Sodium Cloxacillin for Treatment of Mastitis in Lactating Cows

W. T. DAVIS and D. C. MAPLESDEN
Squibb Agricultural Research Center
Three Bridges, NJ 08887
R. P. NATZKE
Department of Animal Science
Cornell University, Ithaca, NY 14850
W. N. PHILPOT
Louisiana Agricultural Experiment Station
Homer 71040

ABSTRACT
Sodium cloxacillin was used to treat 107 cows affected with acute mastitis and 138 affected with subclinical mastitis. Based on the application of conservative bacteriologic criteria, the cure rate was 53% for acute staphylococci mastitis and 65% for subclinical staphylococci mastitis. The cure rate for *Streptococcus agalactiae* infections was 91% in acute conditions and 92% in subclinical conditions. The cure rate was 73% for the acute infections and 88% in cases of subclinical infections when *Streptococcus spp*. "non ag" was the cause. Daily milk production had no effect on response rate. No adverse reactions due to treatment were reported by any investigator.

INTRODUCTION
Bovine mastitis continues to be the greatest cause of economic loss to milk producers (18). The average annual loss has been estimated at $70 per cow, or more than $1 billion per year for the United States. Research has shown that 75% of all cows are infected with mastitis for three-fourths of their milking life and that approximately 90 to 98% of the infections are subclinical resulting in depressed milk production and lowered milk quality (18).

Essential to any mastitis control program is the availability of anti-mastitis udder infusions for treating clinical and subclinical infections in both lactating and dry cows. Over the years, various antibacterial products have been used to control infections of the udder, silver oxide in mineral oil being one of the early preparations (13). Antibiotic preparations for udder infusion were introduced in 1945 (15). Penicillin was one of the first used and is today probably the most widely used (18).

Early treatments were directed entirely toward ameliorating acute mastitis in lactating cows (2, 4, 5, 15, 18, 21). A change in practice followed the realization that subclinical mastitis caused a depression of milk production and that treatment of subclinical cases during the dry period was more effective than treatment of acute cases in lactating cows. Mastitis control programs now seek to treat or remove all infections from the herd and to provide adequate safeguards for minimizing new infections. Nevertheless, a need still exists for effectively treating acute flare-ups of mastitis during lactation.

Effective treatment of the lactating cow requires a product that is effective against *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactia* (the latter two are commonly referred to as *Streptococcus spp*. "non ag"). Since some staphylococci produce penicillinase, which makes them resistant to treatment with the penicillins, it is desirable that an antibiotic employed in mastitis therapy be unaffected by penicillinase. A product for treating lactating cows should be nonirritating when infused into the udder. Antibiotic residues should be eliminated from the udder as quickly as possible after treatment to minimize discarding of milk contaminated with antibiotic. Since the udder is a closed system and susceptible to new infections, it is essential that no new organisms, even those usually considered to be only marginally pathogenic, be infused into the udder with the therapeutic material (9).

Sodium cloxacillin (*ACTACLOX™*) for treatment of lactating cows meets the criteria cited. Based on in vitro data, cloxacillin ap-
pared highly effective against staphylococci and streptococci and is resistant to effects of penicillinase (7). Sodium cloxacillin formulated in an aluminum monostearate gel of Neobee™ is excreted from the udder so that within 48 h after the last treatment any present is below the sensitivity of the analytical method. Milk-out and tissue residue studies were in normal and mastitic cows, young and old. The sterility of the product eliminates any concern about extraneous pathogens from the product being introduced into the udder, provided technique is aseptic during infusion.

In 1961, Doyle et al. (7) described cloxacillin as a semisynthetic penicillin resistant to penicillinase, acid stable, and with useful activity against most gram-positive bacteria. Naylor (14) and his colleagues described the chemistry, toxicology, pharmacology, and microbiology of cloxacillin. Because inherent characteristics of cloxacillin suited it for the treatment of bovine mastitis, clinical work was begun promptly. Wilson, Coats, and Brander (22) reported on the use of cloxacillin in mastitis therapy. Those early clinical trials showed that cloxacillin was highly efficacious against both streptococci and staphylococci and, unlike antibiotics used previously, was active against staphylococci that were resistant to penicillin G.

Cloxacillin is a particularly good choice for the therapy of mastitis because resistance to its action is uncommon; four investigators reported that only 40 of 3018 bacterial isolates were resistant to cloxacillin (6, 8, 10, 11). Another advantage of cloxacillin over many other antibiotics is that it is bactericidal in milk at extremely low concentrations (10).

Efficacy studies were conducted by several workers to determine the optimal dose and the best treatment regimen (2, 3, 4, 21). Since efficacy against streptococcal mastitis is consistently high, efficacy against mastitis caused by Staphylococcus aureus was used as the critical criterion. The optimal dose and regimen determined by the English workers and now commonly in use in the United Kingdom and other countries is 200 mg of sodium cloxacillin, formulated in a long-acting base infused after three milkings at 24 h intervals (1, 2, 3, 4, 21).

The use of this regimen requires disposal of the milk from treated cows for 72 h during treatment and for longer than 96 h after the last treatment. The regimen established in England is not satisfactory for use in the U. S. because the Food and Drug Administration will not allow withdrawal longer than 96 h.

Since its introduction, sodium cloxacillin has been used widely outside of the U. S. as therapy for lactating cows, and its efficacy has been confirmed by several investigators (1, 2, 3, 4, 5, 6, 16, 21). Although the experimental methods used differed (15), data reported by four investigators demonstrated the efficacy of cloxacillin.

**MATERIALS AND METHODS**

Clinical studies began in July 1972 to ascertain the efficacy of sodium cloxacillin in the treatment of lactating cows with mastitis caused by Staphylococcus aureus, Streptococcus agalactiae, or Strep. spp. "non ag." Two conditions were studied: (a) subclinical mastitis (includes chronic cows) in which infection was demonstrated only by pathogenic organisms in milk samples collected aseptically, and (b) acute mastitis in which the attention of the veterinarian was drawn to the cow by some overt evidence of mastitis.

Investigators in seven different geographic locations were chosen because they were either full-time academic investigators involved in mastitis research, or veterinary practitioners specializing in dairy work. All investigators were contacted personally by Squibb veterinarians prior to initiation of the study. At that time, details of the trial and methodology were discussed. Each investigator was provided with (a) Summary of Information for sodium cloxacillin, (b) protocols for the field testing of sodium cloxacillin, (c) case report forms; and (d) supplies of drug.

Each disposable mastitis syringe contained 300 mg of cloxacillin activity as the sodium salt, in 6 ml of a Neobee/aluminum monostearate gel. In the cows selected for treatment, each udder quarter was infused aseptically with the content of one 6-ml tube (300 mg cloxacillin) after each of three consecutive milkings.

A case report form was completed for each cow treated. Data included on the case report form were: owner and cow identification, herd management and hygiene, dates of sample
collection and microbiological reports, clinical observations, investigator's opinion, supportive therapy, and adverse reactions.

Duplicate samples of milk were collected from each quarter, prior to the time of the first treatment, for bacteriologic examination. Three organisms, Staphylococcus aureus, Streptococcus agalactiae, and Strep. spp. "non ag" (including Strep. dysgalactiae and Strep. uberis) were of particular interest. Post-treatment samples were collected in a similar manner at least 21 days after the last treatment. A quarter was infected if a pathogen was in both pretreatment samples. Infected quarters were evaluated as: (1) cured, if the organism was not detected in either of the duplicate post-treatment samples; (2) not cured, if the organism was in both post-treatment samples; and (3) no test, (a) if the post-treatment samples were not in agreement; (b) if the post-treatment sample had been taken less than 21 days after the last treatment; and (c) if additional infusions had been administered prior to the taking of the post-treatment sample.

RESULTS AND DISCUSSION

In subclinical mastitis, five investigators reported the results of treating 138 cows, or 552 quarters. Of these, 230 infected quarters could be evaluated by the established criteria (Table 1) and 36 quarters were rated as "no test."

The treatment of Staphylococcus aureus infection with sodium cloxacillin was evaluated in 74 quarters. Of these, 48 (65%) were cured. Four of the five investigators reported a cure rate greater than 50%. The overall cure rate in these trials is greater than that reported in the literature (1, 5).

The effect of cloxacin against Streptococcus agalactiae infections was evaluated in 74 quarters, of which 68 (92%) were cured. One investigator reported the evaluation of a herd that had 35 cases of Streptococcus agalactiae, but the post-treatment samples were collected 19 days after the last treatment. This was 2 days short of the 21-day minimum imposed by the protocol. Of those 35 quarters, 33 were cured, for a cure rate of 94%. If these 35 quarters had been included in the overall average, the numbers would have been 101 quarters cured of 109 quarters treated (93%).

With or without the quarters reported from this herd, the cure rate for Streptococcus agalactiae

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Quarters</th>
<th>Treated number</th>
<th>Infected number</th>
<th>Cured number</th>
<th>Treated (%)</th>
<th>Cured (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>83.3%</td>
<td>0%</td>
</tr>
<tr>
<td>B</td>
<td>120</td>
<td>60</td>
<td>22</td>
<td>21</td>
<td>77.7%</td>
<td>95.2%</td>
</tr>
<tr>
<td>C</td>
<td>44</td>
<td>18</td>
<td>10</td>
<td>20</td>
<td>66.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>D</td>
<td>188</td>
<td>52</td>
<td>20</td>
<td>32</td>
<td>80.0%</td>
<td>90.6%</td>
</tr>
<tr>
<td>E</td>
<td>160</td>
<td>92</td>
<td>16</td>
<td>25</td>
<td>90.1%</td>
<td>90.1%</td>
</tr>
<tr>
<td>Total</td>
<td>552</td>
<td>230</td>
<td>74</td>
<td>78</td>
<td>84.9%</td>
<td>91.9%</td>
</tr>
</tbody>
</table>

* TABLE 1. Sodium cloxacillin for the treatment of subclinical mastitis in lactating cows. (Condensed clinical summary.)

* Each quarter infused with 300 mg cloxacillin sodium salt in 6 ml of Neobee gel after each of three consecutive milkings.

* Includes only those quarters meeting all criteria for evaluation.
TABLE 2. Sodium cloxacillin for the treatment of acute mastitis in lactating cows.\(^a\) (Condensed clinical summary.)

<table>
<thead>
<tr>
<th>Bacteriologic criteria(^b)</th>
<th>Bacteriologic and clinical criteria</th>
<th>Investigators' opinion</th>
<th>Adverse reactions due to treatment</th>
<th>Louisiana(^d) approved products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (% cure)</td>
<td>Number (% cure)</td>
<td>None/poor (%)</td>
<td>Good/excellent (%)</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>36 52.8</td>
<td>40 50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Strep. ag.</td>
<td>56 91.0</td>
<td>62 85.5</td>
<td>15.9</td>
<td>84.1</td>
</tr>
<tr>
<td>Strep. spp &quot;non ag&quot;</td>
<td>37 73.0</td>
<td>42 71.4</td>
<td>20.9</td>
<td>79.1</td>
</tr>
<tr>
<td>Other</td>
<td>13 61.5</td>
<td>13 61.5</td>
<td>20.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Total</td>
<td>142 73.9</td>
<td>157 70.7</td>
<td>23.3</td>
<td>76.7</td>
</tr>
<tr>
<td>Infected</td>
<td>271(^c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>428</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Each quarter treated with 300 mg cloxacillin sodium salt in 6 ml of Neobee gel after each of three consecutive milkings.

\(^b\)Includes only those quarters meeting all criteria for evaluation.

\(^c\)National Institute for Research in Dairying, England. Results from treatment of cows with 200 mg sodium cloxacillin in a long-cutting base after each of 3 milkings, 24 h apart.

\(^d\)Summary of results over 7 yr with various approved products. Louisiana Hill Farm Experiment Station, LSU.

\(^e\)Includes all quarters in which infection not demonstrated by agreement of duplicate pretreatment bacteriology and samples.
was essentially equal to that reported in the literature (5, 17).

The effect of sodium cloxacillin in *Streptococcus spp.* "non ag" infections was evaluated in 78 quarters; of these, 69 (88%) were cured. The cure rate demonstrated in this test was considerably higher than that expected from reports in the literature (5, 17).

Four quarters infected with "other" organisms (two *Bacillus spp.* and two nonhemolytic *Staphylococcus spp.*) were treated with cloxacillin, and all were cured.

The amount of milk produced by treated cows had no effect on the response to treatment: 83% of 58 cows that were producing between 1 and 11 kg were cured, 85% of 127 cows producing between 11 and 22 kg were cured, and 73% of 44 cows producing between 23 and 34 kg were cured. Only one cow was producing more than 34 kg, and she too was cured.

Since treatment of the lactating cow with mastitis can be evaluated in ways other than the presence or absence of pathogenic organisms, experienced clinicians in the field of mastitis treatment were asked to judge the responses to treatment shown by the cows in this study. Their opinions paralleled closely evaluation of bacteriologic results. Of all cows classified as cured on bacteriologic results, the results of treatment were rated by the investigators as good or excellent in 95% of the cases. These ratings were without prior access to the bacteriologic results. Conversely, of those cows in which the infection had not been cured, 73% of the investigators rated the response as none or poor in 73% of the cases, and as good in 27%.

No adverse reactions were reported by any investigator.

In acute mastitis, five investigators reported treatment of 107 cows affected with acute mastitis. A total of 142 quarters treated met all the criteria for evaluation, as specified in the protocol (Table 2), and 37 quarters were rated as "no test." Acute *Staphylococcus aureus* infection was evaluated in 36 quarters, of which 19 (53%) were cured. The overall cure rate in this trial was approximately twice that reported by other investigators. Dodd (5) had reported a 27% cure rate based on the infusions of 200 mg of sodium cloxacillin formulated in a long-acting preparation, three times at 24-h intervals, and a long withdrawal period. Philpot (17), using products currently approved in the U. S., reported a 25% cure rate.

The investigators' clinical evaluation of the response to treatment of staphylococcal infections was good or excellent in 50% of the cases and none and poor in the other 50%. These evaluations correspond closely to the bacteriologic cure rate.

The effect of cloxacillin in acute *Streptococcus agalactiae* infections was evaluated in 56 quarters; of these, 51 (91%) were cured. This cure rate is comparable to those reported by English workers (2, 3, 4, 5, 6, 22) using cloxacillin (85%) but is higher than Philpot (17) using a variety of approved products (52%).

The investigators' clinical evaluation of the response to treatment of cows infected with *Streptococcus agalactiae* was good or excellent for 84% and none and poor for 16%.

Thirty-seven quarters met all the criteria for evaluation of *Streptococcus spp.* "non ag" infections, and 27 (73%) were cured. This cure rate compares favorably with the 80% cure rate reported from England (5) with cloxacillin and is significantly higher than the 33% cure rate reported from Louisiana (17).

The investigators' clinical evaluation of the response to treatment of *Strep. spp.* "non ag" infections was good or excellent for 80% and none or poor for 20%.

The cure rate did not appear to be related to the amount of milk produced by the treated cows. Of 40 infected quarters from cows producing 1 to 11 kg of milk daily, 73% were cured; of the 58 infected quarters from cows producing 11 to 22 kg of milk per day, 72% were cured; of 40 infected quarters from cows producing 23 to 34 kg of milk per day, 75% were cured; and of 4 infected quarters from cows producing more than 34 kg of milk, all 4 (100%) were cured.

No adverse reactions due to treatment were reported by any of the investigators.

Sodium cloxacillin (300 mg of activity in 6 ml of Neobee gel) infused after each of three consecutive milkings provided efficacious treatment for acute or subclinical mastitis in cows caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Strep. spp.* "non ag."

REFERENCES
1 Brander, G. C. 1963. The use of the new synthetic penicillins in the control bovine mastitis and pig