

Extended Ceftiofur Therapy for Treatment of Experimentally-Induced *Streptococcus uberis* Mastitis in Lactating Dairy Cattle

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ABSTRACT

Streptococcus uberis is an important cause of mastitis in dairy cows throughout the world, particularly during the dry period, the period around calving, and during early lactation. Strategies for controlling *Strep. uberis* mastitis are poorly defined and are currently inadequate. Objectives of the present study were to evaluate efficacy of ceftiofur, a new broad-spectrum cephalosporin antibiotic, for treatment of experimentally induced *Strep. uberis* intramammary infections (IMI) in lactating dairy cows during early lactation and to determine whether extended therapy regimens enhanced efficacy of ceftiofur. Efficacy of extended ceftiofur intramammary therapy regimens was investigated in 37 mammary quarters of 23 dairy cows that developed clinical mastitis following experimental infection with *Strep. uberis* during early lactation. Cows that developed clinical mastitis during the challenge period were allocated randomly to 3 groups representing 3 different ceftiofur treatment regimens: 2-d ($n = 7$ mammary quarters), 5-d ($n = 16$ mammary quarters), and 8-d ($n = 14$ mammary quarters) treatment regimens. For all groups, 125 mg of ceftiofur hydrochloride was administered via intramammary infusion. A bacteriological cure was defined as an experimentally infected quarter that was treated and was bacteriologically negative for the presence of *Strep. uberis* at 7, 14, 21, and 28 d posttreatment. Percentage of *Strep. uberis* IMI eliminated was 43, 88, and 100% for the 2-, 5-, and 8-d ceftiofur treatment regimens, respectively. Both the 5- and 8-d ceftiofur extended therapy treatment regimens had significantly higher bacterial cure rates than the standard 2-d ceftiofur treatment regimen. The bacterial cure rate of the 8-d ceftiofur extended therapy group was marginally better ($P = 0.052$) than the 5-d ceftiofur extended therapy group. Results of this study indicate that ceftiofur therapy was effective for eliminating *Strep. uberis* ex-

perimental IMI, and 5- and 8-d extended ceftiofur therapy regimens were more effective than the standard 2-d treatment.

(**Key words:** mastitis, *Streptococcus uberis*, ceftiofur, extended therapy)

INTRODUCTION

Acceptance and application of mastitis control programs, including teat disinfection, antibiotic therapy, and culling of chronically infected cows, have led to considerable progress in controlling mastitis caused by contagious pathogens, such as *Streptococcus agalactiae* and *Staphylococcus aureus*. However, these mastitis control procedures are less effective against environmental *Streptococcus* species and Gram-negative mastitis pathogens. Studies have shown that, as the prevalence of contagious mastitis pathogens was reduced, the proportion of IMI by environmental pathogens increased (Smith, 1983; Bramley and Dodd, 1984; Oliver and Mitchell, 1984; Smith et al., 1985). Therefore, environmental mastitis can be a major problem in many well-managed dairy farms that have successfully controlled contagious pathogens. Environmental *Streptococcus* species involved in bovine mastitis include *Streptococcus uberis*, *Streptococcus dysgalactiae* ssp. *dysgalactiae*, *Streptococcus equinus*, *Streptococcus equi*, and the *Enterococcus* species (Watts, 1988). Among the environmental streptococci, *Strep. uberis* appears to be the most prevalent (Bramley and Dodd, 1984; Hillerton et al., 1995; Bramley, 1997; Oliver et al., 1997; Jayarao et al., 1999).

Streptococcus uberis is an important cause of mastitis in dairy cows, particularly during the dry period, the period around calving, and during early lactation, which is not controlled effectively by current mastitis control practices. In the absence of antibiotic dry cow therapy, the number of new *Strep. uberis* IMI during the nonlactating period increased markedly, especially during the early portion of the nonlactating period and near calving. Antibiotic therapy at drying off reduced the rate of new *Strep. uberis* IMI during the early nonlactating period,

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but it had no effect on preventing *Strep. uberis* IMI at the end of the nonlactating period (Smith et al., 1985). Many *Strep. uberis* IMI that originate during the nonlactating period and near calving result in clinical and subclinical mastitis during early lactation. Control programs for reducing *Strep. uberis* IMI should focus on periods adjacent to the nonlactating period, where opportunities exist to develop strategies to reduce the impact of *Strep. uberis* infections in the dairy herd.

Ceftiofur is a new broad-spectrum third-generation cephalosporin antibiotic for veterinary use. Ceftiofur inhibits bacterial cell wall synthesis by interfering with enzymes essential for peptidoglycan synthesis (Hornish and Kotarski, 2002). Consequently, this new antibiotic should be effective against a wide variety of mastitis pathogens, including environmental mastitis pathogens such as *Strep. uberis*. Objectives of the present study were to evaluate efficacy of ceftiofur for treatment of experimentally induced *Strep. uberis* IMI in lactating dairy cows during early lactation and to determine whether extended ceftiofur therapy regimens enhanced efficacy.

MATERIALS AND METHODS

Holstein dairy cows ($n = 8$) in their second lactation from The University of Tennessee Middle Tennessee Experiment Station, Spring Hill, TN, and Jersey dairy cows ($n = 15$; 3 first lactation, 6 second lactation, 4 third lactation, and 2 fourth lactation cows) from the Dairy Experiment Station, Lewisburg, TN, were used in this study. Two uninfected mammary quarters of each cow were experimentally challenged with *Strep. uberis* during early lactation at 11 to 41 d after calving.

Streptococcus uberis strain UT888 was used in this study. This organism was isolated originally from a Jersey cow with chronic mastitis and was identified as described previously (Jayarao et al., 1991; Jayarao and Oliver, 1994). This strain of *Strep. uberis* causes mild clinical mastitis following experimental infection and has been studied extensively in our laboratory (Hockett et al., 2000; Rambeaud et al., 2003). *Streptococcus uberis* strain UT888 was revived from storage at -80°C . After incubation, colonies were inoculated into Todd-Hewitt broth (Becton Dickinson and Company, Franklin Lakes, NJ) and incubated for 7 h at 37°C . Following incubation, the broth culture was diluted in sterile PBS (0.01 M, pH 7.4) to provide approximately 1500 cfu/mL. Following the afternoon milking on the day of experimental challenge, 5 mL of inoculum containing *Strep. uberis* in sterile PBS were infused into 2 uninfected mammary quarters of each cow. Quarters of cows selected for bacterial challenge were free from *Strep. uberis* and other mastitis pathogens based upon microbiological analysis of quar-

ter foremilk samples obtained on 2 occasions before challenge. The bacterial suspension was infused using sterile disposable syringes fitted with sterile disposable teat cannulas. Full insertion of the teat cannula through the streak canal was used. Before inoculation, teat ends were cleaned thoroughly with swabs containing 70% ethanol. The infused inoculum was massaged upward into the gland cistern. Teats were immersed in a postmilking teat disinfectant when the previously described procedure was completed.

Quarter foremilk samples for microbiological evaluation were obtained immediately before challenge, daily for the first week following challenge, immediately before antibiotic treatment, and at 7, 14, 21, and 28 d after last treatment of microbiological evaluation. All samples were collected immediately before regular milking using standard procedures described by Harmon et al. (1990). Before sample collection, teats of cows were cleaned thoroughly, dried with individual disposable paper towels, and teat ends were sanitized with swabs containing 70% isopropyl alcohol.

Milk samples were examined following procedures recommended by the National Mastitis Council (Hogan et al., 1999) and essentially as described by Oliver et al. (1994). Briefly, foremilk samples (10 μL) from each quarter were plated onto one quadrant of a trypticase soy agar plate supplemented with 5% defibrinated sheep blood (Becton Dickinson Microbiology Systems). Plates were incubated at 37°C , and bacterial growth was observed and recorded at 24-h intervals for 3 d. Bacteria on primary culture media were identified tentatively according to colony morphologic features, hemolytic characteristics, Gram-stain reaction, and catalase test. Isolates identified presumptively as streptococci were evaluated initially for growth in 6.5% NaCl, hydrolysis of esculin, and CAMP-reaction. Streptococcal organisms were identified to the species level using the API 20 Strep System (bioMérieux Inc., Hazelwood, MO) upon first and last isolation of the organism from infected quarters.

The number of somatic cells in foremilk was determined by the Dairy Herd Improvement Association Laboratory, Knoxville, TN, from samples collected from each infected quarter immediately before antibiotic treatment and at 7, 14, 21, and 28 d after the last treatment. Milk samples (~25 mL) for SCC determination were collected before cows were milked and after conducting the strip cup evaluation and collection of milk for microbiological evaluation.

Qualified farm personnel evaluated the appearance of milk and performed a palpation (physical condition of the udder) evaluation. Farm personnel conducting the milk and udder evaluations were not aware of which treatment the cow received. Appearance of foremilk on

an individual quarter basis was evaluated before milking immediately before challenge, at every afternoon milking for the first week following challenge, immediately before antibiotic treatment, and at 7, 14, 21, and 28 d after the last treatment and scored as follows: 1 = normal, 2 = flakes, 3 = small slugs, 4 = large slugs, 5 = stringy (watery), and 6 = bloody.

A palpation (physical condition of udder) evaluation was conducted after complete milk-out at the milking immediately before challenge, at every afternoon milking for the first week following challenge, immediately before antibiotic treatment, and at 7, 14, 21, and 28 d after the last treatment and scored as follows. 1) Normal. The udder was pliable when totally milked out. Heat, pain, redness, and/or swelling were not detectable; cows exhibited no signs of discomfort. 2) Slight swelling. The udder was less pliable with some firmness, as if not totally milked out. Additional milking or stripping did not return the gland to normal. Redness, heat, and pain were generally not detectable, and cows generally did not exhibit signs of discomfort. 3) Moderate swelling. The udder was definitely firm, reddened, and warm to the touch. The udder did not return to normal size when milked out. The cow generally exhibited signs of discomfort (irritable, performed a stepping motion with feet, and/or kicked) during the prepping and milking procedures. 4) Severe swelling. The udder was very hard, red, hot, and noticeably larger than other quarters before milking with little or no change in size following milking. The cow was extremely uncomfortable and very irritable. 5) Scar tissue. The udder was generally pliable and a hardened lump was palpable and present from milking to milking over time without a change in size or location. No pain, heat, redness, or swelling was associated with this condition. 6) Edema. The udder was swollen, reddened, hard, and often extending forward toward the navel, as well as posteriorly up the rear quarters where the udder attaches to the body.

Mammary glands of cows that developed clinical mastitis following experimental infection with *Strep. uberis* UT888 were allocated randomly to one of 3 ceftiofur treatment regimens as follows: 2-d treatment (n = 7 quarters), 5-d treatment (n = 16 quarters), and 8-d treatment (n = 14 quarters). One nonchallenged mammary quarter in the ceftiofur 5-d treatment group developed clinical mastitis because of *Strep. uberis* and was included in data analysis. Distribution of experimentally challenged mammary quarters that developed clinical mastitis was as follows: 9 right front, 7 right rear, 12 left rear, and 9 left front. Infected mammary glands of each cow were treated with 125 mg of ceftiofur hydrochloride (Pfizer Animal Health, Kalamazoo, MI) per 10 mL plasset administrated via intramammary infusion. Current manufacturers' recommendations call for 2 infu-

sions of ceftiofur per infected quarter at a 24-h interval (standard 2-d treatment regimen). Cows in the 5-d treatment regimen were treated once daily with 125 mg of ceftiofur per infected quarter for 5 consecutive days. Cows in the 8-d treatment regimen were treated once daily with 125 mg of ceftiofur per infected quarter for 8 consecutive days. Milk was discarded for 7 d after *Strep. uberis* challenge and for 14 d during the treatment and posttreatment period from all cows in the study.

Mammary glands were considered to have clinical mastitis when the udder or milk score was 3 or higher on at least one occasion during the first week after challenge. Mammary glands of cows with an udder and milk score of 4 for 2 consecutive days were treated prior to 7 d after challenge, and all infected mammary quarters were treated by 7 d after challenge.

A bacteriological cure was defined as an experimentally infected mammary gland that was treated with ceftiofur and was bacteriologically negative for *Strep. uberis* at 7, 14, 21, and 28 d posttreatment. A clinical cure was defined as an experimentally infected mammary gland that developed clinical mastitis that was treated with ceftiofur and did not exhibit abnormal milk or abnormal udder swelling at or beyond 14 d after last treatment. An infected quarter was considered a combination cure if it was both a bacterial cure and a clinical cure. The percentage of *Strep. uberis* IMI eliminated in quarters receiving extended ceftiofur therapy (5-d treatment regimen and 8-d treatment regimen) was compared with the percentage of *Strep. uberis* IMI eliminated in quarters receiving the standard 2-d treatment regimen. The percentage of *Strep. uberis* IMI eliminated in the 8-d extended therapy regimen was compared with the percentage of IMI eliminated for mammary quarters receiving 5-d extended therapy regimen.

Binary data were analyzed utilizing multidimensional contingency table methodology (PROC FREQ and PROC GENMOD, SAS Version 8.2) that compared cure rates between treatments by use of χ^2 tests. Herds and cows were examined to determine whether they could be eliminated and data pooled without a loss of information. For each binary end point, if it was possible to pool across cows within each herd by treatment group, the resulting 3-factor contingency table was herd \times treatment \times response. To test whether there was a herd effect, a 2-step analysis was conducted. The first hypothesis tested was that there was no herd \times treatment interaction. If this hypothesis was not significant, then the second hypothesis, that there was no herd effect, was tested. If this second hypothesis was also not significant, then herds were considered to be conditionally independent of treatments. In this case, the treatment \times response information could be pooled over herds, and the Likelihood Ratio χ^2 statistic for 2-way tables was the appropriate statistic

to compare treatments. When there was no herd \times treatment interaction, but there was a significant herd effect, treatment comparisons were made adjusting for herds. In this case, the Mantel-Haenszel χ^2 statistic was used with herds as strata. A general linear mixed model (PROC MIXED, SAS Version 8.2) was used to analyze the logarithm of SCC data as a repeated measure on time. The strategy was to identify the most parsimonious model that included herds, treatment groups, the bacterial cure-fail result of the mammary quarter, and the sampling day posttreatment when the observation was taken. These factors, along with their interactions, were considered fixed effects, whereas cows within herd \times treatment group were random. Starting with the “saturated” model that included main effects along with all 2-, 3-, and 4-way interactions, a backward step-wise process was followed in which the model effect that had the highest P value that was not below the significance level of $\alpha = 0.10$ was dropped until all terms remaining had a significant effect on the dependent variable. Main effects and lower order interactions were not eligible for deletion as long as a higher order interaction that included them was still in the model.

RESULTS

Mammary glands of cows with a palpation (physical condition of the udder) and milk score of 4 for 2 consecutive days were treated prior to 7 d after challenge. Two mammary quarters of 2 cows that developed clinical mastitis were treated 4 d after challenge, 7 mammary quarters of 5 cows were treated 5 d after challenge, 5 mammary quarters of 4 cows were treated 6 d after challenge, and 23 mammary quarters of the remaining 12 cows were treated 7 d after challenge.

The primary purpose of this study was to compare bacterial cure rates of extended ceftiofur therapy with the standard 2-d ceftiofur treatment regimen. Results of treatment groups were as follows: 3 of 7 (43%) *Strep. uberis* IMI eliminated in the standard 2-d treatment regimen, 14 of 16 (88%) *Strep. uberis* IMI eliminated in the 5-d treatment regimen, and 14 of 14 (100%) *Strep. uberis* IMI eliminated in the 8-d treatment regimen (Table 1). Both of the extended therapies had higher cure rates than the standard 2-d ceftiofur treatment regimen ($P = 0.014$ for the 2-d treatment regimen vs. the 5-d treatment regimen and $P = 0.001$ for the 2-d treatment regimen vs. the 8-d treatment regimen). The cure rate of the 8-d ceftiofur extended therapy treatment regimen was marginally better than the 5-d extended therapy regimen ($P = 0.052$; Table 1).

A reduction in milk scores and palpation (physical condition of udder) scores was observed at 7 d after the final treatment in cases for which *Strep. uberis* was suc-

Table 1. Pairwise comparison of *Streptococcus uberis* bacteriological cure rates of extended ceftiofur treatment regimens with the standard 2-d ceftiofur treatment regimen.¹

Ceftiofur treatment regimen	No. (%) cured ²	Ceftiofur treatment regimen		
		2 d	5 d	8 d
		P^3		
2 d	3/7 (43)	—	0.014	0.001
5 d ⁴	14/16 (88)	0.014	—	0.052
8 d	14/14 (100)	0.001	0.052	—

¹Data were analyzed using PROC GENMOD in SAS Version 8.2. There was no evidence of intracow variation ($P = 0.460$, 17 degrees of freedom). Therefore, treatment group pooled cure rates over cows within each herd. For the subsequent analysis, herds were conditionally independent of treatments ($P = 0.494$ for test of no herd \times treatment interaction, 2 degrees of freedom; and $P = 0.475$ for the test of no herd effect, 1 degree of freedom. Each test used $\alpha = 0.05$). Treatments were compared using Likelihood Ratio χ^2 statistic.

²Number of bacteriological cures divided by total number of infections.

³One-sided P value comparing treatment listed in row with treatment listed in the column.

⁴One nonchallenged mammary quarter in the ceftiofur 5-d treatment group developed clinical mastitis caused by *Strep. uberis* and was included in data analysis.

cessfully eliminated and where treatment was unsuccessful at eliminating *Strep. uberis*. A further reduction was observed in milk and palpation (physical condition of udder) scores as time after last treatment increased (data not shown). Milk appearance and palpation (physical condition of udder) data of each infected mammary gland were used to determine mammary quarter clinical cures (Table 2). A mammary quarter was considered to be clinically cured by 14 d after last antibiotic treatment if both the palpation (physical condition of udder) and milk score of each challenged mammary gland became “normal” and remained so through 28 d after last antibiotic treatment. Results of treatment groups were as follows: 3 of 7 clinical cures in the standard 2-d treatment regimen, 12 of 16 clinical cures in the 5-d treatment regimen, and 11 of 14 clinical cures in the 8-d treatment regimen. The percentage of clinical cures was 43, 75, and 79% in the 2-d treatment regimen, 5-d treatment regimen, and 8-d treatment regimen groups, respectively. Neither the 5-d treatment regimen nor the 8-d extended ceftiofur treatment regimen was significantly greater than the standard 2-d ceftiofur treatment regimen (Table 2).

Combination cures of extended therapy with the standard 2-d ceftiofur treatment regimen were also determined (Table 3). A mammary quarter was considered to be a combination cure if it was both a bacterial cure and a clinical cure. Results of treatment groups were as follows: 3 of 7 combination cures in the standard 2-d treatment regimen, 11 of 16 combination cures in the 5-d treatment regimen, and 11 of 14 combination cures in

Table 2. Pairwise comparison of clinical cure rates of extended ceftiofur treatment regimens with the standard 2-d ceftiofur treatment regimen.^{1,2}

Ceftiofur treatment regimen	No. (%) cured ³	Ceftiofur treatment regimen		
		2 d	5 d	8 d
2 d	3/7 (43)	—	0.190	0.149
5 d ⁵	12/16 (75)	0.190	—	0.350
8 d	11/14 (79)	0.149	0.350	—

¹An infected quarter was considered clinically cured if it did not exhibit abnormal milk or abnormal udder swelling at or beyond 14 d after last treatment.

²Data were analyzed using PROC FREQ and PROC GENMOD in SAS Version 8.2. There was no evidence of intracow variation at the $\alpha = 0.05$ level ($P = 0.126$, 17 degrees of freedom). Therefore, cure rates were pooled over cows within each herd \times treatment group. For the subsequent analysis, whereas there was no herd \times treatment interaction ($P = 0.498$, 2 degrees of freedom), there was a significant herd effect ($P = 0.015$, 1 degree of freedom). Each of these tests used $\alpha = 0.05$. Treatments were compared using the Mantel-Haenszel statistic with herds as strata.

³Number (%) of clinical mammary quarters cured divided by total number of clinical mammary quarters treated.

⁴One-sided P value comparing treatment listed in row with treatment listed in the column.

⁵One nonchallenged mammary quarter in the ceftiofur 5-d treatment group developed clinical mastitis and was included in data analysis.

Table 3. Pairwise comparison of combination cure rates of extended ceftiofur treatment regimens with the standard 2-d ceftiofur treatment regimen.^{1,2}

Ceftiofur treatment regimen	No. (%) cured ³	Ceftiofur treatment regimen		
		2 d	5 d	8 d
2 d	3/7 (43)	—	0.122	0.053
5 d ⁵	11/16 (69)	0.122	—	0.271
8 d	11/14 (79)	0.053	0.271	—

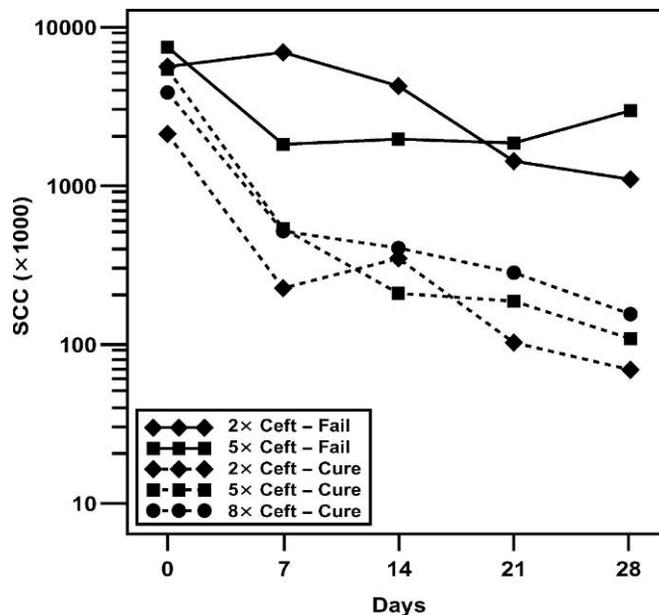
¹An infected mammary quarter was considered a combination cure if it was both a bacterial cure and a clinical cure.

²Data were analyzed using PROC FREQ and PROC GENMOD in SAS Version 8.2. There was no evidence of intracow variation at the $\alpha = 0.05$ level ($P = 0.121$, 17 degrees of freedom). Therefore, treatment group pooled cure rates over cows within each herd. For the subsequent analysis, herds were conditionally independent of treatments ($P = 0.268$ for the test of no herd \times treatment interaction, 2 degrees of freedom; and $P = 0.060$ for the test of no herd effect, 1 degree of freedom). Each of these tests used $\alpha = 0.05$. Treatments were compared using Likelihood Ratio χ^2 statistic.

³Number (%) of clinical mammary quarters with a combination cure divided by total number of clinical mammary quarters treated.

⁴One-sided P value comparing treatment listed in row with the treatment listed in the column.

⁵One nonchallenged mammary quarter in the ceftiofur 5-d treatment group developed clinical mastitis and was included in data analysis.

**Figure 1.** Geometric mean milk SCC from mammary glands experimentally infected with *Streptococcus uberis* following different ceftiofur (Ceft) treatment regimens.

the 8-d treatment regimen. The percentage of combination cures was 43, 69, and 79% in the 2-d treatment regimen, 5-d treatment regimen, and 8-d treatment regimen groups, respectively. Neither the 5-d treatment regimen nor the 8-d extended ceftiofur treatment regimen was greater than the standard 2-d ceftiofur treatment regimen at $\alpha = 0.05$. However, the 8-d extended ceftiofur treatment regimen was marginally greater in combination cures than the standard 2-d ceftiofur treatment regimen ($P = 0.053$; Table 3).

The plot of the geometric mean milk SCC from mammary glands experimentally infected with *Strep. uberis* following different treatment regimens is shown in Figure 1. A reduction in SCC was observed in milk in cases for which *Strep. uberis* was successfully eliminated, and this reduction persisted at all subsequent sampling times. Conversely, SCC in milk did not change in cases where ceftiofur treatment was unsuccessful at eliminating *Strep. uberis*. There was no evidence to suggest that extending ceftiofur therapy to either 5 or 8 d resulted in a greater reduction in SCC than the standard 2-d treatment.

The final parsimonious model included the main effects for herd, treatment, response (cure or fail), and time, along with the 2-way interactions herd \times time and response \times time ($P \leq 0.014$ for all terms). To simplify the application of the algorithm, only data from 3 times (0, 14, and 28 d) posttreatment were used in the analysis. Because treatment did not interact with time, there was

no evidence that extended therapy had any impact on reduction in SCC. Further analysis of the 2 significant interaction terms was conducted in an attempt to identify the effect associated with the significance. For the herd \times time interaction, whereas there was no evidence that herds were different at time 0, there was a significant herd effect at the times 14 and 28 d posttreatment. For the interaction of response \times time, virtually all of the variation was associated with the interaction between response and the 2 time groups: 0 vs. the combined 14 and 28 d times. The SCC for mammary quarters to be cured was significantly different from the SCC for mammary quarters that failed for both time groups; the degree of significance was different with $P = 0.028$ for time 0 and $P = 0.001$ for the pooled 14- and 28-d posttreatment times.

DISCUSSION

Extended ceftiofur intramammary therapy regimens were more effective than the standard 2-d ceftiofur treatment regimen for eliminating *Strep. uberis* experimental IMI during early lactation. However, the 8-d extended ceftiofur treatment regimen was significantly better than the standard 2-d ceftiofur treatment regimen at the $\alpha = 0.05$ level in only one of the 3 cure-rate analyses performed (Tables 1 to 3). One potential explanation was that there were only 37 mammary quarter infections involved, and these infections were spread over 3 treatment groups, resulting in small sample pairwise comparisons. For those analyses in which the 8-d extended ceftiofur treatment regimen was not significantly better than the standard 2-d ceftiofur treatment regimen, the observed proportion of clinically cured quarters for the 8-d extended ceftiofur treatment regimen was 79% compared with 43% for the standard 2-d ceftiofur treatment regimen. For the number of infected mammary quarters available in this study (14 for the 8-d extended ceftiofur treatment regimen and 7 for the standard 2-d ceftiofur treatment regimen), if the observed proportion of clinically cured quarters reflected the true probabilities of cure (79% for the 8-d extended ceftiofur treatment regimen and 43% for the standard 2-d ceftiofur treatment regimen), the study had only 66% power using a one-sided $\alpha = 0.05$ level. If the significance level was relaxed to a one-sided $\alpha = 0.10$, then the study had 79% power of declaring the 8-d extended ceftiofur treatment regimen better than the standard 2-d ceftiofur treatment regimen. Relaxing the α level to 10% would have resulted in the 8-d extended ceftiofur treatment regimen being greater than the standard 2-d ceftiofur treatment regimen with respect to combination cure rates (Table 3).

Ceftiofur therapy resulted in a reduction in palpation (physical condition of udder) scores and milk scores at 7 d after the last treatment in cases for which *Strep. uberis* was successfully eliminated and where treatment was unsuccessful at eliminating *Strep. uberis*. A further reduction was observed in milk and palpation (physical condition of udder) scores as time after last treatment increased. Palpation (physical condition of the udder) and appearance of milk are 2 parameters used by dairy producers to assess whether an antibiotic is effective because microbiological data would not likely be available. If palpation (physical condition of the udder) and appearance of milk were used as parameters to assess the effectiveness of an antibiotic, ceftiofur treatment would have been considered successful because palpation (physical condition of udder) scores and milk scores both decreased following treatment, even if treatment was not successful in eliminating the infection. Thus, palpation (physical condition of the udder) and appearance of milk, while important, are not necessarily effective measures of treatment success. A better measure of treatment success, in addition to microbiological information, is the SCC. The SCC decreased in milk from mammary quarters for which *Strep. uberis* was successfully eliminated. Conversely, the SCC in milk did not change in cases where ceftiofur treatment was unsuccessful at eliminating *Strep. uberis*.

In a companion study (Oliver et al., 2004), Holstein and Jersey dairy cows ($n = 88$) from 3 dairy research herds were used to evaluate efficacy of extended ceftiofur therapy regimens for treatment of subclinical IMI ($n = 166$) during lactation. A bacteriological cure was defined as an infected quarter that was treated and was bacteriologically negative for the presence of previously identified bacteria at 14 and 28 d after the last treatment. Efficacy of ceftiofur therapy against all IMI was 38.8% for the standard 2-d ceftiofur treatment regimen, 53.7% for the 5-d extended treatment regimen, and 65.8% for the 8-d extended ceftiofur treatment regimen. Four of 38 (10.5%) infections in untreated control quarters were cured spontaneously. Significant differences in efficacy were detected between all ceftiofur treatment groups and the untreated negative control group. Differences were also detected between the 8-d extended ceftiofur treatment regimen and the standard 2-d ceftiofur treatment regimen. Results of the study by Oliver et al. (2004) demonstrated that ceftiofur therapy was effective in eliminating naturally occurring subclinical IMI in lactating dairy cows caused by several different mastitis pathogens and that extended ceftiofur therapy significantly enhanced treatment efficacy.

Recently, Oliver et al. (2003) evaluated efficacy of extended pirlimycin therapy with the *Strep. uberis* ex-

perimental infection model used in the present study. The percentage of IMI eliminated in the standard 2-d pirlimycin treatment regimen, 5-d extended treatment regimen, and the 8-d extended pirlimycin treatment regimen was 58, 69, and 80%, respectively. Pirlimycin therapy was effective in eliminating *Strep. uberis* experimental IMI, and the 8-d extended pirlimycin treatment regimen was more effective than the standard 2-d pirlimycin treatment regimen and the 5-d extended pirlimycin treatment regimen. Results of the present study evaluating efficacy of extended ceftiofur therapy against experimental *Strep. uberis* IMI during early lactation are similar to efficacy of extended pirlimycin therapy against experimental *Strep. uberis* IMI during early lactation (Oliver et al., 2003).

Gillespie et al. (2002) demonstrated that extended pirlimycin hydrochloride therapy was more effective against naturally occurring environmental *Streptococcus* species IMI than the standard 2-d pirlimycin treatment regimen. Of the 61 environmental *Streptococcus* species IMI, 41 were due to *Strep. uberis*, 15 were due to *Streptococcus dysgalactiae* ssp. *dysgalactiae*, and 5 were due to *Enterococcus* spp. Following treatment with pirlimycin, 50% of *Strep. uberis* infected mammary quarters in the standard 2-d pirlimycin treatment regimen, 83% of *Strep. uberis* infected mammary quarters in the 5-d extended pirlimycin treatment regimen, and 100% of *Strep. uberis* infected mammary quarters in the 8-d extended pirlimycin treatment regimen were cured. Deluyker et al. (2001) evaluated efficacy of pirlimycin for treatment of naturally occurring subclinical mastitis. In that study, cows either received no treatment, the standard 2-d pirlimycin treatment regimen, or the 8-d extended pirlimycin treatment regimen. A mammary quarter was considered a noncure when the same bacterial species cultured before treatment was isolated in one of 2 posttreatment samples. Cure rates for *Strep. uberis* were 21% for the standard 2-d pirlimycin treatment regimen and 75% for the 8-d extended pirlimycin treatment regimen. Results of the present study evaluating efficacy of extended ceftiofur therapy against experimental *Strep. uberis* IMI during early lactation are similar to those observed following extended pirlimycin therapy against natural cases of *Strep. uberis* during all stages of lactation (Deluyker et al., 2001; Gillespie et al., 2002).

Results of our work (Gillespie et al., 2002; Oliver et al., 2003, 2004) and the study by Deluyker et al. (2001) support the concept that extended therapy is more effective at eliminating natural and experimentally induced *Strep. uberis* IMI than the standard 2-d treatment regimen. Lengthening the duration of antibiotic therapy would appear to increase efficacy of ceftiofur therapy against *Strep. uberis*. This has been demonstrated also

for other environmental *Streptococcus* species and *Staph. aureus* (Deluyker et al., 2001; Gillespie et al., 2002; Oliver et al., 2004).

CONCLUSIONS

Efficacy of extended ceftiofur intramammary therapy regimens was investigated in dairy cows that developed clinical mastitis following experimental infection with *Strep. uberis* during early lactation. Ceftiofur therapy was effective for eliminating *Strep. uberis* experimental IMI, and the 5- and 8-d extended ceftiofur therapy regimens were more effective than the standard 2-d ceftiofur treatment regimen. Bacterial cure rates were 43% for the standard 2-d ceftiofur treatment regimen, 88% for the 5-d extended ceftiofur treatment regimen, and 100% for the 8-d extended ceftiofur treatment regimen. Lengthening the duration of antibiotic treatment would appear to increase efficacy of ceftiofur therapy against *Strep. uberis*.

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