive to cephalonium (15-20 µg/Kg) whilst the LacTek is not, though there has been no documented evidence of test discrepancies as a result of milk being contaminated with this substance. This debate has further been confused by the fact that no MRL for this substance has yet been set in EU legislation which has led to differences of opinion between the veterinary pharmaceutical companies and the milk processors. With an increasing range of substances for which milk may need to be screened and an expanding choice of proprietary tests, the potential for discrepancies seems likely to rise rather than decline.

CURRENT UK SITUATION

It might be said that the UK dairy industry is on the edge of a precipice. Ever since the Delvotest P was introduced to the UK in the mid-1980s, the dairy industry has had a reliable, consistent and uniform approach to the analysis of milk samples for antibiotics. In addition, the Liaison Chemist Service bridged the gap between farm and dairy for the whole of England and Wales and arbitrated in case of disputes regarding antibiotic contamination of raw milk. Following deregulation, there is no national Liaison Chemist Service and UK dairies are free to choose for themselves which tests they wish to use, though if they wish to meet the Blue Book standard a sample must still fail either the Delvotest P or the LacTek β-lactam test.

Most of the major dairy companies have already chosen to replace the Delvotest P with the Delvotest SP, though others are still deliberating. At least one quality testing laboratory has been using Delvotest SP for farmer quality payment purposes for over 2 years whilst another uses different tests for different customers. The third laboratory still requires a milk sample to fail the Delvotest P before it penalises a farmer. The situation should soon be resolved, however, because Gist-brocades has announced its intention to withdraw the Delvotest P from sale in the UK as from October or November 1999.

As far as immune receptor tests are concerned, the current UK situation could be described as a ‘free-for-all’. The choice of an immune receptor test depends on several factors outside the realms of science and technology, cost, ease of use, the need for capital equipment or a maintenance contract and other factors all confounding the difficult decision. Almost all of the immune receptor tests are used in the UK, though their market shares vary considerably.

Following the publication of the Hannah survey of antibiotic test methods (Muir, 1999), the Milk Quality Forum has to decide which tests shall be included in a revised Blue Book. The
original intention was that one or a limited number of tests would be included but it has become very clear that for technical, commercial and even political reasons this is unlikely to be the case. Many dairies have avoided choosing a new test until this occurs. We await the big decision!

REFERENCES

### Table 1.  
Manufacturers' claims for sensitivity and specificity of some antibiotics tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Antimicrobial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td><strong>Microbial inhibitor tests</strong></td>
<td></td>
</tr>
<tr>
<td>Dévoltest P (2½ h)</td>
<td>3†</td>
</tr>
<tr>
<td>Dévoltest SP (2½ h)</td>
<td>2.5</td>
</tr>
<tr>
<td>Dévoltest MCS</td>
<td>2.4</td>
</tr>
<tr>
<td>AIM-96</td>
<td>3.5</td>
</tr>
<tr>
<td>Charm Farm V/MV</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Immune receptor tests</strong></td>
<td></td>
</tr>
<tr>
<td>LacTek 8-L</td>
<td>≤ 4.8</td>
</tr>
<tr>
<td>Dévo X-Press</td>
<td>2.4</td>
</tr>
<tr>
<td>SNAP 8-L</td>
<td>≤ 4</td>
</tr>
<tr>
<td>Beta Screen</td>
<td>1</td>
</tr>
<tr>
<td>Charm II 8-L</td>
<td>2</td>
</tr>
<tr>
<td>Beta STAR**</td>
<td>2.4</td>
</tr>
<tr>
<td>Charm MRL</td>
<td>3</td>
</tr>
<tr>
<td>Penzytin</td>
<td>4-6</td>
</tr>
</tbody>
</table>

All values are µg/Kg  
† 3 µg/Kg = 0.005 IU/ml  
* MRL version  
‡ 2.5 µg/Kg = 0.004 IU/ml  
** 25 and 100 test kits  
NS = Not sensitive
ANTIBIOTIC RESISTANCE IN MASTITIS BACTERIA

C.J. TEALE & G.P. DAVID, VLA Shrewsbury VI Centre, Kendal Road, Harlescott, Shrewsbury, SY1 4HD

SUMMARY
The Veterinary Laboratories Agency (VLA) has created a national antimicrobial database on which it is intended that the results of all sensitivity tests performed in England and Wales at VLA regional laboratories will be collected. Preliminary findings related to bacteria causing mastitis are presented in this paper and briefly discussed with reference to selected published findings from other countries; certain epidemiological aspects are also briefly discussed.

INTRODUCTION
The presence of mastitis bacteria that are resistant to antimicrobials has obvious implications for the treatment of infected animals. More importantly, in the current climate, there could potentially also be implications for the consumer if raw, unpasteurised milk or milk products contained such resistant bacteria. Most milk is pasteurised and further safeguards include the quality standards regarding the maximum total bacterial content of milk and regulations preventing mastitic milk going for human consumption. The udder itself is usually free of bacteria and this has important consequences in relation to microbial ecology. Intramammary preparations that are restricted to the udder will have an affect only on the target bacteria within the udder. This is quite obviously different from the situation in the intestine, where antimicrobial treatment will put a range of commensal organisms under selective pressure, in addition to the target organism. Resistance factors may become established and can be maintained in the commensal intestinal flora; the large numbers of bacteria present can also favour exchange of genetic information (1). The absence of a resident intra-mammary flora means that resistance factors do not in general become established in this organ.

There is currently no internationally recognised reference method for susceptibility testing of mastitis bacteria, though there are proposals at a European level to develop validated reference methods for veterinary pathogens. Each country therefore tends to use its own method: within the VLA the disc diffusion method of the British Society for Antimicrobial Chemotherapy (2) is used, with a uniform cut-off point of 13 mm to distinguish between sensitive and resistant strains. This method is used in all VI Centres (VLA Regional Laboratories) in England and Wales, though methodology is under review pending the results of European initiatives and developments in the medical field. 1999 has seen the creation of a national antimicrobial database on which it is intended that the results of all sensitivity tests, performed in England and Wales at VLA regional laboratories, will be collected. This paper includes some preliminary findings concerning the mastitis bacteria and discusses these with reference to previously published work. Although comparisons are made with published data from other countries, it should be appreciated that differences in methodology mean that these comparisons may not be entirely valid and that any differences observed must be interpreted with caution. Three broad groups of organisms are considered; the streptococci, staphylococci and Escherichia coli/colliforms.

STREPTOCOCCI (not Streptococcus uberis as data unavailable)

VLA National Data - Winter/Spring 1999
**Streptococcus agalactiae**

Total number of isolates: 8
Number showing multiple resistance: 0

<table>
<thead>
<tr>
<th>Disc Concentration</th>
<th>Number Resistant</th>
<th>Percentage Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin 10 IU</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin 10 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxycillin/Clavulanate 2/1 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline 10 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin 5 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neomycin 30 µg</td>
<td>5</td>
<td>63</td>
</tr>
</tbody>
</table>

**Streptococcus dysgalactiae**

Total number of isolates: 56
Number showing multiple resistance: 0

<table>
<thead>
<tr>
<th>Disc Concentration</th>
<th>Number Resistant</th>
<th>Percentage Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin 10 IU</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin 10 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amoxycillin/Clavulanate 2/1 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline 10 µg</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>Erythromycin 5 µg</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Neomycin 30 µg</td>
<td>31</td>
<td>55</td>
</tr>
</tbody>
</table>

It is interesting to note that in the field of human medicine, resistance in *Streptococcus pneumoniae* to penicillin, tetracyclines and macrolides has been observed in Europe and in some areas, multiple antimicrobial resistance has limited the number of antimicrobials that can be used to treat infections with this organism. Vancomycin and the newer fluoroquinolones with an extended Gram-positive spectrum have been suggested as possible candidates (3,4). It is also noteworthy that *Str. dysgalactiae*, with its wider range of habitats on the bovine host than *Str. agalactiae*, shows greater resistance, although only a limited number of the *Str. agalactiae* isolates are included in this preliminary data. The reason for this is not entirely clear and could be due to intrinsic bacterial factors or increased exposure to commensals carrying transferable resistance factors in *Str. dysgalactiae*.

Erythromycin resistance was detected in a low number of *Str. dysgalactiae* isolates; no resistance to erythromycin was detected in the *Str. agalactiae* isolates. Erythromycin and the other macrolides act by preventing bacterial protein synthesis. A common resistance mechanism, encoded by *erm* (erythromycin ribosome methylation) genes, involves methylation of a single adenine in the peptidyltransferase 23S rRNA. More than 30 *erm* genes have been described which all methylate this same adenine residue. The result is a reduction in binding of the 50S ribosomal sub-unit to macrolide-lincosamide-streptogramin antimicrobials. *Streptococcus agalactiae, Str. dysgalactiae* and *Str. uberis* can all carry the *erm* F gene, usually in conjunction with other *erm* genes (B, C or Q) (5). Some 71 streptococci from cases of clinical mastitis in dairy cows in the USA were examined by
Roberts and Brown (6) who found that 7.1% were resistant to erythromycin and/or lincomycin and nine of the isolates hybridised with \textit{erm} gene probes.

Resistance to streptomycin, novobiocin and tetracyclines in \textit{Str. agalactiae}, \textit{Str. dysgalactiae} and \textit{Str. uberis} has also been reported in the USA (7) where multiple antimicrobial resistance has also been observed in the mastitis streptococci (8). Although no penicillin resistance was detected in the American study on 71 streptococcal isolates (6), Polish workers found using a disc diffusion method that 86.7% of \textit{Str. agalactiae}, 84.6% of \textit{Str. dysgalactiae} and 88.5% of \textit{Str. uberis} strains were sensitive to penicillin (9). In a Canadian study, none of 68 strains of \textit{Str. agalactiae} of bovine origin showed resistance to penicillin or erythromycin (10). In New Zealand, resistance to streptomycin in \textit{Str. uberis} and \textit{Str. dysgalactiae} has been described (11). Mutation in the ribosomal protein of streptococci and enterococci can lead to high-level streptomycin resistance and confers complete resistance to any synergy from combination with penicillin (12).

\textbf{STAPHYLOCOCCUS AUREUS}

\textbf{VLA National Data - Winter/Spring 1999}

\textit{Staphylococcus aureus}

Total number of isolates: 211
Number showing multiple resistance: 5

<table>
<thead>
<tr>
<th>Disc Concentration</th>
<th>Number Resistant</th>
<th>Percentage Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin 10 IU</td>
<td>98</td>
<td>47</td>
</tr>
<tr>
<td>Ampicillin 10 µg</td>
<td>96</td>
<td>46</td>
</tr>
<tr>
<td>Amoxycillin/Clavulanate 2/1 µg</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Tetracycline 10 µg</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Erythromycin 5 µg</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Neomycin 30 µg</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Penicillin was first used for the treatment of mastitis in the late1940s. Since then, \(\beta\)-lactamase producing \textit{Staphylococcus aureus} has emerged in a number of countries, including the UK (13). In several countries (e.g. Belgium, Switzerland and UK) levels of penicillin-resistance increased up until the 1970s-1980s, but since that time have shown a decline (13). The reason for this decline is not obvious, though it has been speculated that it could perhaps be due to the introduction of other antimicrobial agents in mastitis therapy, although there was considered insufficient evidence for definitive conclusions (13). In other countries, for example Norway and Sweden, levels of resistance have remained fairly constant at around 10-15\% (13).

Methicillin-resistance has been detected in \textit{S. aureus} from mastitis cases in several countries (14, 15), though not as far as we are aware, from the UK. A survey for methicillin-resistance in bovine \textit{S. aureus} was performed in the UK in 1986 and no resistance was detected in 106 isolates examined. Of these isolates, 69.8\% were \(\beta\)-lactamase producers and resistant to penicillin (16). No resistance to neomycin, erythromycin or novobiocin was detected in 186 \textit{S. aureus} isolates from bovine mastitis examined by Francis and Carroll in 1986 (17). In the Rhône-Alpes region of France in 1988, 60\% of staphylococcal isolates were found to produce a \(\beta\)-lactamase; \textit{S. aureus} strains did not in general possess other acquired mechanisms of
resistance (1). Martel and Vandaele (14) reported that 60% of 1157 coagulase-positive staphylococci isolated in France were resistant to penicillin and 5% of 411 strains were methicillin-resistant. Devriese and Hommez collected 68 methicillin-resistant *S. aureus* strains from Belgian dairy herds in the early 1970s (15). All of the isolates were apparently derived from a single strain, which was considered to be of human origin.

**E. coli**/Coliforms

VLA National Data Winter/Spring 1999

**E. coli**/coliforms from milk

Total isolates: 598  
Number showing multiple resistance: 39

<table>
<thead>
<tr>
<th>Disc Concentration</th>
<th>Number Resistant</th>
<th>Percentage Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin 10 µg</td>
<td>74</td>
<td>12</td>
</tr>
<tr>
<td>Amoxycillin/Clavulanate 20/10 µg</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Cephalexin 30 µg</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>Neomycin 10 µg</td>
<td>36</td>
<td>6</td>
</tr>
<tr>
<td>Tetracycline 10 µg</td>
<td>78</td>
<td>13</td>
</tr>
<tr>
<td>Trimethoprim/Sulphonamide 25µg</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>

The *E. coli* that infect the bovine udder tend to belong to a wide range of somatic antigen types and this is generally taken as evidence that infection does not occur with specific strains (18). Hinton reported that in seven of 30 cows in which milk and faeces were cultured, the O serogroup associated with mastitis was also found in faeces (18). The major source of *E. coli* in the cow’s environment is presumed to be the adult bovine alimentary tract, where most *E. coli* tend to be sensitive (18). One would therefore expect that the resistance of mastitis *E. coli* strains would be low and comparable with levels in strains from the adult alimentary tract (18). It is therefore interesting to compare the levels of resistance in the table above, with levels in calves and cattle older than six months (mainly faecal isolates).
**E. coli/coliforms from calves younger than 1 month old**

Total isolates: 764  
Number showing multiple resistance: 300

<table>
<thead>
<tr>
<th>Disc Concentration</th>
<th>Number Resistant</th>
<th>Percentage Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin 10 µg</td>
<td>561</td>
<td>73</td>
</tr>
<tr>
<td>Amoxycillin/Clavulanate 20/10 µg</td>
<td>184</td>
<td>24</td>
</tr>
<tr>
<td>Tetracycline 10 µg</td>
<td>604</td>
<td>79</td>
</tr>
<tr>
<td>Neomycin 10 µg</td>
<td>332</td>
<td>44</td>
</tr>
<tr>
<td>Trimethoprim/Sulphanomide 25 µg</td>
<td>323</td>
<td>42</td>
</tr>
</tbody>
</table>

**E. coli/coliforms from cattle older than 6 months**

Total isolates: 44  
Number showing multiple resistance: 5

<table>
<thead>
<tr>
<th>Disc Concentration</th>
<th>Number Resistant</th>
<th>Percentage Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin 10 µg</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Amoxycillin/Clavulanate 20/10 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetracycline 10 µg</td>
<td>11</td>
<td>25</td>
</tr>
<tr>
<td>Neomycin 10 µg</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Trimethoprim/Sulphanomide 25 µg</td>
<td>9</td>
<td>21</td>
</tr>
</tbody>
</table>

The tables confirm that the enteric flora of calves is in general more resistant than that of adults, as other workers have found (18). It should be remembered that VLA data relates mainly to field cases of disease and that the isolates from adults are likely to originate from animals with intestinal disease that may well have received treatment with antimicrobials; resistance levels may therefore be significantly higher than in healthy animals. The amoxycillin/clavulanate resistance levels are higher in the mastitis isolates than in the isolates from intestine of cattle older than 6 months. This may be a reflection of selection by intramammary treatment (19) or possibly the fact that cows are often housed at calving in close proximity to young calves in whom levels of resistance in the enteric flora are much higher than in adults. This is an interesting point, since freshly-calved cows, in whom peracute coliform mastitis can occur may be exposed on occasion to organisms showing increased resistance from calves, increasing the difficulties of treatment.
CONCLUSION

The VLA network of regional laboratories throughout England and Wales has for many years provided a diagnostic and investigation service for mastitis cases. The creation of a national antimicrobial sensitivity database will allow trends in the resistance of mastitis bacteria to be monitored and will also provide an alert for emerging bacterial resistance.

REFERENCES

BALANCING MASTITIS AND QUALITY

J. ERIC HILLERTON, Institute for Animal Health, Compton.

SUMMARY

The International Dairy Federation definition of mastitis in the dairy cow dates from 1967 and is based partly on the cell count of quarter foremilk exceeding 500,000 cells/ml. It is argued that this is unsustainable with the current knowledge of udder health, technical advances in controlling mastitis and demands for milk quality. It is proposed that a quarter cell count of 200,000 cells/ml should indicate mastitis and that for practical purposes when the whole cow milk cell count exceeds 400 000 cells/ml the milk is abnormal. These changes will reflect widespread industry practice and be supportive of likely international trading standards.

INTRODUCTION

Milk naturally contains body or somatic cells. These are largely leukocytes and some epithelial cells shed from the lining of the mammary gland. The leukocytes are derived from blood with, very simply, polymorphonuclear cells acting as a primary means of defence against an invasion of the mammary gland, macrophages involved more in immune recognition and lymphocytes responsible for immune memory. There is a plethora of evidence suggesting that the truly healthy gland of the dairy cow has a natural level of 100,000-150,000 cells/ml and more than this indicates secretory disturbance (1).

The commonest disturbances would be a new invasion of foreign material especially bacteria, an established infection usually by bacteria and a longer term response often related to damage or impaired secretory function after elimination of infection. It is possible, but uncommon, for a gland to have a cell count of 200,000-300,000 cells/ml in the absence of infection.

Cell count is affected by animal physiology and particularly breed. The effect of age is largely cumulative from previous responses to infection. Various dynamic factors influence cell count. Cell count is briefly high immediately post-partum until lactation establishes but this rarely lasts more than five days and milk is usually not acceptable for sale until four days post-partum. Cell count is affected by milking frequency, being lower in uninfected glands with more frequent milking after accommodation to the change (2). Uneven milking intervals affect cell count although the effect disappears when daily values are calculated. Cell count may also be marginally higher at the end of lactation, as yield decreases, due to lack of dilution, but stage of lactation has no real effect (3).
CELL COUNT AND INFECTION

Milk cell count has been used extensively as an indicator of the infection status of the mammary gland and in 1967 this was included as a component of the definition of mastitis (4). Infection is the major influence on cell count (5) and cell count is used as an indirect indicator of the prevalence of infection (6).

When the Five Point Mastitis Control Plan was introduced in the early 1970s the cell count of UK milk was greater than 600,000 cells/ml. This equated, from field studies, with more than 55% of all cows being infected in one or more quarters. With good mastitis control and market requirements for good quality milk, a cell count less than 150,000 cells/ml, we now have a UK bulk supply of 160,000-170,000 cells/ml but still 10-12% of all cows are infected in at least one quarter.

Figure 1. Assessment of cytological - bacteriological findings in mastitis diagnosis (4)

<table>
<thead>
<tr>
<th>Cell count per ml milk</th>
<th>Pathogenic organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not isolated</td>
</tr>
<tr>
<td>&lt;500 000</td>
<td>normal secretion</td>
</tr>
<tr>
<td>&gt;500 000</td>
<td>latent infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-specific mastitis</td>
<td></td>
</tr>
<tr>
<td>mastitis</td>
<td></td>
</tr>
</tbody>
</table>

In 1975 Prof. Tolle recognised at the IDF Mastitis Symposium in Reading (7) that the IDF definition had to be improved to take account of the factors described above. It was clear then to the participants of the meeting that the dynamics of the infection process led to variability of both cell count and bacterial recovery such that latent mastitis and nonspecific mastitis were not useful terms. The threshold levels for cell count were based on a population mean ± 2 SD for one measurement of foremilk from an individual quarter. The definition was a guide to diagnosis though 50% of truly infected quarters might at anytime have a cell count less than 500,000 cells/ml (8). It should be noted that strictly the definition applies to mastitis, the inflammatory response, and not necessarily to infection.

The use of cell count and the strict research definition of mastitis based on quarter foremilk, have rightly become part of the assessment of milk quality and this threshold was probably relevant when the bulk supplies of nations involved in extensive milk trading exceeded the threshold of mastitis. There are now significant anomalies possible. The most obvious example is that a bulk supply is unacceptable in many countries when cell count exceeds 400,000 cells/ml. It is possible that all quarters of all cows in the supplying herd have a cell count 401,000-499,000 cells/ml yet none have mastitis!

IS THE THRESHOLD OF 500,000 CELLS/ML STILL RELEVANT?

The threshold of 500,000 cells/ml is clearly not relevant and has not been for a considerable time. In the EU, bulk milk for sale is required to have a cell count less than 400,000 cells/ml. This translates into at least one of every four quarters possibly being infected (6) and mastitic, yet the
milk from these cows is still saleable. A recent survey of national milk quality levels (9) has shown that many IDF member countries now have a national average of approximately 250,000 cells/ml or better in the bulk supply. Within many of these countries buyers encourage individual producers, by financial incentives, to supply milk with a cell count less than 150,000 cells/ml.

It is almost certain that all quarter milk with a cell count less than 100,000 cells/ml contains no micro-organisms derived from the udder. There are temporary situations at the beginning and end of the lactation when this may be true for a higher cell count too. Usually cell count is raised in response to infection and it is almost certain that when cell count exceeds 200,000 cells/ml bacteria derived from the udder can be found. Typically these bacteria are existing in a commensal relationship, in a balance between excessive bacterial growth and overt stimulation of immune defence - pathogenesis. It is irrelevant whether the bacteria are ‘minor’ pathogens or ‘major’ pathogens. The gland is unhealthy and the milk is abnormal. When cell count exceeds 400,000 cells/ml then the balance is upset and the gland is truly diseased.

A grey area exists when cell count may be 100,000-400,000 cells/ml when bacteria are not isolated. This condition is usually induced by an infection that has been resolved and is a state of recuperation or results from longer-term damage to secretory tissue.

These thresholds indicating health, infection and disease are taken from various studies and are approximate. They could be refined but will not be significantly wrong. They need to be considered in terms of the health of the cow, the image of a healthy product and what can be achieved, practically. When whole country bulk milk supplies can average approximately 100,000 cells/ml, for example by Austria and Switzerland, then they are achievable (9).

**SHOULD THE CELL COUNT THRESHOLD DEFINING MASTITIS BE CHANGED?**

The thresholds should be changed because progress in management to control infection, acceptable standards of milk quality and scientific refinement of diagnosis and measurements, mean that the threshold of 500,000 cells/ml is both wrong and unsustainable.

A practical level, relevant to research, quality payments and mastitis control, is necessary. It needs to be based on whole quarter milk and not foremilk, to cope with quality measurements and to be relevant to upcoming automated screening systems, for example real-time in milking systems. It also needs to be easily related to whole udder milk as much management use is now made of readily available and frequently obtained individual cow milk cell counts. Various thresholds are currently in use including

- milk acceptability - national standards based on levels such as 400,000 or 500,000 cell/ml for bulk supplies
- milk quality - payment thresholds for bulk milk at 150,000, 250,000, 400,000 cells/ml etc.
- milk loss - recognition that yield losses start when quarter cell count exceeds 200,000 cells/ml
• mastitis control - levels for intervention, based on monthly individual cow cells count start at 200,000 cells/ml.

However, there is no evidence that any particular cell count per se has any significant effect on human health save that the higher the cell count then the greater the risk of contamination with pathogens and antibiotic residues, and the suspicion that the raw food is produced under poorer standards of hygiene.

The original definition to indicate mastitis was a quarter foremilk value largely for research purposes. A refined value is necessary for this purpose and a value is necessary for quarter whole milk, cow whole milk and the bulk supply in this more advanced dairy industry. The levels cannot be absolute to individual samples. They must cope with various forms of error by calculation from necessary repetition of measurements and averaging of values. Suitable methods are already in place in many countries for bulk supplies where rolling geometric means are employed, individual cow cell counts are used in advising mastitis control by examining sequential samples, and quarter cell counts are used in mastitis diagnosis by consideration of sequential or duplicate samples. Agreement of means and uniform adoption would be a useful agreement and recommendation.

The problems to resolve and agree are

• the threshold levels indicating mastitis and acceptable milk quality at quarter, cow and bulk supply levels

• the sampling frequency and averaging methods to be employed

• the tolerance acceptable on the thresholds.

The consideration may need to take account of the physiological and dynamic influences described earlier. However, there is evidence suggesting that physiological effects are of little consequence in truly uninfected cows (3). Certain other factors should perhaps be excluded. The effect of milking interval would tend to disappear naturally when daily values are calculated. Milking frequency is usually not a problem except for once daily milking and then there is other evidence of secretory disturbance to suggest that the milk is abnormal, and possibly, unacceptable in other ways (10). Seasonality and stress affect cell count in some situations but the major effects are from risk of infection due to increased exposure and so such effects on cell count are most likely to indicate poorer health. Allowance should not be made (11).

WHAT LEVELS?

Absolute values can be suggested from existing scientific knowledge but in many situations these will be difficult to accept for historical and cultural reasons. They are all achievable and practical. Indicators for quarter and cow cell count levels are in Figure 2.

Figure 2. Suggested relationship between average milk cell count, udder health and milk quality
Cell count threshold (‘000 cells/ml)

<table>
<thead>
<tr>
<th>Quarter</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>healthy udder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>disturbance to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>probable infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exudate/disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cow</th>
<th>acceptable</th>
<th>problem (must be &lt;10%)</th>
<th>abnormal (unacceptable ? cows)</th>
</tr>
</thead>
</table>

This suggestion is based on no new information but rather changes the definition of mastitis to what is scientifically supportable and away from a threshold based on a description of what occurs in milk production. The suggestion includes compromise in taking account of the grey areas so allowing for errors in determining the cell count, dynamic effects of physiology and infection, and population effects.

The quarter milk thresholds may be easily applied to research and evaluation of efficacy of therapeutics. The cow milk thresholds are valuable for mastitis control strategies and managing bulk milk quality. No suggestion is made for bulk milk quality as this is a commercial value but it is clear in terms of product quality, value and image that the threshold must be close to 200,000 cells/ml. When the cell count from a cow exceeds 400,000 cells/ml the infected quarter has a cell count of 1,000,000 cells/ml or greater, irrespective of a yield depression (Table 1). This means that this milk is grossly abnormal and that it should not be consigned as a food under much current legislation (10).

Table 1. Predicted quarter cell counts (‘000 cells/ml) when an uninfected quarter has a cell count less than 200,000 cells/ml, the infected quarter yield is 30% or less than the whole udder yield and for a whole cow cell count up to 1,000,000 cells/ml

<table>
<thead>
<tr>
<th>Uninfected quarter cell count yield</th>
<th>Quarter yield</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>600</th>
<th>700</th>
<th>800</th>
<th>900</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>30%</td>
<td>430</td>
<td>600</td>
<td>730</td>
<td>1100</td>
<td>1430</td>
<td>1760</td>
<td>2100</td>
<td>2360</td>
<td>2760</td>
<td>3100</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>600</td>
<td>850</td>
<td>1100</td>
<td>1600</td>
<td>2100</td>
<td>2600</td>
<td>3100</td>
<td>3600</td>
<td>4100</td>
<td>4600</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>1100</td>
<td>1600</td>
<td>2100</td>
<td>3100</td>
<td>4100</td>
<td>5100</td>
<td>6100</td>
<td>7100</td>
<td>8100</td>
<td>9100</td>
</tr>
<tr>
<td>150</td>
<td>30%</td>
<td>320</td>
<td>480</td>
<td>650</td>
<td>980</td>
<td>1320</td>
<td>1650</td>
<td>1980</td>
<td>2320</td>
<td>2650</td>
<td>2980</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>400</td>
<td>650</td>
<td>900</td>
<td>1400</td>
<td>1900</td>
<td>2400</td>
<td>2900</td>
<td>3400</td>
<td>3900</td>
<td>4400</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>650</td>
<td>1150</td>
<td>1650</td>
<td>2650</td>
<td>3650</td>
<td>4650</td>
<td>5650</td>
<td>6650</td>
<td>7650</td>
<td>8650</td>
</tr>
<tr>
<td>200</td>
<td>30%</td>
<td>200</td>
<td>370</td>
<td>530</td>
<td>860</td>
<td>1200</td>
<td>1530</td>
<td>1870</td>
<td>2200</td>
<td>2530</td>
<td>2860</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>200</td>
<td>450</td>
<td>700</td>
<td>1200</td>
<td>1700</td>
<td>2200</td>
<td>2700</td>
<td>3200</td>
<td>3700</td>
<td>4200</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>200</td>
<td>750</td>
<td>1200</td>
<td>2200</td>
<td>3200</td>
<td>4200</td>
<td>5200</td>
<td>6200</td>
<td>7200</td>
<td>8200</td>
</tr>
</tbody>
</table>
The conclusion from this is that milk should not be acceptable in the bulk supply if the cow has a cell count of greater than 400,000 cells/ml. It is difficult to argue otherwise. The situation is even worse where cows have a cell count of more than 1,000,000 cells/ml and the secretion from the infected quarter is likely to be self solid. Practical limitations have to be made as it is often difficult to detect the individual infected quarter quickly. There is a need for better mastitis detection and not least in more automated systems where there is no compliance with the legal requirement to ‘inspect the appearance of the milk’. Chronic cases are readily identifiable from individual cow cell counts and here the goalposts have already changed. The National Milk Records threshold has been applied to separate cows above and below 200,000 cells/ml for some time. This is a highly relevant threshold in line with suggestions here and in common use in good mastitis control programmes.

It may be necessary to tolerate milk from cows with a cell count of 200,000-400,000 cells/ml going in the bulk tank but it has to be appreciated that very many of these cows are infected. There is no way that consigning ‘milk’ from a cow with a cell count greater than 400,000 cells/ml, likely to have a quarter cell count of 1,000,000 cells/ml, is compliant with milk hygiene requirements. This secretion is physically abnormal. It is exudate.

SUMMARY

It is timely to redefine the threshold for quarter milk cell count for the dairy cow indicative of mastitis, probable infection and abnormal milk. It is proposed that this should be 200,000 cells/ml. Tolerance is needed for practical purposes, to cope with the frequency of measurements, accuracy of measurements and transitory effects such that a whole cow milk value of 400,000 cells/ml is proposed. Notwithstanding these values milk cannot be consigned for sale if it is physically abnormal. Considerable scientific information is available to support these proposed thresholds but this should be reviewed fully. It is unlikely that these proposed definitions will have a major impact on bulk milk supplies involved in trade but they are supportive to the suggestion for a world standard for bovine milk cell count (13).

REFERENCES

MASTITIS IN LOW SOMATIC CELL COUNT DAIRY HERDS –
preliminary results from a postal questionnaire survey

E.J. PEELER\(^1\), M.J. GREEN\(^2\), J.L FITZPATRICK\(^3\), K.L. MORGAN\(^4\) & L.E. GREEN\(^{15}\) 
\(^1\)University of Bristol, Department of Clinical Veterinary Science, Langford, Bristol BS40 5DU; \(^2\)Orchard Veterinary Group, Glastonbury; \(^3\)University of Glasgow Veterinary School; \(^4\)University of Liverpool, \(^5\)University of Warwick.

SUMMARY
A questionnaire survey of dairy farmers with low bulk tank milk somatic cell counts (BTMSCC) was carried out to assess the level of mastitis and to quantify risk factors for mastitis. Questionnaires were sent to 3009 producers with an average BTMSCC in 1997 of less than 100,000 cells/ml. One thousand eight hundred thirty nine replies were received, a 61% response rate. The average reported incidence of clinical was 22.8 cases per 100 cows/year. Simple and complex analyses were done to assess the associations between potential risk factors and a binary (yes/no) outcome variable of more than 25 cases of mastitis per 100 cows/year. The following factors, which were associated with an increased risk of disease, remained in the final logistic regression model: leaking milk in the parlour, leaking milk at other times, milking and dry cow straw yard housing (compared to cubicle housing), infrequent mucking out of the calving area (less than once a month), access to an outdoor yard, changing teat liners more often than every 6000 milkings and sometimes or always wearing rubber gloves during milking. The implications of the results for future research and the development of control programmes for mastitis are discussed.

INTRODUCTION
Mastitis is one of the most common diseases affecting dairy cattle. It is estimated that mastitis costs the UK dairy industry £200 million each year (1). However, the incidence of mastitis has been reduced from an estimated 120 cases per 100 cows/year in 1960 (2,3) to the current level of 35-45 cases per 100 cows/year (4,5). This is mainly attributed to the control of contagious pathogens, namely *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, through the introduction of improved control measures, notably the 5 point plan (6,7). The main source of infection for contagious pathogens is the infected quarter, and transmission usually takes place in the parlour. Reduction in the prevalence of contagious mastitis pathogens has been associated with a decline in the average bulk tank milk somatic cell count (BTMSCC) in the UK from 573,000 cells/ millilitre (ml) in 1971 to the most recent estimate of 180,000 cells/ml in 1996 (8). Financial incentives to reduce BTMCC accelerated this decline in the early 1990s.

Environmental pathogens survive in the cow’s environment, i.e in the soil or bedding, which is the main source of infection. The main environmental organisms are *Escherichia coli* and *Streptococcus uberis*. Recent reports from the Veterinary Laboratories Agency (VLA) of the Ministry of Agriculture, Fisheries and Food (MAFF) have highlighted the increased importance of environmental compared with contagious pathogens (quoted in 8). There is evidence from the United States (9,10,11) and the Netherlands (12,13) that low BTMSCC herds suffer a higher level of environmental mastitis, compared with herds with higher BTMCC. It is also well recognised that current control measures for mastitis are considerably less effective in controlling environmental pathogens compared with contagious
pathogens. There has been no large scale study of mastitis in low somatic cell count (SCC) herds in the UK previous to this study. The aim of this work was to identify management and other factors associated with farmer reporting of mastitis in low SCC herds.

Questionnaires are applied commonly in epidemiological investigations to collect information on disease occurrence, associated factors and opinions. They have been used successfully in studies of mastitis (14). A postal survey was chosen as the most appropriate method to collect information from a large number of producers from all parts of Great Britain.

MATERIAL AND METHODS
Selection of farms
There are currently more than 100 processing dairies purchasing milk from producers in Great Britain. Nine dairies were asked to co-operate in this study and each collected milk from at least 400 producers. One declined to participate and a further two were unable to provide the data required. The six dairies which participated finally were supplied by 22,700 producers, approximately 80% of all producers in Great Britain (15). The dairies identified low cell count producers. In this study, low cell count herds were defined as having an average three monthly rolling geometric mean BTM SCC of fewer than 100,000 cells/ml during the period December 1996 to November 1997.

Questionnaire
A pre-tested questionnaire, an explanatory letter and a business reply envelope were posted to all of the 3009 producers who fell into the low cell count category (13% of all milk producers). The dairies either provided a list of their producers or mailed them directly. A post card reminder was posted to non-respondents 3 weeks later, with the exception of farmers supplying one dairy, as the dairy declined to allow their producers to be re-mailed. One month later a second copy of the questionnaire, another explanatory letter and a business reply envelope was posted to non-respondents.

The questionnaire contained 11 pages with 65 questions. It was designed to assess the incidence of clinical mastitis and management practices in 1997 and included questions on milking routine, milking machine maintenance, housing, bedding, dry cow therapy and management of calving cows. The majority of the questions were closed, with space available to note alternatives to the options given. The questionnaire was pre-tested on ten farmers from a local veterinary practice.

Analysis of results
The records were stored and manipulated in “Access” (Microsoft Inc.) and analysed using Epi-Info version 6 (Center for Disease Control, Atlanta, USA) and STATA (Stata Corporation, College Station, USA).

The mastitis incidence was calculated as the reported number of cases of clinical mastitis per 100 cows/year. A binary (yes/no) mastitis variable was created to divide the herds into high (>25 cases per 100 cows/year) and low mastitis incidence categories (≤25 cases or fewer per 100 cows/year). The associations between disease and all potential explanatory variables were screened using bivariable chi² or t-tests as appropriate. Variables with significance probability (p≤0.2) were used in further analysis. They were grouped into 5 categories: milking preparation, milking routine, housing, bedding and management. A second stage of selection was performed within each group of risk factors. The variables were tested in a backward
stepwise elimination logistic regression model, with mastitis as the dependent variable. The statistical significance of each variable was tested using the likelihood ratio. Variables with p≤0.1 were used in further analysis. In the final stage risk factors were combined in a backward stepwise elimination logistic regression model, using p≤ 0.05 as the lower inclusion level. Biologically plausible interactions between the main effects were tested. The goodness of fit of the model was tested.

RESULTS
Response rate
Of the 3009 questionnaires mailed 1838 were returned, a response rate of 61%. One thousand seven hundred and seventy one questionnaires were usable.

Herd characteristics
The average herd size was 78 (s.d. 42). Ninety six percent of herds had Friesian Holstein cows. Cubicles and straw yards were the two main housing systems.

Figure 1. Mastitis incidence

![Mastitis incidence graph]

Mastitis data
The average number of cases of mastitis reported was 22.8 per 100 cows/year, ranging from 0.6 to 147.1 (Fig. 1). The binary mastitis variable split the herds into two groups, 68% herds having 25 cases or fewer per 100 cows/year, and 32% having more than 25 cases. The median mastitis incidence in each group was 13.1 and 36.7 cases per 100 cows/year, for the low and high incidence groups, respectively. Ninety percent of farmers reported that 10% or fewer cows with mastitis were clinically sick, and 5% of farmers reported 30% or more cows as clinically sick. The incidence of mastitis was greater in herds where more than 10% of cases were reported as clinically sick (28.8 compared to 21.4 cases per 100 cows/year, p<.001).
Seventy seven percent of farmers reported keeping mastitis records (15% of the records kept were computerised); and they reported a significantly higher level of mastitis compared with those not keeping records, 24.0 compared with 18.8 cases per 100 cows/year (p<.001). Sixty three percent of farmers reported culling cows for mastitis in 1997, whilst 43% reported culling for high SCC. The average percentage of the herd culled for mastitis and high SCC in 1997 was 2.3% and 1.6%, respectively. Approximately 7% of farmers reported mortality due to mastitis in 1997 (an overall mortality risk of 0.11%).

Table 1. Variables associated with mastitis in the second stage screening (p≤0.1)

<table>
<thead>
<tr>
<th>Association with mastitis</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking preparation variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>always / sometimes wearing rubber gloves</td>
<td>dry wiping with a cloth</td>
<td>Washing udders and drying with a cloth</td>
</tr>
<tr>
<td>post milking teat disinfection by spray</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other milking variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>increasing frequency of liner change</td>
<td>Gathering cows together before milking</td>
<td></td>
</tr>
<tr>
<td>leaking milk on entering the parlour</td>
<td>rotary parlour</td>
<td></td>
</tr>
<tr>
<td>Bedding variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>straw in milking cow accommodation</td>
<td>increasing frequency of mucking out dry cow accommodation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sawdust/wood shavings in calving area</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sawdust/wood shavings in dry cow accommodation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>increasing frequency of mucking out calving area</td>
<td></td>
</tr>
<tr>
<td>Housing variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>milking cows in straw yards</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry cows in straw yards</td>
<td></td>
<td></td>
</tr>
<tr>
<td>access of milking cows to outdoor yard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disinfecting teat end before using dry cow therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaking milk (other than in the parlour)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>high milk yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40% replacement rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>leaking milk before calving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>milking cows once a day for more than 13 day before drying off</td>
<td></td>
<td></td>
</tr>
<tr>
<td>increasing length of dry period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not offering fresh feed after both milkings</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Regression analysis
A total of 52 variables were found to be significantly associated with mastitis from the bivariable analysis. Twenty five variables were significantly associated with mastitis from the second screening stage and were tested in the final regression model (Table 1). Nine variables remained in the final model (Table 2). None of the interactions tested were found to be
statistically significant. A good fit was demonstrated between the observed and expected values.

Table 2. Final regression model for the outcome variable of mastitis (p≤0.05, n=1371)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio</th>
<th>Standard Error</th>
<th>95% C.I.</th>
<th>LRS $\chi^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaking milk (other than in parlour)</td>
<td>1.90</td>
<td>0.24</td>
<td>1.49</td>
<td>2.43</td>
<td>5.12</td>
</tr>
<tr>
<td>leaking milk on entering the parlour</td>
<td>1.84</td>
<td>0.41</td>
<td>1.20</td>
<td>2.85</td>
<td>2.79</td>
</tr>
<tr>
<td>straw yard housing for milking cows</td>
<td>1.44</td>
<td>0.24</td>
<td>1.04</td>
<td>1.98</td>
<td>2.21</td>
</tr>
<tr>
<td>byre/stalls for milking cows</td>
<td>0.55</td>
<td>0.24</td>
<td>0.23</td>
<td>1.31</td>
<td>-1.34</td>
</tr>
<tr>
<td>kennels for milking cows</td>
<td>1.09</td>
<td>0.28</td>
<td>0.66</td>
<td>1.82</td>
<td>0.34</td>
</tr>
<tr>
<td>other milking cow housing</td>
<td>1.70</td>
<td>1.32</td>
<td>0.37</td>
<td>7.79</td>
<td>0.68</td>
</tr>
<tr>
<td>straw yards housing for dry cows</td>
<td>1.34</td>
<td>0.19</td>
<td>1.01</td>
<td>1.77</td>
<td>2.05</td>
</tr>
<tr>
<td>byre/stalls for dry cows</td>
<td>2.04</td>
<td>0.79</td>
<td>0.96</td>
<td>4.36</td>
<td>1.85</td>
</tr>
<tr>
<td>kennels for dry cows</td>
<td>0.80</td>
<td>0.26</td>
<td>0.42</td>
<td>1.51</td>
<td>-0.68</td>
</tr>
<tr>
<td>other dry cow housing</td>
<td>0.48</td>
<td>0.25</td>
<td>0.17</td>
<td>1.33</td>
<td>-1.41</td>
</tr>
<tr>
<td>mucking out calving areas less frequently than once a month</td>
<td>1.38</td>
<td>0.18</td>
<td>1.06</td>
<td>1.80</td>
<td>2.40</td>
</tr>
<tr>
<td>changing liners more often than every 6000 milking</td>
<td>1.36</td>
<td>0.20</td>
<td>1.02</td>
<td>1.81</td>
<td>2.11</td>
</tr>
<tr>
<td>access to outdoor yard</td>
<td>1.34</td>
<td>0.17</td>
<td>1.04</td>
<td>1.73</td>
<td>2.30</td>
</tr>
<tr>
<td>not offering fresh feed after both milkings</td>
<td>1.30</td>
<td>0.16</td>
<td>1.02</td>
<td>1.64</td>
<td>2.17</td>
</tr>
<tr>
<td>wearing rubber gloves always/sometimes when milking</td>
<td>1.28</td>
<td>0.16</td>
<td>1.01</td>
<td>1.63</td>
<td>2.03</td>
</tr>
</tbody>
</table>

DISCUSSION
There is no widely accepted definition of a low somatic cell count dairy herd. Published studies have used BTMSSC of fewer than 150,000 (14,16,17) and 70,000 cells/ml (18) to define low cell count herds. A cut-off value of 100,000 cells/ml in this study resulted in the selection of 13% of herds.
This survey achieved a 61% response rate, which compares favourably with other published questionnaire studies (19,20). It is important to obtain a high response rate because the respondents choose to reply and thus may have different opinions, management styles and disease problems compared to those who do not respond (21). No significant difference in mastitis incidence was found between respondents to the first, second and third mailings. So there was no indication that speed of response was associated with mastitis incidence.

The average herd size was 78 cows and the average annual lactational yield per cow was 6456 litres, compared to the national averages of 75 cows and 5790 litres per cow per year (15). This would indicate that whilst low SCC herds are no different in size to the national herd milk production is more than 10% greater.

The incidence of mastitis reported (22.8 cases/100 cows/year) was considerably lower than other recent reports. DAISY (the Dairy Management System) data from 144 herds for the period 1994-6 revealed an annual incidence of more than 43 quarter cases per 100 cows/year (4). However, this represented 25.9% of cows with 1.6 quarter cases per cow per year. It is possible that farmers in the present survey recalled the number of cows affected rather than the number of cases. Since farmers who kept records reported more cases than those who kept no records, it is possible to hypothesise that recall alone results in an underestimation of mastitis incidence. An alternative explanation is that these low cell count herds experienced lower levels of mastitis. However, this is not supported by other recent work which found a positive association between BTMSSC and mastitis incidence only in herds with a BTMSSC of over 300,000 cells/ml (22), and no overall association between mastitis incidence and BTMSSC (23).

In its simplest form an odds ratio (OR) is the ratio of the chance of a disease occurring in farms exposed to a particular factor and the chance of the disease in farms not exposed. An OR of one implies no association between disease and exposure, a ratio significantly greater than one implies a positive association and a ratio less than one indicates a negative association. Simple comparisons may lead to biased estimates of OR if two, or more, exposures which are themselves associated, are both also determinants of a disease. As a result, multivariate statistical techniques are required. Conditional odds ratios, calculated from partial regression coefficients derived from logistic regression, are adjusted for all the other factors included in the regression model.

The variables which remained significant in the final model developed in this study are now discussed.

Leaking milk on entering the parlour and at other times carried the highest risks (OR 1.84 and 1.90, respectively). Mastitis caused by E. coli has been associated with cows leaking milk before calving (12, 24). In addition, it has been found that checking first streams of milk for mastitis was a risk factor for S. aureus (24) and it was concluded that the practice led to an “increased exposure” to pathogens. Leaking milk on entering the parlour may similarly increase exposure to mastitis pathogens. However, hand stripping to check the first stream of milk may result in transfer of S. aureus from the hands of the milker.

Housing dry and milking cows in straw yards resulted in a significant increased risk for mastitis compared with cubicle housing (OR 1.34 and 1.44, respectively). Other studies have also found that cows housed in straw yards have a higher incidence of mastitis, compared to
other housing systems (22,25). Infrequent mucking out of the calving area (less than once a month) was a significant risk for mastitis (OR 1.38). Cows are particularly susceptible to mastitis immediately before and after calving when environmental pathogens are the main cause (26). An increased incidence of *E. coli* mastitis, but not *S. aureus* mastitis, was found in herds where there was no disinfection of the calving area after parturition (12).

Access to an outside yard was found to be a risk factor for mastitis (OR 1.34). It has been demonstrated that poor sanitary conditions in the exercise yard were associated with increasing risk of the herd being positive for *S. agalactiae* (27). Whereas exercise has been shown to reduce individual cow SCC but not affect mastitis incidence (28). It is likely that the condition of the yard, and not merely access, affects mastitis incidence. Indeed, in this study a low frequency of scraping the yard was significantly associated with a greater mastitis risk in the bivariable analysis (first screening).

Not offering fresh feed after both the morning and evening milking was shown to be a risk factor for mastitis (OR 1.30). In the period immediately after milking the teat canal is patent and thus more vulnerable to penetration by mastitis pathogens. Offering fresh feed after milking may result in cows remaining standing after milking instead of returning to lie down in the sleeping area. Penetration of the teat canal by mastitis pathogens is more likely to occur in cows which lie down whilst the teat ducts are patent, compared with cows which remain standing after milking.

It is recommended that milk liners should be changed every 2500 milkings (29). Thus it may be considered surprising that changing liners more often than every 6000 milkings should be a risk factor for mastitis (OR 1.36). Similarly, it is generally recommended that rubber gloves be worn during milking (29). In this study sometimes or always wearing rubber gloves during milking, compared with never wearing gloves, was found to be a risk factor for mastitis (OR 1.28). It is likely that farmers with a mastitis problem start to wear gloves and change liners more frequently. Alternatively, dairymen may be less aware of soiled gloves compared with soiled hands. There is no evidence that frequent liner change may damage teats. Further investigation is required to clarify the importance of these factors.

CONCLUSION
This is the first large scale study of risk factors for mastitis in low SCC dairy herds in Great Britain. The study highlights aspects of the environment, in particular the housing system, as important risk factors for clinical mastitis. The results presented in this paper largely confirm the findings of similar work from other countries. The OR were small, which can be attributed partly to the fact that major risk factors, such as day to day management changes, stockmanship, genetics etc., were not measured. In addition data from all farms were used. A comparison of low with high incidence farms, omitting farms with a medium mastitis incidence, may have generated higher OR. The main conclusion of this study is that further control of mastitis in low SCC herds may be achieved through reducing exposure to environmental pathogens. Further analysis of the existing data by housing system should assist in developing improved control measures. However, future studies are required to investigate in more detail aspects of the environment which have been identified by this study as important risk factors for mastitis. Six hundred and ninety farmers who returned the questionnaire are currently participating in a mastitis monitoring study. It is expected that this study will generate more detailed data on the epidemiology of mastitis in low SCC herds.
ACKNOWLEDGEMENTS
We thank the dairies for their help in the study. In addition our thanks go to all the farmers who returned the questionnaire and are still participating in this research. This study was funded by the Milk Development Council.

REFERENCES

BREEDING CATTLE FOR MASTITIS RESISTANCE

J.L. FITZPATRICK1, K.E. LOGAN1, F.J. YOUNG1, M.J. STEAR1, D.J. PLATT2 & B.J. MCGUIRK3
1Department of Veterinary Clinical Studies, University of Glasgow Veterinary School; 2Department of Bacteriology, Royal Infirmary, Glasgow; 3Genus Ltd., Westmere Drive, Crewe.

SUMMARY

The development of an immunological assay where cells isolated from blood are stimulated by an *Staphylococcus aureus* antigen *in vitro* is described. Variation was observed in the ability of cells isolated from young cattle, cows and bulls to respond *in vitro* to *S. aureus*, suggesting that differences in immune responsiveness may be measured by this method. Bull progeny groups were shown to have cells with significantly different abilities to respond in the assay. There was a significant negative correlation between the immune response of bulls and their predicted transmitting ability (PTA) for somatic cell count (SCC). In other words, bulls that were high responders in the immune assay tended to have daughters with lower SCC, while bulls that were low responders in the immune assay tended to have daughters with higher SCC. The assay, therefore, shows potential for use as an indicator for selection of bulls whose progeny might be relatively resistant to sub-clinical mastitis caused by *S. aureus*. The approach currently being used to identify genes that may be important in controlling mastitis resistance and some preliminary immunological results relating to mastitis are also described.

INTRODUCTION

Modern breeding programmes for dairy cattle aim to produce more profitable cows. Indices such as PIN (Profit Index) are published to help breeders select bulls whose progeny should be more profitable than average. However, while breeding for increased milk yield and quality is desirable, it is likely that mastitis will continue to increase, although slowly, as these traits are positively correlated with mastitis incidence (1). Relatively recently, attention has also turned to considering disease resistance or susceptibility in breeding programmes for dairy cattle and this is reflected in the development of indices such as PLI (Profitable Life Index) which include longevity in addition to production traits. Some of the major diseases adversely affecting longevity in the UK are lameness, reproductive disease and mastitis (2).

Selection for cows that are generally more resistant to mastitis occurs routinely on farms when cows that are more susceptible to mastitis are selected for culling, based on a high incidence of clinical mastitis, or a persistently high SCC. This practice has been encouraged, particularly in recent years, by the Bovine Spongiform Encephalopathy OTMS, where compensation was given for cows culled at over 30 months of age. As farmers used this opportunity to cull problem cows, including those with persistently high SCC, this Scheme potentially resulted in a greater proportion of cows that may be more resistant to mastitis remaining in the herd. It is also possible, however, that low SCC may indicate that cows were not exposed to mastitis pathogens, rather than necessarily being better at responding to infection caused by them.

In some countries, especially in Scandinavia, considerable work has been done on breeding dairy cattle based on records of clinical mastitis. This can result in a reduction in clinical
mastitis as the trait is heritable, although heritability is relatively low (3) compared to traits such as milk yield. Milk SCC are an indirect measurement of sub-clinical mastitis and as this trait has a reasonably high heritability it can be used to select for a reduction in the SCC of progeny (4). Breeding for low SCC should result in a reduction in clinical mastitis as SCC and clinical mastitis are positively correlated with estimates of between 0.60-0.80 (5). Breeding companies have recently published sires PTA for SCC (6,7). The PTA is an estimate of the additive effect of the bulls’ genes on the trait in question (in the present case: SCC). It is an estimation of the deviation of progeny SCC from the mean population SCC. These data are based on mean lactation SCC of the bulls’ progeny. S. aureus is one of the major causes of sub-clinical mastitis and causes persistent infections, often of long duration (8). It is likely, therefore, that mean lactation SCC better reflects infection with S. aureus than infection with other pathogens, that are either less prevalent (e.g. Streptococcus agalactiae), or often cause intramammary infections of short duration (e.g. Escherichia coli).

It is recognised that while some cows are able to eliminate mastitis pathogens, including S. aureus, others are unable to and remain persistently infected. The immune responses primarily involved in successful elimination of S. aureus are yet to be fully elucidated but neutrophil recruitment and phagocytosis, production of antibody and cell cytotoxicity may all be important at both the local mucosal surface and in the mammary gland. T cells play an orchestrating role in immunity as they are involved in providing help to B cells to produce antibody, they kill cells and produce cytokines that attract and activate many types of immune cells. Milk somatic cells include all these immune cell types and may provide protection at the level of the udder. However, as these cells are derived from the systemic circulation, it is relevant to measure responses in cells isolated from blood, in the first instance.

Collection of data from which PTAs for SCC are calculated is both time-consuming and expensive. There is considerable potential for a predictive assay that may allow bulls to be selected for mastitis resistance in their progeny at an early stage in the bulls’ life, or conversely, to be culled if mastitis susceptibility could be expected in their progeny.

The aim of this project was to investigate if it was possible to measure immune responses, stimulated by S. aureus and measured in vitro in bulls, to predict the ability of cows to resist mastitis, measured by sires’ PTA for SCC. If a correlation between immune response in bulls and sire PTA for SCC were identified, this could potentially be used as a selection criterion for future breeding programmes. Bulls could be pre-selected before entering progeny testing, based on the result of an immunological test, suggesting increased resistance to S. aureus mastitis in their progeny.

METHODS

Blood samples were collected from healthy Holstein Friesian cows kept on commercial farms, or from bulls owned by Genus Ltd. Mononuclear cells (monocytes and lymphocytes) were isolated from blood by density centrifugation and were cultured in vitro, in specially developed media, in the presence of formalin-killed, whole, S. aureus bacteria. The S. aureus bacteria were originally isolated from a case of sub-clinical mastitis and the strain identified using a DNA fingerprinting technique. The cells were cultured with S. aureus bacteria for up to 10 days in vitro and cell proliferation stimulated by the bacteria was quantified by the incorporation of a radioactive nucleotide. The results are expressed either as counts per
minute (cpm) as measured in a 3-counter or as a stimulation index (SI) (cpm in the presence of \emph{S. aureus} divided by cpm in the absence of \emph{S. aureus}).

The sire PTA for SCC were kindly provided by the Animal Data Centre. In the case of PTA for SCC, bulls with high PTA values should have progeny with high SCC and are, therefore, undesirable, whereas bulls with low values of PTA should have progeny with low SCC and are, therefore, desirable. In the present study, bulls owned by Genus Ltd. were selected on the basis of extreme PTA for SCC; five bulls with the highest PTA (mean 13.71 +/- 3.45) and five bulls with the lowest PTA (-9.33 +/- 3.06). The cell proliferation assay induced by \emph{S. aureus} was carried out on cells isolated from these bulls. It was anticipated that choosing these samples of bulls would provide the greatest chance of detecting differences in \emph{S. aureus}-induced cell proliferation.

The cell proliferation assay induced by \emph{S. aureus} was also performed on cells isolated from approximately 60 calves of both sexes, aged approximately 6-9 months. This was to investigate if the immune response could be measured in young animals. As breeding records were available, this allowed investigation of a potential effect of sire on the response measured.

The data were analysed using general linear and mixed models (SAS, Cary, North Carolina). Stimulation indices were logged prior to analysis due to their having a non-normal distribution.

**RESULTS**

**Cows**

Cell proliferation induced by \emph{S. aureus} could be measured from day 5 of culture \emph{in vitro}. The proliferation did not occur in the absence of antigen (Figure 1). Peak cell proliferation occurred on day 9 when antigen was used at a concentration of 0.5x10^5/ml, after which the response declined gradually. The cells proliferating were shown to be T cells, including all the major cell subsets. The peak proliferative response varied among cows, and cows which were either high or low responders in the assay tended to remain either high or low when the assay was repeated (Figure 2).

**Figure 1.** Time course of the immune response in cultured cells from a single Holstein-Friesian cow to \emph{S. aureus} and in the absence of antigen
The assay was then performed on two progeny groups, each group consisting of 5 cows, and each group sired by a different Holstein Friesian bull. The response induced by *S. aureus* were shown to be significantly different between the two progeny groups (*p* < 0.05) on day 9 of culture *in vitro* and the assay was shown to have a repeatability of 73% (Figure 3). This result suggested that genetic control may be playing an important role in explaining the variation in the immune response being measured.

**Figure 3.** Immune response in cell culture of two groups of five Holstein Friesian
dairy cows, one group sired by sire A and one group sired by sire B, to \textit{S. aureus}

![Graph showing immune response in cell culture of ten Holstein-Friesian bulls to \textit{S. aureus}]

**Bulls**  
The kinetics of the responses induced by \textit{S. aureus} in bulls were similar and as variable as those of cows. The results of the proliferative assay performed on the two bull groups chosen on extremes in PTA for SCC is shown in Figure 4. The bulls with high PTA for SCC tended to be low responders (i.e. they had low SI in the assay) while bulls with low PTA for SCC tended to be high responders (i.e. they had high SI in the assay). The responses of the two bull groups were dichotomous and overlapping. Statistical analysis showed a highly significant negative correlation between PTA for SCC and logSI \((r = -0.7)\).

**Figure 4.** Immune response in cell culture of ten Holstein-Friesian bulls to \textit{S. aureus}
Calves
The results of the proliferation induced by *S. aureus*, performed on the male and female calves, are shown in Figure 5. The day of peak proliferation was day 5 when antigen was added at a concentration of $2.5 \times 10^9$/ml. The cattle showed considerable variation in the ability of their cells to respond to *S. aureus* in the assay. Statistical analysis showed there was a highly significant effect of sire ($p<0.001$), again indicating the importance of genetic control of the proliferation induced by *S. aureus*, in male and female cattle.

**Figure 5.** Immune response in cell culture of six Holstein-Charolais calves to *S. aureus*  

DISCUSSION
Future breeding programmes for dairy cattle should ideally include selection for resistance to disease in addition to selection for production and economic traits. Measurement of disease, however, is not easy and difficulties are encountered in ensuring accurate records of incidence and prevalence of diseases on farms. This is particularly so with diseases of long duration and where there is often no exact day of onset, exemplified by many types of lameness. However, measurement of sub-clinical mastitis occurs routinely on many dairy farms through routine recording of individual cow SCC. The usefulness of these data is limited by the lack of knowledge of the bacteriological cause of the sub-clinical mastitis but in the UK, it is considered that SCC generally reflects infection with contagious pathogens, particularly *S. aureus*. The recent publication of sire PTA for SCC has allowed selection of bulls for reduced sub-clinical mastitis in their progeny and it is likely that PTAs for SCC will be combined into an index with economic traits in future.
Collection of data from which PTAs for SCC are calculated is both time-consuming and expensive. There is considerable potential for a predictive assay that may allow bulls to be selected for mastitis resistance in their progeny at an early stage in the bulls' life, or conversely, to be culled if mastitis susceptibility could be expected in their progeny.

The results show that genetic control appears to play an important role in the proliferative response to *S. aureus*. Cows sired by different bulls had different responses and bulls of extreme PTA values for SCC were significantly different from each other. Bulls with the most desirable PTA for SCC were the best responders in the assay, suggesting that the cellular response measured *in vitro* may reflect the ability of bovine cells to respond to and kill *S. aureus*. This, in turn, suggests that it is possible that bulls may pass on superior immunity to their progeny, resulting in cows that are able to successfully eliminate *S. aureus* infection from their udder, or even prevent establishment of infection in the first place. The immune mechanisms whereby this might be achieved are yet to be established, but work is ongoing in this area.

The results also showed variation in the proliferative response to *S. aureus* in both male and female calves. A highly significant sire effect was identified on statistical analysis of the calves’ responses, again indicating the important role of genetics in the response measured.

This project has indicated that it may be possible to use an immune response of bulls, measured *in vitro*, to predict immunity to *S. aureus* in their progeny. By using a combination of immunogenetic and epidemiological approaches, it is hoped that gene loci may be identified that control resistance and susceptibility to *S. aureus* mastitis, and that these may be exploited to reduce mastitis in future, thus increasing economic efficiency of dairy farming and improving dairy cow welfare.

**CONCLUSIONS**

- Cell proliferation induced by *S. aureus* can be measured *in vitro* in calves, cows and bulls, potentially allowing this assay to be used as a measure of immunity to *S. aureus*

- Variation in cell proliferation was shown in calves, cows and bulls

- Bull progeny groups varied in the ability of their cells to proliferate in the presence of *S. aureus*, suggesting that genetic control of the immune response may be important

- Sire PTA for SCC were negatively correlated with the ability of their cells to proliferate in the presence of *S. aureus in vitro*: bulls with the most desirable PTA for SCC had the highest responses to *S. aureus*; bulls with the least desirable PTA for SCC had the poorest responses to *S. aureus*

- This work indicates that there is the potential to use the *in vitro* immune assay in bulls to predict sub-clinical mastitis caused by *S. aureus* in their progeny
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REFERENCES

BENEFITS FROM EARLY REMOVAL OF THE MILKING UNIT

MORTEN DAM RASMUSSEN, Danish Institute of Agricultural Sciences, Dept. of Animal Health and Welfare, Foulum, DK-8830 Tjele, Denmark.

SUMMARY

The milking unit can be detached at a milk flow rate of 400 g/min without having a negative effect on milk yield. Machine-on time is shortened and teat condition improved, and udder health does not seem to be affected. The threshold may be set even higher for cows milked more than twice daily. It is recommended that specific setting of switch points and delay times are evaluated for each farm. A good pre-milking teat preparation, a short, consistent interval until attachment, and calm cows are prerequisites for detachment at high flow rates. Cows will respond with reduced machine-on times, improved teat condition, and complete milking out.

INTRODUCTION

End of milking flow rate based detachers are used worldwide. Most of the new milking parlours are installed with automatic cluster removers (ACR) and it has become very common to use ACR in stanchion barns as well. The first portable ACR were very heavy, but as electronics have improved, ACR units have became lighter and more advanced. Today it is possible to buy ACR computer linked equipment that will give an estimate of machine-on time, milk flow, and milk yield. The claimed advantages of ACR are no over-milking, improved teat condition, labour saving, and a more consistent milking routine. Disadvantages include cost, maintenance, and reliability. The list of requirements of ACR is long and includes: reliable and handy, correct measurement of milk flow rate at end of milking, delay until detachment, adjustable switch point and delay time, initial delay time in the beginning of milking, closure and relief of vacuum before detachment but without drop of the milking unit, minimal influence on milking vacuum, no negative influence on milk quality and udder health, and a test procedure provided.

Traditionally, the cow has been regarded sufficiently milked when the milk flow rate drops below 200 g/min. However, it is still an open question whether this threshold is the best or not. Removal of clusters at higher flow rates will leave more milk in the udder but shortens the machine-on time whereas low threshold values may increase over-milking for the faster milking quarters.

This paper deals with different settings of thresholds and their influence on machine-on time, milk yield, and udder health. Threshold is used as a general term for milk flow rate at detachment whereas switch point is the flow rate at which the delay time until detachment is initiated.
THE INFLUENCE OF OVERMILKING ON TEAT CONDITION AND UDDER HEALTH

Hillerton et al. (1) observed poor teat condition in herds with over-milking. The effects were assessed by scoring teat colour, response to touch, ringing at the base of the teat, and degree of teat orifice closure. Teat colour and ringing at the base of the teat evaluated immediately after detachment showed the biggest differences between types of milking systems. However, effects were confounded with liner type, cluster, and milking system. The interaction between cluster type and over-milking was investigated in a Latin-square design (2). About one third of teats was reddish even with no over-milking but the proportion increased with 2 and 5 min of over-milking. Certain types of liners caused more ringing at the base of the teat and ringing after over-milking with these liners were more pronounced. It was concluded that avoidance of over-milking is especially important with certain milking conditions (2). It appears that as vacuum level increases and massage provided to teats during milking is reduced, the negative effects of over-milking become more pronounced. Lack of massage may be due to selection of a wrong liner for that size of teats, stiff liners, or pulsation failure. The influence of over-milking on udder health has been evaluated at several occasions. However if over-milking is associated with mastitis its effects appear to be small (3). Reverse pressure gradients across the teat canal might be related to bacterial invasion of the teat cistern. Reverse pressure gradients occur only during milking on empty teats (4) and over-milking will therefore increase the possibility for bacteria to enter the teat by this method. On average, front teats will start over-milking at a threshold value of 400 g/min and rear teats at 200 g/min (Rasmussen, unpublished data).

WHAT IS THE TRUE THRESHOLD?

Initiation of detachment at a particular milk flow rate (e.g. 200 g/min) does not ensure that the milk flow rate is that when the milking unit is detached. Apart from the switch point, the actual flow rate at removal of the milking unit depends on the final delay time and the rate of decrease in milk flow towards the end of milking. A long final delay time will cause over-milking for cows with a rapid decrease in flow rate at end of milking. Conversely, actual milk flow rate at removal of the milking unit will be less influenced by the final delay time for cows with a slow flow rate decrease. When different ACR were tested a difference in machine-on time of 1.5 min occurred between a flow rate decrease of 0.15 and 0.60 kg/min² (5). It appears that there is no relationship between chosen switch points of ACR and time of removal of the milking unit (5). Consequently, the switch point indicates the highest milk flow rate that would initiate removal of the milking unit.

There is no International Standards (ISO) test procedure for ACR that will give the correct switch points and delay times or formulas to apply to specific set-ups. Some of the difficulties that interfere with tests are: measuring technique, measuring point in respect to ACR sensors, milk or artificial test fluid, length of hoses, lifting height, and pulsating or continuous flow. Stated threshold values for different types of ACR often differ and what is a threshold of 300 g/min for one type of ACR is not necessarily the same for another type. Delay time is probably the most variable factor among types of ACR because different systems use different parameters and sensors such as electronic measurements, counts of slugs, or mechanical devices. A 10 s delay time for one type of ACR might equate to 2 s for another type. Consequently, changes in switch points and delay times have to be evaluated on each farm.
EXPERIMENTS WITH CHANGES IN SWITCH POINT AND DELAY TIME

There are very few reports on the influence of ACR on milking performance and udder health and only one in a reviewed journal. Threshold values of 200 and 400 g/min were tested in a change over experiment with 16 cows (6). The threshold value of 400 g/min compared with 200 g/min reduced the machine-on time by 0.68 min per day and had no significant effect on milk yield, but increased the amount of milk that could be milked out after automatic cluster removal. Long term studies are still needed to evaluate the influence on udder health.

Rasmussen (7) reported an experiment with 135 freshly calved cows that were split into two treatments. Group 200 cows were milked with an ACR switch point of 200 g/min and a delay time of 18 s. Group 400 cows were milked with an ACR switch point of 400 g/min and a delay time of 12 s. Treatments started four days after calving and lasted 36 weeks for first lactation cows and 12 weeks for older cows. Cows were milked in stanchion barns with high pipeline milking. The main results are shown in Table 1.

Table 1. Milking performance and udder health of cows with automatic cluster removers detaching the cluster at threshold values of 200 or 400 g/min

<table>
<thead>
<tr>
<th>Group getable</th>
<th>First lactation</th>
<th>Older cows</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
<td>400</td>
<td>200</td>
</tr>
<tr>
<td>No. of cows</td>
<td>38</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>Machine-on time, min</td>
<td>5.54*</td>
<td>5.01</td>
<td>7.90*</td>
</tr>
<tr>
<td>Energy corr. milk, kg</td>
<td>22.78</td>
<td>22.73</td>
<td>33.26</td>
</tr>
<tr>
<td>Teat end eversion, %</td>
<td>39*</td>
<td>25</td>
<td>67*</td>
</tr>
<tr>
<td>Teat thickness front, %</td>
<td>3.4</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Teat thickness rear, %</td>
<td>5.5*</td>
<td>1.1</td>
<td>-0.8</td>
</tr>
<tr>
<td>Cell count, log</td>
<td>4.94</td>
<td>4.84</td>
<td>5.11</td>
</tr>
<tr>
<td>Clinical mastitis per 100 cow days</td>
<td>0.17</td>
<td>0.25</td>
<td>0.75</td>
</tr>
<tr>
<td>Cows sub-clinically infected, %</td>
<td>37.0</td>
<td>45.7</td>
<td>40.3</td>
</tr>
<tr>
<td>Cows sub-clinically newly inf., %</td>
<td>16.4</td>
<td>15.3</td>
<td>15.0</td>
</tr>
</tbody>
</table>

* Significantly different from value of Group 400: P < 0.05.

Overall, machine-on time was reduced by 0.52 min (P< 0.05) by increasing the threshold from 200 to 400 g/min. The reduction in machine-on time seemed to be consistent throughout the lactation. Peak milk flow rate was not increased and average milk flow rate of Group 400 cows was slightly higher than of Group 200 cows. Milk yield and treatment group did not influence milk composition. Hind quarters normally have higher milk yield and take longer time to milk than fore quarters; consequently, the reduction in machine-on time of Group 400 could have reduced the proportion of milk in hind quarters, but no such change was detected. Significant differences in scores for teat end hyperkeratosis were established after only four weeks of milking of older cows and after eight weeks of milking of first lactation cows. These differences between treatment groups clearly demonstrate that the last 0.5 min of milking when teats are getting empty of milk is a sensitive period for developing hyperkeratosis. Teat end thickness increased during milking for hind teats of first lactation cows in Group 200 compared with Group 400. The same trend was observed in fore teats, but there was no significant difference in teat end thickness for older cows, although the trend seemed to be reversed. There was no difference in cell counts between the two groups. There was no difference between groups in the number of clinical cases of mastitis of first lactation.
cows or in the sub-clinical udder health status of cows. Older cows of Group 200 developed more clinical cases than Group 400 but this difference was not significant. Percent quarters sub-clinically infected during lactation were 7.4% and 9.5% and the new infection rates 4.2% and 5.8% in Group 200 and Group 400 respectively for cows not having sub-clinical or clinical infections during the first 10 days after calving. These differences were not significant. Those cows that had an infection in the early stage of lactation had 23.1 and 23.5% infected quarters compared with new infection rates of 11.7% and 7.2% (P<0.05) in Group 200 and 400 respectively. It was concluded that the milking unit could be detached at a milk flow rate of 400 g/min instead of 200 g/min without having negative influence on milk yield. Machine-on time is shortened and teat condition improved, and udder health does not seem to be affected (7).

THRESHOLD VALUES WHEN MILKING MORE THAN TWICE DAILY

Lactation milk yield was increased by 14% and 15% by giving cows access to be milked 3 or 4 times daily respectively (8). Machine-on time increased with 40 and 56% and significantly disrupted the teat ends as lactation advanced. Obviously, milking occurred on teats not full of milk. It was concluded that more attention should be paid to the milking conditions with more frequent milking. Hyperkeratosis of the teat orifice is a consequence of too much vacuum for too long a time and too a short time to recover after milking. By not attaching the milking unit when milk will not flow and removing the milking unit at a relatively high flow rate at the end of milking, the influence of milking vacuum on the teat tissue will be minimised. In a short term experiment a switch point of 400 g/min. was not sufficient to improve teat condition of cows milked four times a day but the time period (four weeks) might have been too short (9).

Early removal of the milking unit seems to be a possibility for shortening the machine-on time and preserving teat condition. High switch points of automatic cluster removers function as soon as the initial delay time is passed. Consequently, the milking unit is removed if the flow rate is lower than the pre-set switch point. In the experiment of Ipema and Benders (8) average milk flow rate was just above 1.1 kg/min. in late stage of lactation of cows milked 4 times a day. Only half of those cows would have had a chance to be milked to any extent if the switch point was set to 1 kg/min. The question remains whether the cows could be trained to deliver their milk within a short period of milking.

High threshold values are only practicable if milk flows continuously shortly after attachment. Consequently, cows must be pre-stimulated and milk ejection evoked before attachment of the milking unit. Oxytocin is released as long as milking continues and causes no discomfort to the cow. With twice daily milking pre-stimulation may be responsible for less than 5% of milk yield, whereas release of oxytocin during milking may enable extraction of more than 95% of available milk. A shorter machine-on time shortens the period of oxytocin release. It is not known if this could actually improve the oxytocin release of four times daily milking.

AMERICAN EXPERIENCES WITH HIGH THRESHOLD VALUES OF COWS MILKED 3 TIMES DAILY

The threshold value of automatic cluster removers might be set higher than 400 g/min for cows milked more frequently. An increase of the threshold value to 1.0 kg per min reduced machine-on time but did not reduce milk yield of high producing cows being milked 3 times per day. Setting of the threshold value to 0.5 kg/min for the whole herd did not influence milk yield either (Paetz, personal communication).
Two farms with three daily milkings were studied when the threshold of ACR was increased (10). Herd 1 milked 430 cows in a 2x12 herringbone parlour. The switch point was increased from 300 to 450 g/min and the delay time decreased from 12 to 7 s. Machine-on time decreased from 7.8 to 6.4 min and milk production increased slightly from 39 to 40 kg per cow per day. Managers of the herd reported that they could easily milk at least 70 more cows with the same labour cost at the new ACR settings. Herd 2 milked more than 700 cows in a 2x10 herringbone parlour. The switch point was increased stepwise from 200 to 900 g/min and the delay time decreased from 15 to 3 s. Machine-on time decreased from 7.4 to 6.2 min and milk production increased from 34 to 37 kg per cow per day. A random sample of fresh and mid to late lactation cows showed less than 100 g of milk left in all four quarters after detachment. Both dairies reported less stepping and kicking of the cows in the parlour, especially of first lactation cows, with earlier detachment.

These American field reports show successful application of early detachment of the milking unit for cows milked three times daily. The reported increases in milk yield are probably confounded with change of other factors as well but shows that the potential is there to harvest the milk in less time.

CISTERNAL CAPACITY AND MILK YIELD

The milk yield increase by milking three times a day compared with twice is reported to be 5-25%. The increase in milk yield is a response of 1) removal of the feed back inhibitor (FIL), 2) increased cell differentiation, and 3) an increase in the number of cells. First lactation cows respond in terms of percentage better to more frequent milking than older cows. Moreover, cows bred for low milk yield increased milk yield more with three daily milkings than do cows bred for high milk yields (11). The percentage of cisternal capacity might well explain why cows react differently to better milking. First lactation cows have lower cisternal capacity than older cows, and the cisternal capacity of first lactation cows increases (or at least maintains) during lactation whereas older cows decrease their capacity (12). Cows with low cisternal capacity are more sensitive to inadequate milking conditions than cows with a higher percentage, and this might explain why first lactation cows respond better to 3 daily milkings than older cows.

In a more frequent milking experiment where cows were selected for milk yield it was suggested that there has been a parallel selection of cows with a low and a high percentage of cisternal capacity respectively (11). Cows with a higher proportion of cisternal capacity are more efficient milk producers because the feed back inhibitor is active in alveolar milk only. An overall selection of cows with high milk yields and a relatively high percentage of cisternal capacity might also explain why cows 30 years ago increased milk yield with good pre-stimulation but do not today. Consequently, there is less influence of milk left as strip yield on milk production of a modern high producing dairy cow. In a half-udder experiment with moderate producing East-German cows milk yield increased by 7% in the first lactation in the udder half where machine stripping was performed and by up to 11% in the fourth lactation (13). Incomplete milking with twice daily milkings decreased milk yield even of goats with a high proportion of cisternal milk (14). Deliberately under-milking causes loss of milk yield but this phenomenon should not be confused with high settings of threshold values where cows are used to this method. If the amount of cisternal milk left after an early removal of the milking unit is less than the storage capacity of the milk lobes and cistems, milk will not be forced back in to the alveoli as the milk lobes contract after milking. The switch level of automatic cluster removers might then be set higher than 400 g/min for cows milked more frequently.
WHEN AND HOW?

Over-milking might easily be determined by observing some of the following parameters: teat colour and ringing at the base of the teat after detachment, restless or kicking cows during the late flow rate period, nervous first lactation cows, and long milk hoses or claws are empty milking. The response might be to increase threshold values and/or decrease delay time in small steps. It is also necessary to position the claw well so adjusting the load more evenly on the teats, and to use a consistent milking routine in respect to each individual cow. First lactation cows are the future, not the two old cows that are having problems with complete emptying of the udder. It is important to monitor cows with chronic udder infections and to monitor milk yield and strip yield. Strip yields of 100 ml per cow do not cause a decrease in milk yield but the proportion of cows with more than 250 ml of strip yield should not be greater than 10%.

It takes longer to milk out the last kilogram if pre-milking teat preparation is conducted less efficiently or even omitted. Consequently, a good pre-milking teat preparation, a short, consistent interval until attachment, and calm cows are a prerequisite for detachment at a high flow rate. Cows will respond with a shorter machine-on time, excellent teat condition, and proper milking out.

REFERENCES


E. COLI MASTITIS - THE PAST, THE PRESENT AND THE FUTURE

T.O. JONES BVSc, C.Biol, MIBiol, FRCVS, VLA Sutton Bonington, The Elms, College Road, Sutton Bonington, Leics. LE12 5RB

SUMMARY

1. The challenge of Escherichia coli udder infection will remain so long as cows produce faeces.

2. E. coli mastitis is a disease of wet environments. Infection is via the teat end, in a variety of situations.

3. The outcome of a case of E. coli udder infection is determined by the inflammatory response of the cow rather than the pathogenicity of the invading strain.

4. There is a case for not treating mild cases of E. coli mastitis with antibiotics if an on-farm test for E. coli endotoxin in mastitic milk proves fast and reliable. Perhaps there is a place for the farm laboratory, similar to the already accepted farm workshop.

5. E. coli udder infection towards the end of the dry period, with infected quarters presenting as clinical cases of mastitis in the following lactation, is an important aspect of the problem.

6. Vaccination should protect against acute clinical E. coli mastitis.

7. We need to find some way of effectively separating cows from their excreta, although E. coli mastitis problems are not restricted to herds with obvious bad hygiene.

“In science you can say what you like, so long as you can prove it”.

Absolute truth is something of an abstract concept. In the field of mastitis there are facts established by experimental work completed under controlled conditions with statistically valid results repeated independently by different workers, sometimes in different countries and in different years. Opinions, usually based on experience, are very common and all should be considered. They can sometimes be tested by simple logic or scientific investigation. The degree of certainty with which an opinion is expressed is not necessarily a measure of its accuracy.

For practical purposes there is no limit to knowledge. A vast amount has been published on E. coli and on mastitis during this century. Handling knowledge is a major problem and much might be achieved by correct application of what we already know, rather than further expensive research.
PRE-HISTORY

The mammals inherited the earth from the dinosaurs about 65 million years ago: humans and what can be described as cattle first appeared in the Pliocene Era, between 0.5 and 2 million years ago: the earliest evidence to date of domestication is around 6400 BC (1). _E. coli_, being found in the colon of all mammals, will also have evolved millions of years ago. The long association is likely to be a safe one under natural conditions (although pathological strains of _E. coli_ which cause enteritis have evolved) and it appears that _E. coli_ mastitis results from accidental infection (2). Strains of _E. coli_ recovered from cases of mastitis are the same as those in the cow’s environment, and would presumably attempt growth in the same way in milk in a jug as in a cow’s udder.

HISTORY

_E. coli_ (3), was first recorded in bovine mastitic milk in 1896 (4). Kitt (5) reproduced the disease by “lightely sticking” (sic) _E. coli_ to teat orifices. There are at least 35 references to _E. coli_ bovine mastitis before 1935 (6). Murphy and Hanson (7) described a three year investigation into coliform udder infection recording 79 infections in the 120 cow herd; 31 were first detected in colostrum and a proportion of known infected quarters developed clinical mastitis early in the following lactation. 59.4% of total isolates were _Aerobacter aerogenes_ and 14.5% _E. coli_. Schalm and Wood (8) described acute “coliform” (_A. aerogenes_) mastitis in a California dairy herd in sawdust yards following penicillin “blitz” treatment to eradicate _Streptococcus agalactiae_. They postulated that resulting low milk cell counts allowed udder invasion of _A. aerogenes_ from sawdust bedding and their consequent experiments tended to confirm this (9).

Up until the late 1960s, the majority of cases of subclinical and clinical mastitis in the UK were caused by _Staphylococcus aureus_ and _Str. agalactiae_. The source of both was cow’s udders, and infection spread at milking time. What later came to be called the “five point plan” was initiated in the UK and copied in many other countries and proved successful in controlling these two infections. No other pathogen has taken their place in cow’s udders. The five point plan has not been successful in controlling coliform mastitis. There has been a very substantial rise in the incidence of _E. coli_ mastitis since 1960, and it is the most common cause of fatal mastitis (10,11).

LOW CELL COUNTS

It appears impossible to initiate _E. coli_ mastitis in a quarter with an established _Str. agalactiae_ sub-clinical infection but easy to do so in a low cell count quarter, suggesting that constitutive defences are ineffective (9). (The concept of a pathogen implies an organism which can partially overcome or evade the body’s constitutive defences). The concept of the udder which is “on its guard” responding quickly to invasion by bacteria, compared to a quiescent udder has been proposed (12). The question of whether this could occur in the national herd has been raised on numerous occasions. There is certainly evidence that low cell counts can predispose to herd _E. coli_ mastitis problem (13,14); but a large number of what used to be called low cell count herds have no serious _E. coli_ mastitis problem.

BREEDING
There has been a general selection of cows for faster milking rate, which is associated with mastitis susceptibility, and is inherited (15). Long term it is likely that many diseases will be controlled by breeding disease resistant animals. Higher yielding cows in four UK dairy herds tended to develop *E. coli* mastitis, but there was no correlation with yield *per se* (16).

**MILKING PROCEDURES**

*E. coli* can enter cow’s udders in a variety of situations (17). *E. coli* does not colonise healthy teat skin (18). The teat duct is apparently the major barrier to intramammary infection (19).

When three groups of cows’ teat ends were contaminated with *E. coli* broth cultures (109 cfu’s/ml) before, during and after milking, for three weeks: 23 of 120 quarters (11/30 cows) became infected, 10 developing clinical mastitis (20). There was no difference in infection incidence between the three groups.

A significant incidence of fatal coliform mastitis in suckler cows was recorded in Northern Ireland in a 1992 survey of cow mortality (10). These cases cannot be blamed on the milking machine!

**POST MILKING TEAT DISINFECTION**

This is part of the five point plan mastitis control plan. Effective post milking teat disinfection is thought to sterilise the proximal teat canal. There are organisms normally present there as commensals (e.g. *Campylobacter bovis*) and a number of workers have postulated that these organisms could have a protective effect. For a bacterium to become established on a surface, it must first displace the established flora. There is no evidence that *E. coli* establishes in the teat canal prior to invasion of the udder. There is some evidence that post milking teat disinfection with an iodophor increased the rate of *E. coli* mastitis (21). A more comprehensive Dutch survey (22) calculated a decrease in clinical *E. coli* mastitis of 71% if post milking iodophor teat dipping was discontinued.

**DIAGNOSIS**

Although acute clinical *E. coli* mastitis can be diagnosed fairly confidently by the practising veterinary surgeon, and any peracute mastitis in a low cell count herd is likely to be environmental in origin, it is impossible to be 100% certain without laboratory confirmation. Usually this is based on isolation of *E. coli* in pure culture from mastitic milk. A high proportion of *E. coli* mastitis, particularly cases in the latter half of lactation are not acute, and the organism may have disappeared from the udder before clinical mastitis is detected. Even in some cases of peracute mastitis, numbers of organisms can be very low or absent. In these latter cases, the isolation of only three organisms from one drop of milk is significant. Aliquots of this milk can be incubated in nutrient broth and sub-cultured. If *E. coli* is isolated in pure culture, it is considered to have caused the mastitis. When organisms cannot be recovered, the sample could be tested for the presence of endotoxin. In fact this is the ideal test - *E. coli* mastitis is caused by endotoxin, not merely by the presence of the organism in the udder. The Limulus Amoeboocyte Lysate Test can be used to detect endotoxin in mastitic milk (27).
Contamination of samples is a major hazard in confirming *E. coli* mastitis because the organism is normally present in the cow’s environment. It can be particularly complicating when detecting sub-clinically infected carrier cows. The author developed a system of collecting samples from suspect quarters after the cows had been milked, when the teat canal would have been thoroughly flushed out. In some cases, milk had to be incubated at 37°, or incubated in nutrient broth and sub-cultured. If *E. coli* was recovered on culture on a number of consecutive occasions, it was taken to indicate persistent infection.

**THERAPY**

Mastitis is caused by endotoxin released from the walls of disintegrating *E. coli*. The majority of cases of *E. coli* mastitis, particularly in the second half of the lactation, are mild and cases self cure. Mild cases can return to complete normality. There is considerable evidence that antibiotics do not significantly alter the course of peracute *E. coli* mastitis. However, it would be a very brave veterinary surgeon who would not administer antibiotics, and there are also publications supporting the use of antibiotics. Administration of non antibiotic therapy may depend on the clinical assessment by the attending veterinary surgeon - i.e. cows with different presenting signs may need different therapy It is likely that antibiotics are wasted in the treatment of mild *E. coli* mastitis. In Norway, mastitic milk is tested on farm for *E. coli* endotoxin. If a positive reaction is found then no antibiotics are given. There could well be a future for this approach in this country. The test does cost money, and takes time. The danger of not treating mild coccal mastitis is that it will become established, and the herd bulk milk cell count will rise even if cases remain mild.

**THE DRY PERIOD**

The conclusion from English experimental work in the 1970s was that the dry udder was generally refractory to *E. coli* infection (23). A number of workers in the USA starting in 1943 (7), described the appearance of *E. coli* infection during the late dry period. Culturing milk samples from all newly calved cows for coliforms, and treating positive quarters with antibiotics to prevent clinical episodes in the subsequent lactation became a recommendation in veterinary textbooks by the early 1980s (c.f. 24); also sealing teats of “easy milkers” in the last third of the dry period. Only 6% of “coliform” clinical mastitis cases found during lactation occurred in quarters sub-clinically infected at calving in five UK herds (25). Very extensive work in Somerset confirmed the general eradication of existing *E. coli* udder infections at drying off and the appearance of new infections detected from two weeks prior to calving (26). Of all enterobacterial mastitis occurring in the first 100 days of lactation, 52% arose in quarters previously infected during the dry period, with the same strains of bacteria. Current recommendations include the application of teat seals in the final weeks of the dry period, rather than culture and therapy.

It is extremely important to avoid introduction of bacteria into the udder with dry cow therapy. Some organisms, including *E. coli*, but notably Pseudomonad spp., are resistant to some of the antibiotics used in dry cow therapy and will cause serious clinical mastitis.

**FEEDING/DIET**

Anecdotal observations suggested that low fibre, high protein silage, predisposed to *E. coli* mastitis in Norway (28). Controlled trials (29) did not confirm that a high protein diet predisposed to mastitis, although perhaps this was because it was balanced with carbohydrates.
E. coli mastitis often follows herd diarrhoea problems (30). Widely different faecal E. coli counts in cows on different diets were found in a herd investigation (31), the newly calved animals receiving the most concentrates having the highest counts and the most E. coli mastitis. A comprehensive study (32) revealed only slightly higher faecal E. coli counts in high yielding cows. There appears to be no correlation of late pregnancy diet with environmental mastitis (33). E. coli mastitis correlated with feeding lush spring and autumn grass in a zero grazed herd (34).

Fatty liver does not influence the severity of E. coli mastitis (35), but E. coli tend to persist in udders of cows with livers containing more than 28.3% fat. Vitamin E supplementation reduces the incidence of E. coli mastitis and selenium supplementation its duration (36); and a delayed neutrophil influx into milk and less efficient intracellular killing of E. coli was found in experimental mastitis in selenium deficient cows (37).

ELIMINATIVE BEHAVIOUR (DEFAECATION, DUNGING)
“Cattle deposit their excreta haphazardly with respect to location” (38). They make little or no effort to avoid walking through or lying in soiled areas except in so far as a freshly wet area may be cold and therefore uncomfortable. There is little to indicate that cattle are voluntarily in control of the passage of waste materials. Although defaecation may occur whilst the animal is walking or lying down, it most commonly happens when the animal is standing.

Animals whose young are born not fully formed (usually carnivores or omnivores) tend to live in “homes”, and avoid contaminating them with excreta. This does not apply to animals which evolved on grassland, and whose offspring are able to walk within hours of birth.

One of the great quantum leaps in the control of human disease was separation of excreta from drinking water and food. A number of disease problems in cattle are related to exposure to dung - a variety of foot conditions in housed cattle, environmental mastitis, worms and salmonellosis. Caged laying birds do not suffer from coccidiosis and parasitic worms in the same way as those on free range. In some racehorse studs, faeces are collected every day from paddocks as still the most effective method of controlling worms. It would be a major breakthrough if some efficient system of separating cows from their excreta could be devised. Presumably it would involve training, and there is the danger of welfare problems. A European method used for stalled cattle consists of an electrified wire across the top of cow stalls. A cow standing up and arching her back prior to defaecation and urination receives an electric shock; she will in future stand back in the channel prior to defaecation/urination. There was evidence of cleaner cattle, and better foot conditions, but also an increased incidence of anoestru and other problems. The system has been banned on welfare grounds in some countries. However, if one can clearly present farmers with a defined problem they may devise an acceptable solution.

BEDDING
Bramley and Neave (39) correlated sawdust bedding “coliform” levels with udder new infection rate and suggested that mastitis problems arose when bedding “coliform” counts reached or exceeded 10^3/gm. Klebsiella pneumonia caused 6/7 of the mastitis cases and predominated in the bedding. Bedding “coliform” numbers were static at 22°C, increased between 30-44°C and fell rapidly at 50°C. In more extensive studies a strong correlation was found between sawdust bedding K. pneumonia counts and K. pneumonia mastitis, but no
similar correlation for *E. coli* in the same herds (40,41). When sawdust bedding *E. coli* levels were maintained at 10^9/gm for 4 weeks there was an increased teat end contamination but no new “coliform” udder infections (42).

Milk leaking onto bedding from teats of high yielding cows may provide an excellent substrate for *E. coli* proliferation (43).

High yielding cows lie for longer periods than low yielders, especially in cold weather, and their body heat stimulates multiplication of *E. coli* in bedding (32). Visual assessment of bedding is a very inaccurate measure of bacterial numbers present.

Peri-parturient mastitis was associated with wet bedding in 90 Midlands herds (33). The correlation between dry bedding and absence of mastitis was high. High humidity, due to poor ventilation, overcrowding or weather increases bedding water content (44). A strong correlation between rainfall and sawdust bedding bacterial numbers was found for *K. pneumonialae* but not for *E. coli* (41). High humidity can be a major problem: optimal natural ventilation in cow housing is not sufficient to solve the problem in many parts of the country. Faecal *E. coli* counts of 10^8 have been recorded (23) and Faull *et al.* (34) described *E. coli* mastitis problems in a zero grazed herd receiving no bedding.

*E. coli* mastitis used to be considered a disease of housed cattle (45, 46), but in recent years a substantial incidence occurs during the summer. This could reflect changes in calving patterns (most dairy cows used to calf in October, and acute *E. coli* mastitis is a disease of early lactation), increased supplementary feeding during the summer or year round housing of high yielding cows.

**VACCINATION**

Historically, vaccination against mastitis has not been successful. Protective antibodies that appear in serum following vaccination do not appear in normal milk. They do enter the udder when it becomes inflamed, (when blood components enter milk). Thus they do not protect against intramammary infection, and a degree of mastitis (raised milk cell count) occurs before protection.

There are a very large number of serotypes of *E. coli*, as classified by surface antigens, and production of individual vaccines for every one would not be practical. However, the inner layer of the cell wall is common to the various serotypes of *E. coli*, (and all the Enterobacteriaceae). Naturally occurring outer cell wall deficient (rough mutant, “R”) coliforms are now used for vaccine production. These are the *E. coli* JF5 vaccine (47) and the Re-17 mutant *Salmonella typhimurium* bacterin toxoid (48). They appear not to prevent intramammary infection, but vaccinated herds have a decreased incidence of clinical mastitis due to “coliform” infections. It would appear therefore that in a herd with high *E. coli* challenge, even if vaccinated, cell count problems would remain. There would be a strong case for use of vaccines in herds with a clinical coliform mastitis problem. In one trial (49) a total of 67% of the Gram negative bacterial infections present at calving in control cows became clinical during the first 90 days of lactation compared to 20% in vaccinated cows. The vaccines appear to be safe, and do not have side effects such as suppressing milk yield.
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BEDDING SYSTEMS AND MASTITIS
JOHN HUGHES, Oakfields, Calverhall, Whitchurch, Shrops, SY13 4PJ

SUMMARY
During recent years a change seems to have taken place in the proportion of different types of pathogens causing clinical mastitis. Environmental pathogens associated with bedding appear to predominate on many farms. Straw yards are often preferred to cubicles for welfare and comfort but appear to present an increased risk of intramammary infection. During an attempt to assess the risk of mastitis, when straw was used as a bedding material, it became obvious that the nutritional status of the cows, as indicated by the consistency of the faeces, is the origin of the major environmental challenge to cows. This occurs whether the cows are housed in cubicles or straw yards.

MASTITIS PATHOGENS
Despite the dramatic fall in bulk milk cell counts over recent years, and the lower incidence of contagious mastitis caused by Staphylococcus aureus, Streptococcus agalactiae and Streptococcus dysgalactiae, mastitis continues to be one of the most important diseases of dairy cows. The financial losses incurred remain unaffordable as is the distress and disappointment when a really good animal is ruined for at least a lactation.

Emphasis is now on environmental pathogens, principally Escherichia coli and Streptococcus uberis. Traditionally they have been bracketed, but in practice they are distinct in many important ways.

Escherichia coli
This organism is derived from faeces and survives in dirty and wet conditions. Soiled bedding, feed passageways and collection yards are contaminated. The bacteria survive poorly on skin and are rapidly eliminated from the mammary gland. They are viable at 15-45°C and grow optimally at 37°C. They thrive in acidic conditions but can tolerate alkalinity up to pH 9.5.

E. coli rarely causes sub-clinical mastitis but are a major cause of clinical mastitis. Infections can vary in severity from the mild case to a systemic toxemia resulting in death. Often the most severe cases occur in recently calved cows. E. coli is poorly controlled by teat disinfection and dry cow antibiotic treatment. Prevalence appears highest in low cell count herds.

Invasion of the mammary gland may occur when milk droplets impact on a dirty teat end due to irregular vacuum fluctuations during milking. Selection for faster milking cows having a wider streak canal may have increased susceptibility.

Streptococcus uberis
This organism is more ubiquitous than E. coli. It can be isolated from teat skin, lips, vagina and faeces of the cow as well as causing sub-clinical mastitis. It seems to survive well in bedding and tolerates a wider temperature and pH range. Intramammary infections are reported most commonly near calving but sub-clinical infections can have a long duration. Infections are often intractable to antimicrobial therapy. It has been suggested that the prevalence of chronic Str. uberis infections is increasing.
Both *E. coli* and *Str. uberis* are always present in the bovine environment and proliferate in most bedding materials, especially straw. Controlling exposure to these pathogens by limiting environmental survival remains vital for the dairy farmer.

**BEDDING MATERIALS**

*Cubicles*

The UK dairy farmer usually only has access to sand, sawdust or straw for bedding. Research has clearly shown that the best material is washed sand. It is inert and provides no means for bacteria to grow and so minimises exposure of the teat end.

There is considerable evidence that both *E. coli* and *Str. uberis* readily invade the mammary gland during the dry period and cause new infections. Often these are only apparent at calving or soon afterwards when clinical mastitis occurs. There is a very strong argument for careful control of bedding of dry cows. This could be housing dry cows and heifers in well designed, sand-bedded cubicles and transferring them at calving into a box bedded with 15 cm (6 in) sand topped with clean straw.

Traditionally, calving outside in a paddock has been considered the safest option but in wet spring and autumn conditions sand cubicles are better.

Although sand has considerable advantages as a bedding material, large scale use can create problems with storage and handling of slurry. The sand in the bed must be conditioned daily and replenished often enough to keep it level with the kerb. One 1000 cow herd in Australia now uses a tractor mounted with a spiked arm to harrow daily the sand cubicles.

The common bedding alternatives, straw and sawdust, may provide a deep and soft bed but they have always presented a risk of more environmental mastitis. Straw and sawdust can, however, be used relatively safely as litter on mats covering concrete beds. Then there is little depth to the bedding and less bacterial contamination. As neither *E. coli* nor *Str. uberis* multiply in material above pH 9.5, solid beds dusted daily with lime before adding sawdust or chopped straw can be fairly safe.

**STRAW YARDS - A STUDY**

Straw yards as a housing system for dairy cows offer many advantages for welfare, especially in the control of lameness and the reduction of stress. The downside is the increased risk of environmental mastitis. A study has been undertaken, funded by the Milk Development Council and carried out in association with the Liverpool University Faculty of Veterinary Science, on selected farms in Cheshire. This has evaluated straw as a bedding material for the loose housing of dairy cows. Preliminary findings are reported here.

The type and quality of straw used to litter loose yards is obviously important. Barley straw seemed superior to wheat straw but much depends on the harvesting, storage and transport. Ideally straw should be stored under cover and moisture content should not exceed 15%. Although bales often appear normal there were occasions when these normal, bought-in, bales tested at more than 30% moisture (Figure 1, # middle of bale, > edge of bale)). Not only is such material useless for bedding but it is extremely expensive since more than one third of the
purchase is water! Devices are available to test the moisture content of straw and these seem to be good investment. Overall a moisture content of 15% or less is needed. The straw needs to be dried if the moisture is 15-20% of weight. Bales with more than 20% moisture must not be used.

Manual distribution of straw in the yard is an unpleasant task for those doing it as well as being wasteful and inefficient. Mechanical shredders condition the straw better, leaving it open and fluffy which makes an important contribution to keeping cows clean. Best use of straw is made by applying one third of the daily quantity in the morning and two thirds at night. The overall quantity for 180 days housing should be 2.5 tonnes per cow.

When examined weekly from the start of a new bed to the time of cleaning out there was virtually no occasion when the top surface did not support a population of *E. coli* and *Str. uberis* of at least 10⁶ per gram. This is considered a level at which there is a significant exposure to the teat ends. *E. coli* and *Str. uberis* survive and multiply at 15-45°C. It was found that within 14 days of establishing a new bed the layer of bedding immediately below the surface (5-7.5 cm, 2-3 in) reached optimum growth temperature (37°C) and remained at that temperature until the next clean out. The more heavily contaminated beds, generally accommodating high yielding, freshly calved cows, were quicker to heat up. The sub surface strata increased in depth as the weeks progressed to the next clean out and generated a considerable amount of heat. The heat carried the moisture as it passed to the more surface layers. After 4-6 weeks the base layer was relatively dry.

This ‘rising damp’ effect indicates the importance of good ventilation in a straw yard. The rising heat and moisture need to be dispersed as quickly as possible if the surface of the bed is to remain dry and cool. When the heat and moisture rise and the moisture is allowed to condense and drip back on to the bed the consequences are obvious. Even with the best means of natural ventilation problems can arise, e.g. on very still days in winter when the relative humidity outside is very high there is no escape of moisture from the building. On these occasions the surface of the bed can reach 98-100% relative humidity - a very sweaty bed develops. It is possible that the peaks
of environmental mastitis coincide with these occasions. It may be necessary, as in many countries where cows are housed for long periods, that mechanical air extraction is necessary.

An attempt was made to control bacterial growth in the bed surface by means of additives. Lime was used as the primary product to raise the pH to a level inhibitory of growth by E. coli and Str. uberis. Using control and test beds, increasing amounts were added over a six-month period and the response measured in terms of bacterial numbers. Unfortunately, to raise the pH sufficiently to limit the growth of E. coli and Str. uberis a daily application of lime of approximately 400 g/m² was required. At this level it was not acceptable to the operators spreading this on the bed. There was a major problem in treating adequately and evenly with such a large volume of lime each day. The situation is different in cubicles and a sufficient application can be made.

Nutritional aspects
When considering all of the aspects of bedding and mastitis it was clear in this study that the dominating factors, affecting the condition of the bedding, the cleanliness of the cows and the pathogen load, were the stage of lactation of the cows and their nutritional status. This is shown by the consistency of the faeces. The dung pat from a healthy and fit cow should be firm enough to stand with a dip in the centre and a petalled surround. Correctly fed cows producing 9000 litres of milk will produce faeces of this consistency. Unfortunately, when formulating rations, there has crept in an acceptance that high yields are synonymous with thin faeces, often diarrhoetic cows. Animals in this state tend to have soiled tails and paint faeces on to the flanks and udder. These faeces also splash on to legs and the udder especially when cows are crowded in passageways and collecting yards. Straw yards and cubicles can be pristine clean yet the cows are unacceptably dirty and always highly exposed to mastitis pathogens.

Cow cleanliness
During the study the state of cleanliness of the cows in some high yielding groups became a matter of concern. Although all herds were under similar management, there was an interesting difference in the overall cleanliness of cows between the individual herds. Also, a marked difference was noted in the condition of freshly calved animals and those just before calving. Because of these factors, it was considered necessary to find some way of comparing cleanliness of cows in the different straw yards being observed.

Looking at individual cows it is easy to define a cow as clean or dirty but much more difficult to provide a herd assessment for statistical comparison. The method devised provides a score from 1 to 5 for cows in each herd. The scores relate to flanks, legs, udders and tails (Table 1).
Table 1.  Percentage of cows at cleanliness scores 1 or 2, 3 and 4 or 5

(a) dry cows

<table>
<thead>
<tr>
<th>Herd</th>
<th>n</th>
<th>Flank</th>
<th>Leg</th>
<th>Udder</th>
<th>Tail</th>
<th>Cow</th>
</tr>
</thead>
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<td>5</td>
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<td></td>
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<td>2</td>
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(b) high yielding cows

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<th>Udder</th>
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On each farm the dry cows were substantially cleaner than early lactation cows although management was similar. The difference is likely to be associated with the volume and consistency of faeces. Herds 2, 3 and 5 had an unacceptable proportion of dirty cows and this may be related to nutritional status. Hygiene apart, if environmental mastitis incidence is related to exposure to faeces then herds 2, 3 and 5 were especially at risk.

Space allocation

To minimise the exposure to mastitis pathogens in the bedding the stocking rate for straw yards, actual bedded area available, should vary with stage of lactation rather than simply cow numbers. High yielding, freshly calved cows need at least 6.5 m² (70 ft²) each, 5.57 m² (60 ft²) is adequate for mid and late lactation cows and dry cows can remain clean on 4.6 m² (50 ft²). This space is related to the quantity and consistency of faeces and the quantity of urine produced.

There is a similar relationship in cubicle housing for the cleanliness of cubicles, bedding and cows. An increasing number of herds have limited grazing and are housed for longer. Cows are now bigger and consume more food. In consequence, the traditional movement and feeding passageways are too narrow. Movement passageways now need to be 3 m (10 ft) wide and feed passageways 4.6 m (15 ft) wide if cows are to avoid splashing of the legs and udder and the cubicle beds are not to be soiled from feet. When the cow lies one foot invariably touches the udder and so teats may only be as clean as the feet. Slatted floors and more use of automatic scrapers may also help.
CONCLUSIONS

1. A greater proportion of clinical mastitis is caused by environmental mastitis when cell count is reduced.

2. All traditional bedding materials, with the possible exception of sand, are reservoirs of environmental mastitis pathogens and exposure can only be reduced by good management.

3. The major factor influencing the level of exposure is suggested as the nutritional status of the cow measured by the consistency of faeces.

4. A successful and safe bedding system requires good ventilation, adequate space, a good diet and efficient slurry control especially if sand bedding is not used.

ACKNOWLEDGEMENTS

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MANAGING DRY COWS TO CONTROL MASTITIS

K. LARRY SMITH & J.S. HOGAN, Department of Animal Sciences, Ohio Agricultural Research and Development Center/The Ohio State University, 1680 Madison Avenue, Wooster, Ohio 44691, U.S.A.

SUMMARY
The dry period is critically important to the control of mastitis in dairy herds. Infections that are new in the dry period have a marked influence on herd SCC and the incidence of clinical cases of mastitis. Total dry cow therapy is essential to control the contagious pathogens and the environmental streptococci. Dry cow products are of little value for control of the coliform bacteria. Mastitis control is achieved by either decreasing the exposure of teat ends to pathogens or increasing the resistance of cows to infection. High quality housing is essential to reduce exposure of teat ends to environmental pathogens and the control of environmental mastitis during the dry period. The essential elements of good housing are that the housing should keep cows, clean, cool, dry and comfortable particularly during the mammary gland transition periods of drying off and calving.

INTRODUCTION
The dry period is critical to mastitis control in dairy herds. Many of the infections present early in lactation and much of the clinical mastitis occurring at calving and the first 90 to 120 days of lactation are due to infections new during the dry period (1,2,3,14). In addition, high somatic cell count problems during the first third of lactation are often due to dry period infections. Because the problems occur in lactation, many dairy producers overlook the importance of the dry period in their mastitis control program. Dry period mastitis control is essential if the goal is the production of high quality milk and maximum performance from cows. The goal of dry cow management should be to have cows calve with fewer infections than were present at drying off (14).

EPIDEMIOLOGY OF MASTITIS PATHOGENS DURING THE DRY PERIOD
More than 100 different types of microorganisms have been reported as a cause of bovine mastitis. From an epidemiological perspective, the organisms can be grouped as contagious pathogens, environmental pathogens and the skin flora opportunists (4). The contagious pathogens of primary importance are Staphylococcus aureus, Streptococcus agalactiae and in some herds Streptococcus dysgalactiae. The environmental pathogens are a heterogeneous group of microorganisms but the coliform bacteria and the environmental streptococci (primarily Streptococcus uberis) are responsible for most of the environmental mastitis problems in dairy herds. In addition, the environmental pathogens Arcanobacterium pyogenes (responsible for summer mastitis) and species of Serratia and Pseudomonas cause economically important dry period infections in many herds. The skin flora opportunists are species of staphylococci other than S. aureus and are often designated the coagulase negative staphylococci (CNS). Typical CNS infections only modestly elevate somatic cell counts (SCC) and clinical cases are relatively infrequent and mild. Successful dry period mastitis control must take into consideration the differing epidemiology of these three groups of mastitis pathogens.
Contagious pathogens
A common characteristic of the contagious pathogens is that the primary source of the pathogen in a dairy herd to infect uninfected mammary quarters is infected cows and quarters in that dairy herd (1). The only known source of *Strep. agalactiae* in dairy herds is the infected mammary gland and because of this fact, *Strep. agalactiae* is called an obligate parasite of the mammary gland. There are sources of *S. aureus* in dairy herds other than infected mammary glands but infected mammary glands are the primary source in most dairy herds (4). Other sources include man, flies, bedding, teat lesions, non-bovine animals and equipment. The relative importance of these “other sources” likely becomes more important as the percent quarters infected in the dairy herd is reduced by control methods.

Uninfected teats are most likely to be exposed to the contagious pathogens during the milking operation and at drying off many teats will be contaminated particularly when post-milking teat dipping is not practiced. In the absence of dry cow therapy the rate of new infection by the contagious pathogens will increase dramatically during the first two weeks of the dry period (2). Rates of new infection are then very low until calving and the start of regular milking and renewed exposure to the contagious pathogens.

Environmental pathogens
The important sources of environmental pathogens is not infected quarters in the dairy herd but the environments in which the cows are living and is in contrast to the contagious pathogens (5). As a result the control practices of post-milking teat dipping and total dry cow therapy are less effective control measures for the environmental pathogens. The prevalence of both the environmental streptococci and the coliform bacteria is generally highest at calving and decreases as lactation progresses. This clearly indicates the dry period as the origin of many new environmental pathogen infections (6,7,8,9).

Studies of the epidemiology of the environmental streptococci during the dry period have shown that rates of new infection are markedly elevated during the first two weeks of the dry period and during the two week period prior to calving (3). The latter is in contrast to the contagious pathogens where rate of new infection is elevated only during the period following drying off.

Rates of new infection by the coliform bacteria are 4 to 5 fold greater during the dry period than during lactation (3). Again, rate is not constant across the dry period but is elevated during the two weeks following drying off and the 2 weeks prior to calving. Mammary glands are highly resistant to coliform infections during the mid dry period when glands are fully involuted and this resistance is likely due to both elevated concentrations of immune factors in the secretions of the fully involuted mammary gland and the formation of a keratin plug in the streak canal that effectively prevents microorganisms from gaining entrance to the interior of the gland. The keratin plug is not present early in the dry period and disappears prior to calving in most cows and contributes to the high rates of new infection during these time periods. The secretions of fully involuted mammary glands contain high concentrations of immunoglobulins and the iron binding protein lactoferrin. Lactoferrin has been shown to inhibit the growth of coliform bacteria and a synergistic action between lactoferrin and antibody has been demonstrated (10,11).
Interestingly, the epidemiology of the coliform bacteria during the dry period differs among genera (8). The two primary genera/species of coliform bacteria that cause bovine mastitis are *Escherichia coli* and *Klebsiella pneumoniae*. Both the involuting and involuted mammary glands are very resistant to new infection by *E. coli* (12), most likely due to the presence of high concentrations of lactoferrin. However, the prepartum mammary gland is highly susceptible to new *E. coli* infection and is in part due to the fact that the concentration of lactoferrin decreases dramatically during the process of lactogenesis. As a result, the vast majority of *E. coli* infections present at calving and during early lactation have their origin during the two weeks prior to calving. Rates of new infection by *Klebsiella* spp. and other genera of Gram-negative bacteria are high at both ends of the dry period and their epidemiology differs markedly from that of *E. coli*.

**Coagulase negative staphylococci**

The CNS have often been referred to as minor pathogens because infections tend to be mild with minimal economic loss (4). These infections only modestly elevate SCC and infrequently cause clinical mastitis. The predominant species of CNS associated with intramammary infection are a part of the normal skin flora of cows and as such the exposure of teat ends is very high. The CNS are generally the leading cause of intramammary infection in herds practicing post-milking teat disinfection and total dry cow therapy (13). They are also almost always the leading cause of intramammary infection in heifers at first calving and thirty percent of quarters infected at calving in heifers is not uncommon.

Many new CNS infections occur during the dry period. While dry cow therapy is not without effect, the percent quarters infected at calving is almost always greater at calving than at drying off even when dry cow therapy is used. Cows at first calving have a higher percentage of quarters infected with CNS than cows of other parities and little difference is noted between parities two or greater.

**DRYING OFF COWS**

Cows in the U.S. are typically dried off to achieve a dry period of 45 to 60 days. Dry periods of shorter or longer duration generally result in reduced milk yield in the subsequent lactation. Dry periods of less than 45 days may also result in antibiotic residues in milk when cows with such short dry periods are treated with dry cow antibiotic preparations. The most recommended method of drying off cows is abrupt cessation of lactation. Typically cows will be producing 15 kg to 25 kg per day at the time of drying off.

On occasion, producers are concerned about drying off high producing cows by the abrupt cessation of lactation method. When such concerns are expressed, most mastitis research workers in the U.S. recommend that production be driven down by reducing the amount and quality of the feed being fed, restricting water intake or by physically moving the cow in an effort to drive down production prior to drying off. Intermittent milking or skipping milking is not recommended. Recommended procedures are that the last milking is decided, the cow milked out completely, teat ends are thoroughly cleaned with cotton swabs soaked in alcohol, intramammary dry cow therapy is administered, the teats dipped in an effective teat dip and the cow taken to dry cow housing.

**DRY COW THERAPY**
Dry cow therapy together with post milking teat dipping are the critical elements of mastitis control in U.S. dairy herds (1,2). In the U.S. dry cow therapy is generally administered as total dry cow therapy (77% of producers) while a small percentage (15%) will practice selective dry cow therapy. Approximately 9% of producers use no form of dry cow therapy. The advantages of total dry cow therapy over selective therapy are: 1) all infected quarters are treated at drying off; 2) many new infections are prevented during the first week or two of the dry period; and 3) there are no testing costs or labor costs associated with efforts to determine which quarters or cows should be chosen for selective dry cow therapy. Proponents of selective dry cow therapy would site as advantages: 1) reduced cost of antibiotics; 2) reduced impact on development of bacterial antibiotic resistance; 3) reduced probability of damaging streak canals during the infusion process; and 4) reduced likelihood of introducing pathogens into uninfected glands during the infusion process.

Total dry cow therapy achieves two important goals for control of contagious pathogens in dairy herds (1). Firstly, prevalence of infected quarters in the herd is significantly reduced as all infected quarters are treated and cure rates following dry cow therapy are almost always higher than cure rates during lactation. Reduced prevalence of infected quarters leads to lower exposer of uninfected teats to the contagious pathogens and reduced rates of new infection. Secondly, infusion of antibiotics into all quarters prevents many new infections from occurring during the early part of the dry period. In the absence of total dry cow therapy, the rate of new infection by contagious pathogens increases 5 to 7 fold during the first 2 weeks of the dry period compared to rates during lactation (2). The prevalence of quarters infected with contagious pathogens will increase from drying off to calving in the absence of dry cow therapy and will decrease when total dry cow therapy is administered. When heifers calve with unacceptable numbers of *S. aureus* infections, antibiotic therapy at approximately 60 days prior to calving with products formulated for dry cows may be warranted.

The impact of dry cow therapy on control of environmental mastitis pathogens is greatly reduced compared to the contagious pathogens (3). However, the high rate of new infection by the environmental streptococci during the first two weeks of the dry period can be significantly reduced by using total dry cow therapy. Total dry cow therapy does not eliminate the high rate of new infection in the two weeks prior to calving. Apparently, the antibiotics infused at drying off do not persist to the period around calving in concentrations sufficient to kill or inhibit streptococcal growth. Data have consistently shown that the percent quarters infected with the environmental streptococci will increase from drying off to calving when cows were not dry treated or selective dry cow therapy was used while the percent quarters infected at calving will generally be lower or no greater when total dry cow therapy was administered.

The dry cow therapy products available in the U.S. are of no value in the control of coliform infection during the dry period (3). The antibiotics approved for use in dairy cows in the U.S. are uniformly ineffectively against the coliform bacteria.

The majority of CNS infected quarters at drying off are apparently cured by total dry cow therapy. However, spontaneous cure rates are know to be very high. Despite the elimination of infections existing at drying off, the prevalence of infected quarters at calving is generally greater than the
prevalence at drying off (13). The majority of CNS infections present at calving are new in the dry period and management techniques to prevent these new infections are not known.

VACCINATION

The predominant coliform or Gram-negative bacteria involved in bovine mastitis in most herds is *E. coli* and the majority of these infections are new in the late dry period and early lactation (8). This clustering of *E. coli* infections around the time of calving lends itself to control by the Gram-negative vaccines based on core antigen technology (15,16). Vaccines based on the *E. coli J5* mutant are widely available and heavily used in the U.S. The immunization scheme most often recommended is vaccination at drying off, 30 days prior to calving and within 7 days of calving. The timing of the immunization attempts to maximize immunity during the peripartum period. The vaccines reduce severity of infection and clinical signs but have not been shown to reduce the rate of new infection (16).

No vaccines are as yet commercially available that can be used to successfully immunize cows and control the environmental streptococi. The environmental streptococi of greatest significance to dairy herds is *Str. uberis* and a *Str. uberis* vaccine is being tested in England and maybe of value in environmental streptococcal mastitis control (17).

There are no vaccines currently available that have been shown to be of value for control of the contagious pathogens during the dry period.

NUTRITION

Research of the past ten years has clearly shown that the dietary intake of the vitamins E, A, β-carotene and the trace minerals selenium, copper and zinc affect the immunological systems of the cow and deficiencies are clearly related to increased rates of new infection during both the dry period and during lactation (18). The majority of published evidence is for vitamin E and selenium and both vitamin E and selenium are important components of the antioxidant defenses of tissues and cells (19). Inadequate dietary concentrations lead to reduced phagocytic cell function and recruitment of phagocytic cells to sites of infection including the mammary gland. The risk of low blood and tissue concentrations of vitamin E and selenium appears to be greatest around calving, a period of known high susceptibility to the environmental pathogens. Diets of dry cows are often low in vitamin E as poor quality forages are frequently fed to dry cows. In addition, forages in many parts of the world are deficient in selenium. Inadequate concentrations of vitamin E and selenium during the dry period have been associated with increased prevalence of infected quarters at calving, higher somatic cell counts during lactation and increased cases of clinical mastitis with greater severity.

Dietary recommendations for dry cows are 1000 IU supplemental vitamin E per cow per day. Cows on good green pasture or fed fresh green forages are not likely to benefit from vitamin E supplementation. Blood plasma values of less than 3.5 μg per ml have been associated with impaired phagocytic cell function and increased intramammary infection. American Holstein cattle need 6 to 7 mg of selenium per day during the dry period and typically whole blood selenium concentrations should be 0.2 μg per ml but not greater than 0.5 μg per ml.
HOUSING
Housing facilities and management practices on farms contribute to the contamination of environments and the exposure of teats to the environmental pathogens (5). Dry cow housing is frequently substandard compared to that of the lactating cows on many dairies (20) and poorly designed facilities can contribute to increased incidence of environmental mastitis. Facilities should be designed to maximize cow comfort and minimize stress and physical injuries during all seasons of the year. High levels of pathogen exposure during periods of heightened susceptibility lead to increased herd mastitis and these conditions frequently come together during the dry period.

Organic bedding materials can contain large numbers of both the coliform bacteria and the environmental streptococci and are a significant source of teat end contamination in the environments of dairy cows (21). Organic bedding materials retain moisture and act as a food source for bacteria. Seasonal effects on bacterial numbers occur in most herds with significantly higher numbers in summer compared to winter. Wood products such as sawdust are known to promote coliform mastitis while straw bedding is a good source of the environmental streptococci. Heavy packs of bedding that contain large quantities of moisture favor the growth of pathogens and poor ventilation and overcrowding will exacerbate these conditions on farms.

Pastured cows are generally thought to be at reduced risk for environmental mastitis when compared to cows in confinement housing. However, conditions do exist in pastures that can lead to high levels of exposure to the environmental pathogens. Areas under shade trees where cows congregate can produce conditions of high exposure and pastures that are over grazed or grazed during periods of heavy rain may also lead to conditions of exposure similar to housed cattle.

Long straw used in maternity stalls or as bedding for loose housed cattle can be the source of considerable exposure of teats to the environmental streptococci. Problems with cows calving with environmental streptococci are often associated with cows calving on straw bedding packs that are heavily soiled with feces and urine. Herd problems with high somatic cell counts not associated with the contagious pathogens are frequently the result of high levels of infection caused by the environmental streptococci. Such herds are often found to have cattle in loose housing bedded with deep straw packs, again heavily soiled by feces and urine. In the northern part of the U.S. these problem herds are often associated with poorly ventilated barns and often occur in late spring when outside temperatures are warming, before cows go out to pasture or before barns can be thoroughly cleaned.

Our opinion is that dry cows should be on well groomed pastures or housed in free stalls bedded with washed sand. Sand is the ideal bedding for dairy cows, it is inorganic and does not support the growth of the environmental pathogens. Calving areas should be clean and dry and the bedding material of choice for the calving area is clean long straw. Calving pens should be thoroughly cleaned between cows.

TEAT DIPPING AND TEAT SEALS
Barrier teat dips have been introduced into the U.S. market place and claims for these products are that they control environmental mastitis during the dry period (22). Claims are that the barrier dips will remain on the teats for up to two weeks and will prevent the environmental pathogens from coming into contact with the teat end. There is little data to support such claims and there is little or no data to show that these dips prevent environmental pathogen infections during the early or late dry period. These dips are generally expensive and their interaction with the contagious pathogens is not known.

Teat seals are being investigated in New Zealand (23) and Ireland (24). Teat seals contain a very dense material that is infused into the teats following the last milking of the lactation. The material sits in the bottom of the teat and prevents pathogens from gaining entrance to the interior to the mammary gland. Recent studies have attempted to improve upon the teat seal by addition of a non-antibiotic antibacterial substance (24). The work has promise and could provide a means of dry period mastitis control that is not dependent on the use of antibiotics.

CONCLUSIONS
The dry period is critical for mastitis control in dairy herds. Total dry cow therapy is highly recommended for control of the contagious pathogens and the environmental streptococci. The impact of total dry cow therapy on CNS is marginal and there is little if any positive effect on control of coliform infections. Dry period vitamin and trace mineral nutrition can affect the resistance of dry cows to mastitis and in many dairy herds supplemental vitamin E and selenium are beneficial. Vaccines for coliform bacteria based on core antigen technology can be helpful in problem herds. Efficacious vaccines for the environmental streptococci or the contagious pathogens are not presently commercially available. Teat seals containing non-antibiotic antibacterial compounds are being tested and appear to have promise. Finally, housing is a major factor in environmental mastitis control and particular so during the dry period. Housing should be designed to keep dry cows clean, cool, comfortable, and dry. The ideal housing for dry cows is a well-ventilated structure with correctly designed free stalls, bedded with sand. Calving areas must be clean and dry.
REFERENCES