Efficacy of Intramammary Tilmicosin and Risk Factors for Cure of *Staphylococcus aureus* Infection in the Dry Period

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ABSTRACT

The objective of this study was to evaluate the efficacy of intramammary tilmicosin, administered at drying-off, for eliminating *Staphylococcus aureus* infection, and to identify risk factors for *S. aureus* cure during the dry period. A total of 219 naturally infected cows, representing 308 quarters, were randomized to receive either one of two treatments at drying-off. Cows received either an intramammary infusion of 500 mg of benzathine cloxacillin, or a sterile solution containing 1500 mg of tilmicosin. All cows had quarter milk samples taken aseptically three times before dry-off, and at wk 1, 2, and 4 of the subsequent lactation. Overall, 62% of cows and 67.5% of quarters infected with *S. aureus* cured during the dry period. The cure following administration of tilmicosin was 67.3 and 72.5% for cows and quarters, respectively. By comparison, the cure achieved with cloxacillin was 56.9 and 62.9% of cows and quarters. Cows receiving tilmicosin were 2.1 times more likely to cure. The cure rate for cows decreased as the linear score on the last DHI test increased, and as the amount of *S. aureus* being shed increased. Quarters that cultured positive multiple times before drying-off were less likely to cure. *Staphylococcus aureus* infections located in front quarters of the udder were 2 times more likely to cure than those in hind quarters. Results of this study demonstrate that intramammary tilmicosin at drying-off is efficacious in curing existing *S. aureus* during the dry period. Risk factors associated with the cure of *S. aureus* were identified.

(**Key words:** *Staphylococcus aureus*, dry cow therapy, tilmicosin)

Abbreviation key: CNS = coagulase-negative staphylococci, CP5 = capsular polysaccharide 5, DCT = dry cow antibiotic therapy, LS = linear score, OR = odds ratio.

INTRODUCTION

The goal of mastitis control during the dry period is to have as few infected quarters as possible at the next calving (Eberhart, 1986). Achieving this goal involves eliminating existing IMI present at the end of lactation, as well as preventing new IMI during the dry period. At present, the most effective means of achieving these objectives is to administer long-acting dry cow antibiotic therapy (DCT) immediately after the last milking (Eberhart, 1986). The use of DCT can reduce the incidence of new IMI by 50 to 75%. This reduction in IMI is more pronounced early than later in the dry period (Smith et al., 1985). In addition, approximately 70 to 98% of infections present at drying-off are eliminated during the dry period following DCT (Natzke, 1981; Smith et al., 1985). However, compared with elimination of other pathogens, DCT is generally acknowledged as being less successful in eliminating IMI caused by *Staphylococcus aureus*. The cure rate of *S. aureus* has been reported to be approximately 50% (Browning et al., 1990; Osteras et al., 1994, 1999). Despite this average, actual cure rates of *S. aureus* may vary between 20 to 78% (Cummins and McCaskey, 1987; Erskine et al., 1994; Nickerson et al., 1999; Soback et al., 1990; Sol et al., 1994).

With very few exceptions, cure of major mastitis pathogens following DCT is not likely a random event. Variation in cure rate may be explained by pathogen, cow, and herd factors. It would be of considerable importance to the dairy industry to be able to predict which cows were more likely to cure (Schukken et al., 2001). In an attempt to augment the effectiveness of DCT, various protocols and products have been tested (So-

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back et al., 1990; Hogan et al., 1995; Erskine et al., 1998; Nickerson et al., 1999). One such treatment that has recently been investigated is the use of tilmicosin.

Tilmicosin is a semisynthetic macrolide antibiotic, currently approved for the treatment of bovine respiratory disease. This drug possesses properties that might prove favorable in combatting dry period IMI, especially subclinical *S. aureus* infections. These properties include the observed in vitro interactions of the antibiotic with bovine phagocytes and epithelial cells (Scorneaux and Shryock, 1999). Tilmicosin is rapidly accumulated in bovine macrophages and mammary epithelial cells. The uptake in mammary gland cells is dependent on cell viability, temperature, and pH, but is not influenced by either metabolic inhibitors or an anaerobic environment (Scorneaux and Shryock, 1999).

In recently published research, tilmicosin was compared to intramammary cephalixin benzathine as a treatment of *S. aureus* at dry-off (Nickerson et al., 1999). The tilmicosin was either administered intramammary (1500 mg) or via a subcutaneous injection at 5 mg/kg of BW on the day of drying-off, and again 4 d later. The reported quarter cure rates for IMI caused by *S. aureus* at 28 d post calving were: 78.1% intramammary cephalixin, 74.2% intramammary tilmicosin, and 9.1% subcutaneous tilmicosin. Intramammary tilmicosin was concluded to be as effective as cephalixin benzathine.

In addition to new treatment regimes, considerable attention has been concentrated on identifying specific risk factors that influence the cure of *S. aureus*, such that DCT might be specifically aimed at only those cows with a high probability of cure (Sol et al., 1994). This implies that a manager would have to decide to remove cows from the herd that have a low probability of cure. There are significant quarter level and cow level predictors for cure of *S. aureus*. Cure rate has been shown to be strongly influenced by the location of the infected quarter in either the front of the hind of the udder, the age of the cow, the percentage of samples that were culture positive for *S. aureus* before drying-off, the SCC of infected quarters and the total number of quarters that are infected (Sol et al., 1994; Osteras et al., 1999).

The efficacy of intramammary tilmicosin to eliminate *S. aureus* has recently been reported from data collected in two herds, with the majority of cows having experimental infections (Nickerson et al., 1999). The objective of this paper is to present the results of a DCT field trial that evaluated the efficacy of intramammary tilmicosin phosphate to eliminate naturally occurring *S. aureus*, and identified risk factors associated with achieving cure of subclinical *S. aureus* IMI during the dry period.

MATERIALS AND METHODS

Herd Selection

For a period of 1 yr, beginning in July 1999, cows in 75 commercial and two research dairy herds located in three eastern Canadian provinces participated in the field trial [Ontario (n = 39), Quebec (n = 34), Prince Edward Island (n = 4)]. Herds were selected based on proximity to the veterinary college in their province, to allow for weekly visits by a trained technician. Herd size ranged from 27 to 168 cows with an average of 64 cows. Cows that had not received antibiotic treatment in the last 3 wk and that were 1 mo from the time of scheduled drying-off were eligible for enrollment. The range of prevalence of *S. aureus* in these herds was 0 to 36% of cows. Data from only those cows that had at least one quarter infected with *S. aureus* are reported in this paper, to discuss the efficacy of intramammary tilmicosin against, and risk factors associated with, cure of *S. aureus*. All other cows in these herds were also randomized to treatment schedules, which formed the basis of other experiments to document the infection status of cows at drying-off and freshening, elimination of pathogens other than *S. aureus*, and the incidence of new IMI during the dry period.

Sampling Time Line and Treatment Randomization

A study technician collected quarter milk samples aseptically from all eligible cows starting between 28 (d -28) and 22 (d -22) d before scheduled drying-off, and again between 21 (d -21) and 14 (d -14) d before drying-off. Drying-off date was calculated to ensure cows would have a minimum 60-d dry period for residue avoidance concerns. Cows that had at least one quarter that yielded ≥ 1 cfu per 0.01 ml of milk of *S. aureus* on either of these first two samples, were defined as infected. These cows were sampled for a third time at drying-off (d 0) and randomly assigned to receive, in all four quarters, either intramammary benzathine cloxacillin (Dry Clox, Ayerst Laboratory, Guelph, Ontario) or a novel intramammary formulation of tilmicosin phosphate (supplied by Provel, Division of Eli Lilly Canada Inc., Guelph, Ontario, Canada). The intramammary infusion of DCT was done by the technician as soon as possible after the last milking, and was administered by the partial insertion technique. Following the subsequent calving, all cows were sampled three times during the first month of lactation at 3 to 9, 10 to 16, and 24 to 30 DIM. Quarters that were infected before the dry period were considered to be cured if they were culture negative for *S. aureus* on all three samples taken in the first month of lactation. A cow was

defined as cured if all infected quarters before drying-off were culture negative on all three samples after freshening.

Herd Management and Other Data Collection

During one of the scheduled visits to each farm, the technician at each site administered a comprehensive herd survey. Data collected included housing style, milking hygiene practices, management of the environment for both lactating and dry cows, as well as routine drying-off procedures used by each individual farm. Management factors used to determine appropriate drying-off time, and specific practices such as abrupt versus intermittent cessation of milking, gram-negative core-antigen vaccination, and teat-end protection were collected. Individual cow DHI data for the last three DHI tests before and including the month of drying-off, were collected. The data consisted of dry period length, SCC, linear score (LS), 305 mature equivalent milk, and 24-h yields of milk, fat and protein.

Bacteriological Procedures

Teats were aseptically prepared before collection of all samples according to National Mastitis Council sample collection and handling guidelines (NMC, 1999). Samples were frozen and shipped to the Mastitis Research Laboratory at the University of Guelph. All laboratory procedures were performed by the same individuals, who were blinded to treatment, and in accordance with NMC recommendations (NMC, 1999). Briefly, depending on the day of arrival at the lab, samples were either thawed at room temperature or stored at -20°C for a period of not longer than 5 d, and then thawed the first day of the following week. An inoculum of 0.01 ml of milk was plated onto Columbia base agar containing 5% sheep blood. Plates were incubated at 37°C and examined for bacterial growth at 24 and 48 h. Colonies were tentatively identified, and a presumptive diagnosis of staphylococci, streptococci, coliform, or other pathogens was made, based on colony growth, morphology and appearance, pattern of hemolysis, catalase reaction, and Gram staining. Staphylococcal isolates were tested for coagulase production with the tube coagulase test. Streptococcal isolates were further subcultured with agar containing esculin. Gram-negative bacteria were plated on MacConkey agar to facilitate identification. Gross appearance and reaction to citrate were used to differentiate *Escherichia coli* and *Klebsiella* spp. For each positive quarter, the number of cfu per 0.01 ml of milk was reported in one of four categories: 1 to 5, 6 to 10, 11 to 50 or ≥ 50 cfu. A quarter was considered infected with coagulase-negative staphylo-

cocci (CNS) if greater than or equal to 11 cfu per 0.01 ml were isolated. The isolation of CNS was reported when the organism grew with another pathogen. A sample was considered contaminated if three or more colony types were present on a plate. Since all samples were frozen, quarter SCC determination was not performed.

Statistical Analysis

Data generated from the quarter milk cultures, herd management surveys and individual cow DHI records, were stored in a Microsoft Access database (Microsoft Access 2000). Only those herds that participated in the trial for its entire length, and that had *S. aureus* isolated from at least one cow during that time, provided the data analyzed in this study. Records for 308 quarters infected with *S. aureus* from 219 cows on 59 farms were extracted from the database and imported into SAS version 8.01 (SAS, 1999). There were 18 farms in which cows infected with *S. aureus* were either not identified, or cows were identified but they did not complete the trial. Descriptive statistics were generated using the univariate and frequency procedure in SAS (PROC UNIVARIATE, PROC FREQ, SAS v.8.01). Differences in cure rates were tested with a chi-square (χ^2) analysis. Least square means for linear scores (LS) at drying-off were calculated (PROC GLM) and differences examined using a Student's *t*-test. Logistic regression for cure or no cure of *S. aureus* infection was modeled by fitting a generalized linear model using the GENMOD procedure, with the logit link function, and a binomial error distribution. Because quarters within a cow are not independent, correlation within cows was accounted for using generalized estimation equations (Liang and Zeger, 1986; Barkema, 1997). A compound symmetry correlation structure was used. Data were analyzed by quarter, cow, and herd levels, acknowledging that there was clustering of quarters within a cow, as well as of cows within a herd. The variance components at both the herd and cow level were evaluated to decide whether cow and herd effects would be considered as either random or fixed effects in the final model. Since herds had a very low variance component, the most appropriate and best fitting model included herd as a fixed effect and cows within herds as a clustering variable. In fitting herds as a fixed effect, a herd size variable was created to identify each herd that had more than 100 cows. This allowed comparison between these herds, as well as to those that were smaller in size. This distinction was necessary, as the model did not converge when a parameter was estimated for each individual herd. The choice of herd size was based on inherent differences in management strategies and housing styles associated with larger herds.

A variable to quantify the degree of shedding was created using the reported colony-forming units on each positive sample. The original results from the laboratory were reported in categories, thus the variable created was categorical. A bacteriology result of 1 to 5 cfu was given a code of 1, 6–10 cfu a code of 2, 11–50 cfu coded 3, and greater than 50 cfu coded 4. Hence, if a cow had three positive samples that were coded 3, 3, and 4, the shedding code assigned to that cow was 10. To assess the effect that the month of drying-off had on the cure rate, a season variable was created using 3-mo intervals. Spring was designated as March–May, Summer as June–August, Fall as September–November, and winter included December to February. A univariate model with each independent variable of interest was evaluated first, with all significant variables at $P < 0.20$ allowed to enter the multivariable model. Relaxing the P value for entry into the model was used as a method to ensure all covariates, with a potential to exert significant main effects as well as important confounding effects, were considered. A backwards step-wise procedure was used to determine the final model. Statistical significance was declared at $P < 0.05$, but variables with $P < 0.10$ were allowed to stay in the model based on a tendency towards significance, and if these associations were deemed to be of biological importance. All biologically plausible two-way interactions were tested. The estimated regression parameters were converted to odds ratios.

RESULTS

The total number of cows dried off that were defined as infected with *S. aureus* positive was 288. The percentage of quarter samples that were contaminated was 13.5%. After excluding cases where protocol was not followed or cows were lost to follow-up sample collection, (samples not taken, cows having received other antibiotic treatment, cows aborting in the dry period or having been sold), as well as excluding all contaminated quarters, 219 cow records, representing 308 infected quarters, were available for analysis of treatment efficacy and risk factors for elimination of *S. aureus* infection. Complete DHI records were not available for all 219 cows, as some herds did not subscribe to this milk recording service. Descriptive data pertaining to the infected quarters and cows are presented in Tables 1 and 2. Of the 219 *S. aureus* positive cows, 154 (70.3%) had only one quarter infected. The percentage with two, three, or four infected quarters were 21.5, 5.5, and 2.7%, respectively. The average LS as reported on the last DHI test in the month of drying-off was 5.3. There was a significant difference in the mean LS of cows based on the number of quarters infected (Table 1). A calculation

of the mean prevalence of *S. aureus* within herds was made, by using the number of cows identified as infected in a herd divided by the total herd size. The mean was found to be 7% in the 59 herds with a range of 1.2 to 36%.

The majority (58%) of the 308 quarters identified as infected cultured positive on all three samples taken before drying-off. There was a linear decline in the percentage cure with the number of times a quarter cultured positive (Figure 1). The cure rate for quarters that cultured positive on one sample was significantly higher than quarters that cultured positive multiple times. The distribution of the location of the infected quarters was approximately equal between front and hind quarters (47 and 53%, respectively).

The overall cure of *S. aureus* after DCT was 136 of the 219 cows (62%), and 208 of the 308 quarters (67.5%). The cure rates achieved with intramammary tilmicosin was 67.3% of cows and 72.5% of quarters (Table 2). By comparison, the cure achieved with intramammary cloxacillin was 56.9% of cows and 62.9% of quarters. The results of unconditional univariate analysis determined that this difference in cure rates between treatments was not statistically significant at the $P < 0.05$ level for either cow or quarter cure rates (Table 2).

Cure rates were seen to be higher in cows that were finishing their first lactation, that had a LS of less than 5 on their last DHI test. There was a statistically significant difference in the cure rate based on LS on last DHI test: 70.9% of cows cured that were at $LS < 5$, compared with 56.4% that were at $LS \geq 5$ ($P < 0.05$). The highest cure rate was observed in cows that had three quarters infected (83.3%). The percentage of cows that cured with one, two, or four quarters infected were 65.6, 46.8, and 50%, respectively (Table 2).

Logistic Regression Analysis

Quarter level. The logistic regression model for cure of quarters is shown in Table 3. Variables predicting the probability of bacteriologic cure in an infected quarter included the number of samples that cultured positive, location of the quarter and the use of tilmicosin DCT (Table 2). The fixed effect herd size variable was forced into the final model. When accounting for the other variables in the model, the use of tilmicosin DCT resulted in a higher probability for cure of *S. aureus* ($P = 0.04$). The odds ratio (OR) for cure comparing tilmicosin DCT to cloxacillin was 1.8. The probability of cure increased when the infection was in a front quarter (OR = 2.3). There was a decreased risk of cure with an increasing number of positive samples before drying-off (OR = 0.6). A higher LS, based on DHI testing at the cow level, tended to decrease the probability of cure at

Table 1. Descriptive data of cows and quarters infected with *Staphylococcus aureus* prior to drying-off.

Category	Quarters	Cows
Observations (no.)	308	219
Times an individual quarter cultured positive prior to drying-off (no.)		
1	62 (20.1%)	
2	67 (21.8%)	
3	179 (58.1%)	
Quarter location		
Front	145 (47%)	
Hind	163 (53%)	
Isolation of CNS ¹ as well as <i>S. aureus</i> in the same quarter sample	11 (3.6%)	
Quarters infected per cow dried off (no.)		
1		154 (70.3%)
2		47 (21.5%)
3		12 (5.5%)
4		6 (2.7%)
Mean lactation (range)		2 (1–9)
Parity 1		63 (28.8%)
Parity 2		57 (26%)
Parity 3+		99 (45.2%)
Mean LS ² last DHI test (95% CI):		
Overall and by the number of quarters infected with <i>S. aureus</i>		
Overall		5.3 (5.1–5.5)
1 qtr		5.1 ^a (4.8–5.3)
2 qtr		5.7 ^b (5.4–6.1)
3 qtr		5.9 ^{abc} (4.8–6.9)
4 qtr		6.8 ^c (5.8–7.7)

^{a,b,c}Means with different superscripts are significantly different at $P < 0.05$.

¹CNS = Coagulase-negative staphylococci.

²LS = Linear SCC.

Table 2. Descriptive results of the effect of parity, number of quarters infected, LS¹ on the 1st DHI test, and treatment on cure of *Staphylococcus aureus* in 219 cows and 308 quarters.

Factor	Total	Cure (cow)		Cure (qtr)	
		No.	%	No.	%
Parity					
1	63	42	(66.7%)		
2	57	34	(59.7%)		
3+	99	60	(60.6%)		
Quarters infected ²					
1	154	101	(65.6%)		
2	47	22	(46.8%)		
3	12	10	(83.3%)		
4	6	3	(50%)		
LS ¹ at Drying-off					
< 5	86	61	(70.9%) ^a		
≥ 5	133	75	(56.4%)		
Intramammary DCT ³ —qtr					
Tilmicosin	149			108	(72.5%) ^b
Cloxacillin	159			100	(62.9%)
Intramammary DCT ³ —cow					
Tilmicosin	110	74	(67.3%)		
Cloxacillin	109	62	(56.9%)		

^a $P < 0.05$.

^b $P < 0.10$.

¹LS = Linear SCC.

²Quarters infected = number of quarters per cow defined as positive for *S. aureus* before drying-off.

³DCT = Dry cow antibiotic therapy.

Figure 1. Percentage cure of quarters infected with *Staphylococcus aureus* during the dry period, relative to the number of times that the quarter cultured positive prior to drying-off.

the quarter level ($P = 0.08$). LS was a significant risk factor for cow level cure ($P = 0.05$). The presence of a CNS infection in the same quarter infected with *S. aureus* was associated with a much higher OR for cure (5.1). The effect was nonsignificant but may be biologically important due to its magnitude. The goodness of fit of the model was assessed by the departure of the model deviance from the degrees of freedom. The deviance of this final model is 308 with 289 df, which indicated acceptable fit.

Cow level. The logistic regression model for cure of cows is shown in Table 4. Risk factors for bacteriological

cure of cows included LS on the last DHI test, bacterial shedding, season of drying-off, as well as intramammary treatment with tilmicosin. At the cow level, intramammary tilmicosin remained a significant factor in the model ($P = 0.03$). A cow treated with tilmicosin has a 2.1 times greater chance of curing *S. aureus*, compared with a cow treated with cloxacillin. The variable 'shedding' was significant ($P = 0.0002$), indicating that as the degree of *S. aureus* shed increased, the probability of cure decreased. Clearly, an equivalent shedding variable could be generated from large numbers of bacteria shed from one quarter or from moderate numbers shed from multiple quarters. Therefore, the interaction of shedding and number of infected quarters was tested and found to be significant ($P = 0.002$). This interaction indicated that lower shedding per infected quarter had a somewhat better probability of cure. There was a significant effect, at the cow level, for season to impact the probability of cure. In comparison to cows that were dried off in the spring season (March-May), cows that were dried off during the winter (December-February) were 4 times more likely to cure *S. aureus* infection ($P = 0.004$). Age of the cow and dry period length were not significant risk factors for *S. aureus* cure either at the quarter or cow level. With 198 df and a deviance of 221, the fit of the model was acceptable.

DISCUSSION

Administration of DCT at the end of lactation remains one of the cornerstones of an udder health management program. Previous research has demonstrated that the dry period represents a time of increased susceptibility to new IMI and that DCT can eliminate and prevent new infections during this time. However, the

Table 3. Results of final logistic regression model for the probability of cure of quarters infected with *Staphylococcus aureus* during the dry period.

	β estimate	SE	Wald 95% confidence limits		P-value	Odds ratio
Intercept	2.4595	0.74	1.015	3.904	0.0008	
Herds ¹	...	...	...	...	...	
LS ² at Dry-off	-0.1968	0.11	-0.419	0.025	0.08	0.8
DCT ³						
Tilmicosin	0.6190	0.29	0.03	1.203	0.04	1.8
Cloxacillin	...	...	...	...	...	...
Front Quarter	0.8429	0.24	0.373	1.313	0.0004	2.3
No. cultures positive ⁴	-0.4615	0.20	-0.847	-0.077	0.02	0.6
CNS infection ⁵	1.6447	0.93	-0.171	3.460	0.08	5.1

¹Fixed effect of herds forced into the model.

²LS = Linear SCC.

³DCT = Dry cow antibiotic therapy.

⁴Number of quarter cultures prior to drying-off from which *S. aureus* was isolated.

⁵CNS infection = >10 cfu/0.01 ml milk coagulase negative staphylococci isolated from same quarter as *S. aureus*.

Table 4. Results of final logistic regression model for the probability of cure of cows infected with *Staphylococcus aureus* during the dry period.

	β estimate	SE	Wald 95% confidence limits		P-value	Odds ratio
Intercept	3.4579	1.13	1.249	5.666	0.002	
Herds ¹	...	...	...	...	...	
LS ² at Dry-off	-0.2406	0.12	-0.482	0.0005	0.05	0.78
DCT ³						
Tilmicosin	0.7522	0.35	0.066	1.438	0.03	2.1
Cloxacillin	...	...	...	...	...	
Season of dry-off						
Summer	0.8299	0.47	-0.100	1.76	0.08	
Fall	0.6504	0.43	-0.185	1.486	0.13	
Winter	1.4880	0.52	0.479	2.498	0.004	4.4
Spring	...	...	...	...	...	
Shedding (CFU) ⁴	-0.3275	0.09	-0.501	-0.154	0.0002	0.72
No. Quarters Positive ⁵	-0.3971	0.56	-1.498	0.704	0.48	
(Shedding)*(No. Qtr.) ⁶	0.0832	0.03	0.031	0.136	0.002	

¹Fixed effect of herd forced into the model.²LS = Linear SCC.³DCT = Dry cow antibiotic therapy.⁴Variable created to assess the amount of colony forming units of *S. aureus* shed.⁵Number of quarters from which *S. aureus* was isolated in a cow.⁶Interaction term of shedding by the number of quarters positive.

rate of elimination of existing *S. aureus* mastitis following DCT at the end of lactation is variable. Tilmicosin phosphate is a semisynthetic macrolide antibiotic, which has been shown to have important interactions with bovine phagocytes and epithelial cells, demonstrating a potential role in its clinical efficacy against intracellular organisms. One mechanism, believed to aid in *S. aureus* evasion of cure during DCT, is by the intracellular accumulation of *S. aureus* within phagocytes. Hensen et al. (2000) recently studied the location of *S. aureus* after experimental infection, both by gram staining and immunochemical staining of tissues with antibodies against capsular polysaccharide 5 (CP5). In both early and chronic stages of these infections, *S. aureus* was located in the lumen of alveoli, associated with epithelium, and within phagocytic cells. It was concluded that invasion by *S. aureus* of the interstitium, as well as expression of CP5, augment its resistance to host defense mechanisms. Likewise, Nickerson and Heald (1981) demonstrated that both under natural chronic infection, and in experimental infections, *S. aureus* could be observed in neutrophils or macrophages. Therefore, if an intramammary DCT product had the ability to be accumulated within these same cells, an improved efficacy against mastitis caused by *S. aureus* may be attainable.

Benzathine cloxacillin was used as the positive control in this field trial, as it has been commercially available and used for approximately 20 yr (Keefe, 1980). Cloxacillin is a semisynthetic penicillin. When it was first evaluated as a DCT, the rates of elimination of *S.*

aureus when administered at drying-off were 85 and 77% in controlled and field trial experiments, respectively. One year later, Ziv et al. (1981) published efficacy data comparing three antibiotics at drying-off, one of which was a derivative of benzathine cloxacillin. This data revealed a mean cure rate of *S. aureus* from all three treatments during the dry period of 81.4%. The cure rate for benzathine cloxacillin was 83.1%.

The most recently published efficacy data for the use of tilmicosin at drying-off was reported by Nickerson et al. (1999), who used cephalixin benzathine as a positive control. This study demonstrated that an experimental intramammary formulation of tilmicosin was equally as effective as cephalixin benzathine for eliminating *S. aureus* mastitis at both the cow and quarter levels, with tilmicosin achieving 64.3 and 78.1% cure rates, respectively. The main difference between Nickerson's study and the current field trial, other than the positive control used, was the nature of the infections. The previously cited numbers were obtained following dry cow therapy of 44 cows, the majority of which had infections that were experimentally induced. The cure rates for both intramammary treatments were consistently higher for experimental infections than those caused naturally (Nickerson et al., 1999). The current study was done in commercial and research farms in three geographical regions of Canada, with cows that were naturally infected, and identified through the culture of quarter milk samples collected prior to drying-off.

An important consideration that must be addressed whenever efficacy data for therapy of mastitis patho-

gens, especially for cure rates of *S. aureus*, are reported, are the definitions of infection and treatment cure. In this current study, the definition of infection before drying-off was at least one of three samples being culture positive (≥ 1 cfu/0.01 ml) for *S. aureus*. Based on the fact that approximately 80% of the infections in this trial actually cultured positive on multiple samples before drying-off, confidence that these quarters and cows were indeed infected with *S. aureus* seems reasonable. Furthermore, these animals were probably a good representation of naturally infected cows in commercial herds. However, there were 20.1% of quarters in this dataset that had only a single isolation of *S. aureus*, and there was also an underlying assumption that when *S. aureus* was not isolated, that an infection did not exist. Intermittent and cyclical shedding of *S. aureus* does occur (Sears et al., 1990). Sears et al. (1990) studied the shedding patterns of *S. aureus* and the implication of shedding patterns when evaluating the efficacy of antimicrobial drugs. They concluded that the cyclical pattern of shedding necessitates consecutive samples. The probability of at least one sample being positive from a *S. aureus*-infected quarter when three samples are taken was estimated at 98% (Sears et al., 1990). Based on this fact, both the definition of infection and the definition of cure in this trial were made on three consecutive samples. The decision to extend the sampling out of 30 DIM in the subsequent lactation was deemed appropriate, by earlier research which established that it may take up to 28 d for a *S. aureus* infection to reappear (Newbould, 1974). Another more recently identified aspect of *S. aureus* cure and sampling, is the identification of the particular strain of *S. aureus* through DNA technologies (Zadoks, 2000). This technology was not incorporated into this research trial. Thus, there is a possibility that some treatment failures in efficacy trials, such as the current study, may indeed be misclassified and are actually a combination of a cure and a new infection caused by an entirely different strain of the same organism (Schukken et al., 2001).

Regardless of the efficacy of any one treatment regime of therapeutic approach, emphasis should be placed on identifying cows that have a high probability of cure. Because antibiotic use on dairy farms is under increasing scrutiny, considering opportunities for more rational and selective use of DCT is of value (Schukken et al., 2001). The results of this study indicate that risk factors for cure of quarters infected with *S. aureus* are the number of positive cultures, the location of the quarter, and possibly a coexisting CNS infection ($P < 0.10$). *S. aureus* IMI was less likely to be cured in quarters that were culture positive more than once. Also, cure of *S. aureus* was less likely to occur when the infection was in a rear quarter. These risk factors have been previously

reported (Sol et al., 1994; Osteras et al., 1999). Indeed, there is a possibility that the number of positive samples may be a surrogate measure of severity or chronicity. Also there is a possibility that only a single isolation of *S. aureus* may not truly have been an infection. The repeatability of the significance of the location of the quarter is surprising. Osteras et al. (1999) established even more specifically the predictive failure of an infection with a major pathogen in the right hind quarter, not just a hind quarter. The authors hypothesized in that study that more persistent infections in the right hind quarter might result from hygienic contamination caused by laterality or side-specific interference of blood circulation. No information was gathered in this study to refute or support this claim, or to offer any other hypothesis either. The repeatability of this finding however, should be the attention of further work.

The linear score of the cow in the month before drying-off had a tendency to significantly impact the quarter level cure rate ($P = 0.08$). As the linear score increased, the probability for cure of an infected quarter decreased (OR = 0.8). There was no expectation that a cow level measure such as LS would have that strong of an influence at the quarter level. However, this is likely because 70% of infected cows in this trial only had one quarter infected with *S. aureus*. Similar to the number of positive samples, a high LS is probably indicative of a more severe infection. Sol et al. (1994), also found that an increasing SCC decreased the probability of quarter cure. The other biologically significant finding at the quarter level was the tendency for an existing infection caused by CNS to increase the probability of cure ($P = 0.08$). This finding is based on only a very small number of quarters that had both CNS and *S. aureus* isolated. The literature indicates that minor pathogen infections have a protective effect against the establishment of *S. aureus* infections (Nickerson et al., 1994; Lam et al., 1997; Schukken et al., 1999). Because these infections were identified at the same time as the *S. aureus*, this may not be a readily plausible explanation for the effect seen here. Competition between the two organisms has been described and can result in a shorter duration of *S. aureus* infection (Lam et al., 1997). However, the mechanism producing the protection effect is not fully understood. General activation of the immune system, competition for binding sites and alterations in teat canal keratin are offered as possible explanations (Rainard et al., 1988; Nickerson et al., 1994; Lam et al., 1997).

Risk factors for cure of cows infected with *S. aureus* included LS on the last DHI test before drying-off and the amount of bacteria being shed by the cow. As LS and the amount of bacteria shed increase, the likelihood of a cure diminished. The other factor found to be sig-

nificantly associated with the cure rate of *S. aureus*-infected cows in this research was the season of drying-off. Cows dried off from December to February had a significantly higher cure rate. Biologically satisfying reasons for this effect are not apparent. There was not a significant association with the number of quarters positive for *S. aureus*, as a determinant on its own. However, there was an interaction between the number of quarters and shedding, and as reported in Table 2, the highest proportion of cure was observed in the 12 cows that had three quarters infected (83.3%). However, Sol et al. (1994) reported that cows with more than one quarter infected were 0.57 times less likely to cure. Our result is similar to the finding of Nickerson et al. (1999), who also reported that cure rates for intramammary tilmicosin were not influenced by either the number of quarters infected or by the lactation number. There was also no association between the probability of cure and dry period length. A positive correlation between a SCC measure and dry period length can reduce the effect of both variables individually (Sol et al., 1994). LS on the last DHI test was significant in the model for cure at the cow level. Thus, the finding that dry period was not a risk factor may be reasonable since these two variables were moderately correlated in this data ($r = 0.56$).

CONCLUSIONS

The results of this field trial, conducted in 75 commercial and two research dairy farms, demonstrate that intramammary tilmicosin was efficacious in eliminating existing *S. aureus* mastitis during the dry period. Significant risk factors were identified that were associated with bacteriological cure. Quarters that were culture positive more than once, and that were located in the rear of the udder, were less likely to cure. Significant factors for cow cure rates were low LS in the month prior to drying-off, and shedding low numbers of bacteria. This study has demonstrated the efficacy of a new antibiotic as a dry cow therapeutic product in the struggle against *S. aureus* mastitis. At present, an intramammary formulation of tilmicosin for therapy of cows at drying-off is not available. Of equal importance to choosing the appropriate product, is the identification of risk factors which can aid in the decision of which cows can be expected to respond favorably to dry cow antibiotic therapy. This study confirmed the usefulness of LS before drying-off, culture history, and the location of *S. aureus* infection, for incorporation into mastitis control and therapy decision-making processes.

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