Factors Affecting Cure and Somatic Cell Count After Pirlimycin Treatment of Subclinical Mastitis in Lactating Cows

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ABSTRACT

This study investigated the associations of both bacteriological cure and quarter somatic cell count (SCC) after intramammary antibiotic treatment with treatment duration, cow characteristics, and pretreatment bacteriology and SCC. For the purpose of this paper, data from 2 treatment groups in each of 2 multi-location studies were selected. These studies were conducted to evaluate the efficacy of daily intramammary infusions with 50 mg of pirlimycin hydrochloride for the treatment of subclinical mastitis. Data from study 1 allowed for comparison of a group of cows that received pirlimycin intramammarily for 2 d with a group that received no treatment, and study 2 provided data for comparison of pirlimycin for 2 d with pirlimycin for 8 d. Quarter milk samples from cows with a high monthly SCC were tested for bacteriology and SCC. If one or more quarters had both a positive bacteriology and an SCC $\geq 300,000$ cells/mL, the cow was enrolled and randomly allocated to a treatment group. Enrolled cows were monitored for clinical mastitis and other disease for 4 wk after treatment initiation. At 3 and 4 wk after treatment initiation, milk samples were taken from each enrolled quarter to determine the SCC and conduct a bacteriological culture. Bacteriological culture results were interpreted such that quarters where the same bacterial species was cultured before treatment and found in at least 1 of the 2 posttreatment samples were considered a failure. The analysis of SCC used a mixed linear model (SAS proc mixed) and the analysis of bacteriological cure used a mixed logistic model (SAS glimmix macro). Bacteriological cure rate was significantly higher for lower parity, lower number of colonies in the pretreatment culture, longer treatment duration, and for streptococci compared with *Staphylococcus aureus*. However, treatment regimen affected bacterio-

logical cure differently in major than in minor pathogens and there was a significant interaction of treatment regimen with stage of lactation. Posttreatment SCC was significantly higher with increasing parity, in rear quarters, and with shorter duration of treatment. In the group of second and third parity animals, posttreatment SCC was more reduced in front quarters than in rear quarters. Also, the difference in posttreatment SCC between younger and older cows increased with higher pretreatment SCC. In conclusion, when predicting bacteriological cure following treatment of subclinical mastitis during lactation both treatment regimen and other risk factors need to be considered. The other risk factors may vary with treatment regimen. Posttreatment SCC was associated with treatment regimen, other risk factors, and interactions among the other risk factors; but these other risk factors did not vary significantly with treatment regimen.

(Key words: subclinical mastitis, pirlimycin, lactating cow, somatic cell count)

Abbreviation key: CURE = bacteriological cure, FAIL = no bacteriological cure, lnSCCpre = natural logarithm of pretreatment SCC, lnSCCpost = geometric mean of SCC at 21 and 28 d after treatment initiation, Pirli2 $\times$ = 2-d pirlimycin treatment, Pirli8 $\times$ = 8-d pirlimycin treatment.

INTRODUCTION

Bacterial IMI frequently persist for an extended time while causing subclinical inflammation of the mammary gland. Subclinical mastitis causes decreased milk production and lower milk quality; and contagious bacteria may spread to other quarters. Nevertheless, as long as bacterial infections remain subclinical they often go untreated during the lactation. Low cure rate has been cited as a reason why mastitis in its subclinical appearance should not be treated during the lactation. Treatment is instead postponed until a clinical flare-up occurs or until dry-off, a time when antibiotics are often routinely used to treat all quarters in all cows. With increasing pressure to deliver milk with a low bulk milk SCC, it may not be viable, economically, to

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wait until dry-off before measures are taken to lower the prevalence of IMI, alongside measures to reduce the incidence of new IMI. This may explain the high culling rates of high SCC cows in herds with a bulk milk SCC close to the upper legal limit (Barkema et al., 1998). Treatment of subclinical mastitis during lactation could represent an alternative to culling. However, responsible use of antibiotics demands that its use be limited to cases in which cure rates can be expected to be sufficiently high. Thus, there is an interest in identifying effective treatment regimens and other factors that affect cure.

Risk factors for bacteriological cure of *Staphylococcus aureus* infections following antibiotic treatment were reported previously. They included age, number of infected quarters, quarter location, stage of lactation, and severity of the inflammation (Wilson et al., 1972; Poutrel, 1978; Ziv and Storper, 1985; Owens et al., 1988; Sol et al., 1997). More recently, Dingwell et al. (2003) reported that the intensity of bacterial shedding was negatively associated with *Staph. aureus* cure following antibiotic treatment at dry-off. However, Wilson et al. (1972) did not find these factors to be significant for streptococcal species identified at that time, except for the degree of inflammation. Hence, it is worthwhile to determine whether previously identified risk factors for *Staph. aureus* cure apply to other bacterial species that also often cause subclinical mastitis.

Extending the duration of treatment with β -lactams resulted in significant increases in bacteriological cure rates of *Staph. aureus* (Wilson et al., 1972; Ziv and Storper, 1985). Similarly, higher cure rates for infections caused by *Staph. aureus* and streptococci were reported following treatment with a lincosaminide (Gillespie et al., 2002; Oliver et al., 2003). Whereas treatment regimens that, on average, result in higher cure rates are desirable, it is nevertheless worthwhile to verify whether there are subpopulations that do not respond well to such treatment and to identify risk factors associated with a lower response.

Finally, treatment success should lead to reduction in SCC. Whereas previous studies investigated risk factors for bacteriological cure, risk factors for posttreatment SCC have, to our knowledge, not been investigated thus far.

The objective of this study was to determine whether risk factors for bacteriological cure and posttreatment SCC varied with bacterial species and with treatment regimen efficacy.

MATERIALS AND METHODS

For the purpose of this paper, data from 2 multi-location studies were analyzed. These studies were con-

ducted in Member States of the European Union to evaluate the efficacy of daily intramammary infusions with 50 mg of pirlimycin hydrochloride for the treatment of subclinical mastitis. They complied with the guideline on Good Clinical Practice for the Conduct of Clinical Trials for Veterinary Medicinal products of the European Commission's Committee for Veterinary Medicinal Products and with animal welfare regulations applicable in the countries where the trials were conducted. Data from study 1 allowed for comparison of a group of cows that received pirlimycin for 2 d (**Pirli2** $\times$) to a group that received no mastitis treatment (untreated), whereas study 2 provided data for comparison of Pirli2 $\times$ with pirlimycin for 8 d (**Pirli8** $\times$).

Herds

There were 54 herds in study 1 and 57 herds in study 2. The herds were located in the Netherlands, Sweden, Denmark, Spain, Italy, France, Germany, and the United Kingdom. Only herds with a regular history of subclinical mastitis were included. Farms were required to have proper record-keeping capabilities and cow identification, and be free of officially notifiable diseases.

Enrollment

In selected herds, the principal investigator monitored cows for subclinical mastitis using routine cow SCC results. A cow was considered to have subclinical mastitis (and thus potentially qualify for enrollment) if the SCC was greater than 250,000 cells/mL twice in the past 2 mo or greater than 400,000 cells/mL once in the past month (Dohoo and Meek, 1982). Somatic cell count results from milk samples taken less than 72 h postpartum were not considered for this purpose (Barkema et al., 1999). Cows were excluded from the study if they had teat lesions, were systemically ill, had clinical mastitis (IDF, 1987), had received antibacterial or anti-inflammatory therapy in the previous 30 d, were of sixth or higher parity, had reached a low milk yield, or were to be dried off within the next 30 d. In study 2, quarters with palpable udder lesions were also excluded. Pretreatment aseptic milk samples were collected from each quarter of the remaining cows to determine SCC and the presence of IMI, and the number of colonies was enumerated. Milk samples were refrigerated or frozen and shipped to in-country diagnostic laboratories within 24 h. Laboratory procedures and interpretations for bacteriological culture of milk were consistent with National Mastitis Council Guidelines (1990). Briefly, from each sample, one inoculum (0.05 mL of milk) was plated on Columbia base agar con-

taining 5% sheep blood. Plates were incubated at 37°C and examined for bacterial growth at 24 and 48 h. Before study initiation, uniformity among laboratories in the identification of the bacterial strains was demonstrated through a test conducted by the Quality Assurance Unit of the Veterinary Laboratories Agency (Loughborough, UK). For each bacterial species, the number of colonies on the plate was recorded as 1 of the following 3 categories: actual number (≤ 10 cfu), ++ (11 to 100 cfu), +++ (> 100 cfu). Quarters were enrolled if the SCC was $\geq 300,000$ cells/mL and a mastitis pathogen was identified. Final enrollment occurred within 8 d of milk sampling. Enrollment was limited to a maximum of 20 cows per treatment group at each location.

Blocking, Randomization, Masking, and Treatment

Cows in a herd were ranked first by increasing parity and second by decreasing duration of lactation within parity. Next, treatments were randomly allocated to cows (not quarters) within blocks, consisting of the closest cows that were available for enrollment. Because treatment intervals and durations were different among the treatment groups, treatment administrators were not blinded. However, laboratory personnel who determined SCC and bacteriologic status were blinded to treatment assignment. Cows were infused using appropriate technique in each of the quarters that met the inclusion criteria immediately after milking. Cows were not milked for at least 8 h after infusion.

Clinical Observations and Definition of Bacteriological Cure

Cows were monitored for clinical mastitis and other disease in all 4 quarters for 30 d after enrollment. The principal investigator conducted clinical examinations on all cows at 22 to 23 and 29 to 30 d after enrollment. At these visits, sterile milk samples were taken from each enrolled quarter to be cultured for determination of SCC. If cows required treatment of clinical mastitis or another disease, monitoring was discontinued.

Bacteriological cure (**CURE**) was defined as absence of the bacterial species present pretreatment in both of the posttreatment milk samples from that quarter. Presence of the pretreatment bacterial species in at least one posttreatment sample was considered a failure (**FAIL**).

Statistical Analysis

The analysis of SCC used a mixed linear model (SAS Version 8.2, Proc Mixed, SAS Institute, Cary, NC), and analysis of CURE used a mixed logistic model (SAS

Version 8.2, Glimmix Macro). Study, herd, and cow were considered random effects.

Somatic cell count was measured on each quarter and so the model was based on the quarter within cow. Bacteriological cure was measured on observation within quarter (i.e., a quarter could have multiple bacterial species isolated) and so the model was based on the observation within quarter. For the SCC analysis, when multiple bacteria were identified then the following order of precedence was used: *Staph. aureus*, *Streptococcus uberis*, other streptococci, CNS, *Corynebacterium bovis*, and other. For example, if *Staph. aureus* and *C. bovis* were identified in the same quarter, it was reported as *Staph. aureus* in the SCC analysis. Similarly, the number of colonies used would follow the same order of precedence.

For SCC, the natural logarithm of the somatic cell count divided by 100 was the variable analyzed. An initial analysis was conducted to determine how SCC changed between time of treatment and 21 and 28 d after treatment initiation. This analysis included the effects of treatment and CURE for each quarter. Two contrasts were computed: the first tested whether the SCC geometric mean at d 21 and 28 (**lnSCCpost**) was different from the pretreatment SCC (**lnSCCpre**), whereas the second tested whether the change from pretreatment differed by treatment duration. This analysis was also conducted for CURE and FAIL quarters.

The second analysis was conducted to determine the significant covariates associated with SCC and CURE. A forward stepwise method was used to include/exclude covariates (Table 1). The random effects were included in all models. At the first step of the stepwise procedure all covariates were tested in the model one at a time, the one resulting in the smallest *P* value was included in the model. The next step was to retest all the covariates in the model that included the covariate from step one. This procedure was continued until no additional covariates were significant ($\alpha = 0.05$). Once the main effects model was determined, a similar process was used for 2-way interactions. Each of the possible 2-way interactions was included in the model one at a time, keeping the one with the smallest *P* value. This was repeated until none of the remaining interactions was significant. Because the sample size in at least one of the categories of 3-way or higher-order interactions were quite small, these effects were not tested. Finally, the interactions between treatment and any other risk factors not included in the model were tested for inclusion in the model.

RESULTS

There were 901 quarters from 397 cows (study 1) and 554 quarters in 325 cows (study 2) correctly enrolled

Table 1. Covariates used in the analysis by study and treatment group.

Covariate	Class	Study 1		Study 2	
		Untreated (n = 184) ³	Pirli2× ¹ (n = 192) ³	Pirli2× (n = 164) ³	Pirli8× ² (n = 130) ³
Cowside covariates					
Parity	1	16% ⁴	18%	21%	21%
	2 to 3	34%	41%	56%	55%
	≥4	51%	41%	23%	24%
Lactation stage (d)	≤100	13%	13%	12%	17%
	101 to 200	37%	35%	35%	34%
	>200	51%	52%	52%	49%
	Mean (SD)	203 (90)	206 (87)	216 (107)	210 (107)
Number of quarters affected	1	39%	43%	42%	47%
	2	28%	33%	33%	46%
	>2	33%	24%	25%	7%
Quarter location	Front	39%	39%	49%	36%
	Rear	61%	61%	51%	64%
Laboratory covariates					
Pretreatment SCC (×10 ³ cells/mL)	300 to <500	19%	22%	16%	13%
	500 to 1000	27%	27%	30%	26%
	>1000	54%	51%	54%	61%
LnSCCpre ⁵	Mean (SD)	7.14 (0.92)	7.01 (0.85)	7.23 (0.95)	7.24 (0.94)
Bacterial species ⁶	<i>Staph. aureus</i>	34%	40%	42%	41%
	<i>Strep. uberis</i>	20%	14%	10%	9%
	Other Streptococci	12%	7%	26%	18%
	CNS	12%	23%	15%	20%
	<i>C. bovis</i> ⁸	19%	17%	4%	4%
	Other	18%	13%	8%	12%
	Number of colonies ⁷	1 to 10	22%	26%	12%
	11 to 100	52%	45%	62%	60%
	>100	41%	42%	22%	14%
	Unknown	—	—	10%	14%

¹Pirli2× = 2-d Pirlimycin treatment.²Pirli8× = 8-d Pirlimycin treatment.³n = Number of quarters.⁴Percentage of quarters falling in this category.⁵LnSCCpre = Natural logarithm of pretreatment SCC.⁶Because a quarter could be infected with different bacterial species, the percentages add to over 100.⁷Inoculum size = 0.01 mL.⁸*C. bovis* = *Corynebacterium bovis*.

for which clinical observations were available during the entire monitoring period or until clinical mastitis occurred. The incidence of quarters with clinical mastitis during the monitoring period was 4.6% for the untreated and 4.9% for the Pirli2× groups in study 1, and 1.8% for Pirli2× and 5.2% for Pirli8× in study 2. Quarters with clinical mastitis during the observation period or with incomplete data were excluded from statistical analysis. This left 376 quarters in 245 cows (study 1) and 294 quarters in 203 cows (study 2). The most frequently isolated bacterial species was *Staph. aureus* (Table 1). The distribution of parity, stage of lactation, number of enrolled quarters per cow, proportion of front vs. rear quarters, and pretreatment SCC level showed no major differences among treatment groups within study (Table 1). Study 1 included more cows of fourth and higher parity than study 2.

Bacteriological Cure

Bacteriological cure results by treatment group are in Table 2. The final model of the stepwise analysis, its parameters, and its least square means are in Tables 3, 4, and 5. Treatment, bacterial species, and their interaction were significant (Table 3). As expected, CURE rate was lower for *Staph. aureus* than for the other bacterial species. When compared with Pirli2×, CURE rates were significantly higher following Pirli8× and lower in the untreated group, for *Staph. aureus*, *Strep. uberis*, and other streptococci (Table 5). Cure rates for Pirli2× and Pirli8× did not differ significantly for CNS, whereas for *C. bovis* and other bacterial species, Pirli2× CURE did not differ significantly from the other 2 treatment groups. There was also a significant interaction of treatment regimen with lactation stage ($P = 0.008$, Table 3). In the untreated group, the CURE percentage

Table 2. Geometric mean SCC ($\times 10^3$ cells/mL) for all quarters, and for bacteriological cures and failures.¹

Day after treatment initiation	Untreated			Pirli2×			Pirli8×		
	All (n = 184)	Cures (n = 33) 18% ²	Failures (n = 151) 82%	All (n = 356)	Cures (n = 125) 35%	Failures (n = 231) 65%	All (n = 130)	Cures (n = 78) 60%	Failures (n = 52) 40%
0	1385 ^a	1594 ^a	1356 ^a	1276 ^a	1199 ^a	1281 ^a	1445 ^a	1667 ^a	1219 ^a
Average (d 21 and 28)	1075 ^b	831 ^b	1149 ^a	802 ^b	378 ^b	1172 ^a	446 ^b	326 ^b	746 ^b
Interaction <i>P</i> value ³	0.082	0.091	0.501				<0.001	0.030	0.016

^{a,b}Values in the same column with a different superscript were significantly ($P < 0.05$) different.

¹Analysis was at the quarter level, thus bacteriological cure was collapsed for observations within quarter such that any failure was considered a failed quarter.

²Percentage quarters falling in this category.

³Interaction *P* value compares the difference from d 0 to d 21/28 for untreated group versus Pirli2×

 and for Pirli2× versus Pirli8×, for each category of cure. For example, the reduction in SCC in Pirli8×-treated quarters that were considered bacteriological failures is a significantly ($P = 0.016$) bigger reduction than the reduction for Pirli2×-treated quarters that were considered bacteriological failures.
Table 3. Logistic model for bacteriological cure.

Effect	df	Variance estimate	Denominator df	<i>F</i> -value	<i>P</i> -value
Study	1	0.0204			
Herd (study)	100	0.8959			
Treatment	2		313	6.24	0.002
Parity	2		313	11.21	<0.001
Lactation stage	2		313	0.45	0.638
Treatment $\times$ lactation stage	4		313	3.50	0.008
Cow (herd $\times$ treatment)	313	3.3153			
Bacteria	5		260	4.79	<0.001
Number of colonies	2		260	5.97	0.003
Treatment $\times$ bacteria	10		260	3.38	<0.001
Residual	260	0.5014			

Table 4. Parameters from logistic model¹ for bacteriological cure.

Covariate	Level	Treatment		
		Untreated	Pirli2×	Pirli8×
Intercept		-3.6634	-1.8189	+0.1667
Parity	1	+1.5807	+1.5807	+1.5807
	2 to 3	+1.3787	+1.3787	+1.3787
	≥ 4	+0	+0	+0
Number of colonies	1 to 10	+1.2036	+1.2036	+1.2036
	11 to 100	+0.6927	+0.6927	+0.6927
	>100	+0	+0	+0
Bacteria	<i>Staph. aureus</i>	-1.9304	-0.7121	-1.1278
	<i>Strep. uberis</i>	+0	+0	+0
	CNS	+0.6316	+0.2763	-2.8355
	<i>C. bovis</i>	-0.3512	-1.7046	-1.1268
	Other Streptococci	-0.9740	+0.5028	+1.3539
	Other	+1.4579	+0.4907	-1.0769
Lactation stage (d)	≤ 100	+1.9507	-1.0216	-1.2012
	101 to 200	+1.2413	-0.6073	+0.1768
	>200	+0	+0	+0

¹Model adds down a column, i.e., the estimated logit transformed bacteriological cure for a parity-1 cow with Pirli2×

 treatment, 1 to 10 *Staph. aureus* colonies, and >200 DIM is $-1.8189 + 1.5807 + 1.2036 - 0.7121 + 0 = 0.2533$. Back-transforming gives an expected cure percentage = $100 \times \exp(0.2533) / [1 + \exp(0.2533)] = 56\%$.

Table 5. Least square means from logistic model for bacteriological cure with parity 1,¹ number of colonies 1 to 10,² and lactation stage >200 d.³

Bacteria	Treatment	LSMean	%Cure ⁴	Odds ratio	95% confidence interval (CI)
<i>Staph. aureus</i>	Untreated	-2.8094	6%	0.05	(0.01, 0.21)
	Pirli2×	0.2533	56%		
	Pirli8×	1.8232	86%	4.81	(1.29, 17.9)
<i>Strep. uberis</i>	Untreated	-0.8791	29%	0.16	(0.03, 0.75)
	Pirli2×	0.9655	72%		
	Pirli8×	2.951	95%	7.28	(1.02, 52.0)
CNS	Untreated	-0.2475	44%	0.23	(0.05, 0.98)
	Pirli2×	1.2417	78%		
	Pirli8×	0.1155	53%	0.32	(0.07, 1.58)
<i>C. bovis</i>	Untreated	-1.2303	23%	0.61	(0.12, 3.00)
	Pirli2×	-0.7391	32%		
	Pirli8×	1.8243	86%	12.98	(0.82, 205)
Other Streptococci	Untreated	-1.8531	14%	0.04	(0.01, 0.22)
	Pirli2×	1.4682	81%		
	Pirli8×	4.3049	99%	17.06	(2.23, 131)
Other	Untreated	0.5788	64%	0.42	(0.09, 1.84)
	Pirli2×	1.4561	81%		
	Pirli8×	1.8742	87%	1.52	(0.22, 10.3)

¹For Parity 2 to 3 subtract 0.2021 (95% CI: -0.5054, 0.9095) from the LSMeans; for Parity ≥4 subtract 1.5807 (95% CI: 0.7930, 2.3685) from the LSMeans.

²For Number of Colonies 11 to 100 and >100 subtract 0.5109 (95% CI: -0.0714, 1.0932) and 1.2036 (95% CI: 0.5092, 1.8981) from the LSMeans, respectively.

³For Untreated add 1.9507 (95% CI: 0.4274, 3.4744) or 1.2413 (95% CI: 0.0395, 2.4431) to the LSMeans for Lactation Stages ≤100 or 101 to 200, respectively; for Pirli2×

⁴To obtain percentage cure, back-transform the LSMeans to 100*exp (LSMeans)/[1 + exp (LSMeans)].

decreased as lactation progressed. The trend was opposite for Pirli2×

Considering all possible combinations of the different levels for the various significant risk factors (Table 5), percentage cure for *Staph. aureus* following Pirli8×

Somatic Cell Count

Somatic cell count results by treatment group are shown in Table 2. The geometric mean SCC did not differ between d 21 and 28 posttreatment and were averaged for all analyses. LnSCCpost was significantly lower than LnSCCpre in all treatment groups. This decrease was numerically smaller ($P = 0.082$) for the untreated group (22%) than for Pirli2×

Figure 1. Lowest and highest percentage of *Staphylococcus aureus* bacteriological cure for each parity × treatment group, as predicted by the logistic regression model.

tion ($P = 0.104$), $\ln\text{SCCpre}$ ($P = 0.184$), stage of lactation ($P = 0.121$), bacterial species ($P = 0.243$), number of colonies ($P = 0.143$), and parity ($P = 0.124$).

In the final model, the main effect of parity was masked to some degree ($P = 0.106$) by its significant interaction with $\ln\text{SCCpre}$ (Table 6). $\ln\text{SCCpost}$ increased with age and with higher $\ln\text{SCCpre}$, and their

interaction is due to a shallower slope of $\ln\text{SCCpre}$ on $\ln\text{SCCpost}$ in younger cows (Table 7). In other words, the penalty for high $\ln\text{SCCpre}$ is less in young cows than in older cows. Quarter location (rear/front) and its interaction with parity were also significant. The interaction is due to the higher $\ln\text{SCCpost}$ in rear than in front quarters in the parity 2 to 3 group, whereas

Table 6. Mixed linear model for posttreatment somatic cell count.

Effect	df	Variance estimate	Denominator df	F-value	P-value
Study	1	0			
Herd (study)	104	0.0565			
Treatment	2		338	10.33	<0.001
Parity	2		338	2.26	0.106
Cow (herd × treatment)	338	0.7443			
$\ln\text{SCCpre}$	1		216	71.84	<0.001
Quarter location	1		216	5.15	0.024
Parity × quarter location	2		216	6.96	0.001
Parity × $\ln\text{SCCpre}$	2		216	3.08	0.048
Residual	216	0.6240			

Table 7. Parameters from mixed linear model¹ for posttreatment SCC.

Covariate	Level	Parity		
		1	2 to 3	≥4
Intercept		+4.1827	+2.3864	+2.4153
Treatment	Untreated	+0.7006	+0.7006	+0.7006
	Pirli2×	+0.5567	+0.5567	+0.5567
	Pirli8×	+0	+0	+0
Quarter location	Front	-0.0279	-0.5702	+0.0272
	Rear	+0	+0	+0
lnSCCpre		+lnSCCpre × 0.2390	+lnSCCpre × 0.5445	+lnSCCpre × 0.5573

¹Model adds down a column, that is, the estimated SCC for Parity-1 cow with a Pirli2×-treated front quarter that had a pretreatment SCC value of 500,000 is $4.1827 + 0.5567 - 0.0279 + \ln(500,000/1000) \times 0.2390 = 6.1968$; back-transforming: $\exp(6.1968) \times 1000 = 491,175$.

this difference did not exist for the other parity groups (Table 8). Front quarters in the parity group 2 to 3 responded similarly to quarters in first-parity cows whereas rear quarters responded more like quarters in cows of parity group 4 to 5 (Table 8).

Figure 2 shows for each treatment by parity group combination, the lowest and highest posttreatment SCC when considering all the possible combinations of significant risk factors of the final model (Table 8). The results show that under both these best and worst cases, Pirli8× treatment was about 50% lower than under the respective circumstances in the untreated group.

DISCUSSION

Parity affected both CURE and lnSCCpost, regardless of the treatment regimen. In addition, there was a significant interaction between parity and lnSCCpre for lnSCCpost, i.e., the higher the lnSCCpre, the larger the differences between the parity groups in lnSCCpost. Parity probably impacts posttreatment SCC reduction in several ways. First, bacteriological cure rate decreases with age. Second, the magnitude in SCC reduction that can be achieved, following cure, is less in older cows because their pretreatment SCC was noted to be lower (Deluyker et al., 2001).

Table 8. Least square means for posttreatment SCC with Pirli2× treatment.¹

lnSCCpre ²	Quarter location	Parity	LSMean ³	95% Confidence interval (CI)
6.4	Front	1	6.24	(5.86, 6.62)
		2 to 3	5.92	(5.66, 6.18)
		≥4	6.57	(6.28, 6.85)
	Rear	1	6.27	(5.93, 6.61)
		2 to 3	6.49	(6.25, 6.73)
		≥4	6.54	(6.28, 6.85)
7.0	Front	1	6.38	(6.04, 6.72)
		2 to 3	6.25	(6.02, 6.49)
		≥4	6.90	(6.63, 7.17)
	Rear	1	6.41	(6.11, 6.71)
		2 to 3	6.82	(6.61, 7.04)
		≥4	6.87	(6.64, 7.11)
7.8	Front	1	6.58	(6.21, 6.94)
		2 to 3	6.70	(6.46, 6.94)
		≥4	7.35	(7.05, 7.64)
	Rear	1	6.60	(6.27, 6.94)
		2 to 3	7.27	(7.04, 7.49)
		≥4	7.32	(7.05, 7.59)

¹For Untreated, add 0.14 (95% CI: -0.11, 0.40) to the LSMean; for Pirli8× subtract 0.57 (95% CI: 0.29, 0.85) from the LSMean.

²6.4 (601,845) is the 25th percentile, 7.0 (1,096,633) is the median, and 7.8 (2,440,602) is the 75th percentile of pretreatment SCC.

³To back-transform the LSMean: $\exp(\text{LSMean}) \times 1000$.

Figure 2. Lowest and highest posttreatment quarter SCC for each parity × treatment group, as predicted by the linear regression model.

The significant association of number of colonies present pretreatment with CURE did not differ between treatment regimens ($P = 0.411$). It might be a result of detection level differences (Sears et al., 1990), whereby the likelihood of a positive culture result is higher with high levels of shedding, or it may be real, i.e., that high level of shedding reduces likelihood of CURE. Because in the present study, a quarter was considered a CURE if in both posttreatment samples the original bacterial species could not be isolated, it is unlikely that the false-negative rate was high (Sears et al., 1990). Following treatment of mastitis during lactation, Owens et al. (1999) reported a statistically significant reduction of the *Staph. aureus* concentration present in milk. This was accompanied by a transient decrease in SCC that had returned to near pretreatment levels by d 28 posttreatment. In contrast, in the present study there was no evidence of a posttreatment return to high pretreatment SCC levels for cured quarters. Dingwell et al. (2003) reported number of colonies to be a risk factor for CURE with dry cow therapy. The present results suggest this is also the case for lactation therapy of subclinical mastitis.

The progressive increase in CURE with progression of lactation previously reported for *Staph. aureus* was only present in this study for Pirli2x. In the untreated group, the percentage spontaneous CURE decreased as lactation progressed, whereas in the Pirli8x group, an effect of treatment by stage of lactation was only apparent for the first 100 DIM, the period of peak milk production, when compared with the remainder of the lactation. Wilson et al. (1972) hypothesized that the stage of lactation effect was due to a more rapid elimination of the antibiotic with high milk yield in the first part of the lactation. This effect may be less important with treatment of clinical mastitis, where milk production is generally more affected (Sol et al., 2000). The significant interaction between treatment and stage of lactation in the present study suggests that this phenomenon varies with treatment regimen.

Whereas treatment regimen was significant for both CURE and $\ln\text{SCC}_{\text{post}}$, there was a significant interaction of treatment with bacterial species for CURE. However, the lack of a significant association of $\ln\text{SCC}_{\text{post}}$ with bacterial species cultured ($P = 0.217$) indicates that the effect of treatment regimen on inflammation

reduction did not differ across bacterial classes. This is further illustrated by the finding that following Pirlis $\times$ there was a significant SCC decrease in quarters that did not cure bacteriologically (Table 2). The low CNS bacteriological cure rate and its failure to increase with longer duration of treatment could be due to reinfections with CNS (Timms and Schultz, 1984). It may be worthwhile to identify the most common CNS at the individual species level, particularly as some species could cause more inflammation than others (Laevens et al., 1997). Similarly, the lack of a difference in CURE rate between treatment regimens for *C. bovis* is not surprising because no extra measures were taken to prevent new infections during the study.

The significant effects of parity, stage of lactation, and number of colonies present pretreatment on CURE did not differ ($P \geq 0.414$) between bacterial species. These additional risk factors explain why for example, the *Staph. aureus* bacteriological cure rates varied substantially within each parity by treatment combination (Figure 1).

For lnSCCpost, parity and treatment duration were again significant factors. In addition, quarter location, lnSCCpre, and the interaction of parity with lnSCCpre and quarter location were significantly associated with lnSCCpost. Effects of parity, quarter location, and lnSCCpre have been attributed to chronicity of infection (Wilson et al., 1972; Sol et al., 1997). The fact that within parity group 2 to 3, lnSCCpost in front quarters decreased to the level of quarters in parity-1 cows, whereas the lnSCCpost in rear quarters of those cows remained at a level similar to the higher values in quarters of parity-4 to 5 cows, supports this hypothesis (Table 8, Figure 2).

It is noteworthy that the P value of statistical significance for the interaction between treatment regimen and several other risk factors (quarter location, lnSCCpre, stage of lactation, and parity) was in the range of 0.104 to 0.184 for both CURE and lnSCCpost, suggesting that other risk factors for CURE and lnSCCpost may vary with treatment regimen. This aspect probably merits further research.

CONCLUSIONS

Treatment regimen affected bacteriological cure differently in major than in minor pathogens. Regardless of treatment regimen and the species isolated pretreatment, bacteriological cure rate was higher in younger animals and when fewer colonies had been isolated pretreatment. Stage of lactation also affected bacteriological cure, but its effect varied with the treatment regimen.

Posttreatment SCC was associated with treatment regimen and with other factors, i.e., pretreatment SCC, quarter location, and the interaction of parity with quarter location and pretreatment SCC. These other factors did not vary significantly with treatment regimen.

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