PEER REVIEWED

Comparative Efficacy of a Novel Intramammary Dry Cow Antibiotic to Eliminate Subclinical Mastitis in the Dry Period

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Abstract

Although there is increasing concern over routine administration of long-acting antibiotics to all cows at the end of lactation, the practice is required to eliminate subclinical infections that are present at drying-off. Alternatives to dry-cow therapy (DCT) to prevent new infections from occurring in uninfected quarters in the dry period are increasingly common. However, until a cost-effective and reliable test becomes available to determine which cows and quarters are not infected at drying-off, administering DCT to all quarters and all cows remains the recommended practice. The efficacy of a novel intramammary treatment containing tilmicosin, administered at drying-off to eliminate mastitis pathogens other than *Staphylococcus aureus*, was compared to intramammary cloxacillin. Data from 406 infected quarters, representing 238 cows that either received intramammary tilmicosin or benzathine cloxacillin, were analyzed. A total 11% of quarters had a major mastitis pathogen recovered prior to the dry period. The overall cure rate of mastitis pathogens following administration of dry-cow antibiotic therapy was 89.2%. The cure achieved with intramammary tilmicosin and cloxacillin was 87.2% and 90.9%, respectively. There was no significant difference in the cure rate between the two treatments, either in overall cure, or cure of any specific pathogen. Results of the final logistic regression models to determine factors associated with the probability of infected quarters to cure during the dry period re-emphasize the importance of dry period length, season of drying-off and general environmental exposure. It was concluded that intramammary tilmicosin is as efficacious as benzathine cloxacillin for eliminating existing infections caused by pathogens other than *S. aureus* during the dry period. No commercial preparation of intramammary tilmicosin is currently available.

Résumé

Bien qu'il existe de plus en plus de doute relié à l'utilisation routinière d'antibiotiques à longue action chez toutes les vaches en fin de lactation, cette utilisation est requise pour éliminer les infections sous-cliniques présentes durant le tarissement. Des alternatives à la thérapie en début de tarissement sont de plus en plus utilisées pour la prévention de nouvelles infections dans les quartiers non-infectés durant la période du tarissement. Toutefois, en attendant qu'un test solide à un prix raisonnable devienne disponible pour déterminer quelles vaches et quels quartiers ne sont pas infectés au tarissement, la thérapie en début de tarissement pour tous les quartiers et toutes les vaches demeure la seule pratique recommandée. L'efficacité d'un nouveau traitement intramammaire, contenant de la tilmicosine administrée en début de tarissement pour éliminer les pathogènes causant la mammite autres que *Staphylococcus aureus*, a été comparée à l'administration

intramammaire de cloxacilline. Les données de 406 quartiers, provenant de 238 vaches traitées avec une administration intramammaire de tilmicosine ou de cloxacilline benzathine, ont été analysées. Un pathogène majeur de la mammite a été isolé dans 11% des quartiers en début de tarissement. Le taux de guérison global des pathogènes de la mammite suite à la thérapie antibiotique en début de tarissement était de 89.2%. Le taux de guérison atteint suite à l'administration intramammaire de tilmicosine était de 87.2% et de 90.9% pour la cloxacilline benzathine. Il n'y avait pas de différence dans le taux de guérison entre les deux traitements de même que dans la guérison des pathogènes en général ou de certains pathogènes en particulier. Les résultats du modèle final de régression logistique, utilisé pour déterminer les facteurs associés avec la probabilité de guérison des quartiers infectés en début de tarissement, remettent de nouveau l'accent sur la longueur de la période de tarissement, la saison de tarissement et l'exposition générale aux facteurs de l'environnement. On conclu que le traitement intramammaire avec la tilmicosine est aussi efficace que celui avec la cloxacilline benzathine dans l'élimination des infections causées par des pathogènes autres que *S. aureus* en début de tarissement. Il n'existe pas de préparation intramammaire de tilmicosine sur le marché présentement.

Introduction

The goal of mastitis control during the dry period is to have as few infected quarters as possible at the next calving.[9] Achieving this goal involves eliminating intramammary infections (IMI) present at the end of lactation, as well as preventing new IMI from occurring 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 following the last milking.[3,9,13] As such, administration of DCT remains one of the key components of the National Mastitis Council (NMC)-recommended mastitis control program. However, a continuing controversy involving DCT revolves around the necessity to treat all quarters of all cows at the end of lactation. Momentum for this debate has increased with growing public awareness and concern for the issue of antibiotic resistance, residue avoidance, and the reduced prevalence of IMI in dairy herds that have implemented effective udder health management practices. Until such time that a cost-effective, fast and reliable test becomes available to identify uninfected cows and quarters at the end of lactation, the administration of DCT will remain one cornerstone of dry-period udder health management.

The use of DCT can reduce the incidence of new IMI by 50 to 80%, especially early in the dry period.[2,9] In addition, historical reports indicated that approximately 70 to 98% of infections present at drying-off were eliminated during the dry period following DCT.[15,26] However, it is generally acknowledged that DCT is less successful at eliminating IMI caused by *Staphylococcus aureus*. The cure rate of *S. aureus* has been reported to be approximately 50%.[6,8,19,20] In an attempt to augment the overall effectiveness of DCT, and improve cure rates for *S. aureus* during the dry period over conventional therapy, new intramammary products have been tested.[5,7,8,16] One such treatment recently investigated is tilmicosin.

Tilmicosin is a semi-synthetic macrolide antibiotic, currently approved for the treatment of bovine respiratory disease. Tilmicosin was recently compared to intramammary cephapirin benzathine as a treatment for *S. aureus* at dry-off.[16] That study primarily focused on cure of experimentally challenged and known *S. aureus*-positive cows. Efficacy data for tilmicosin against pathogens other than *S. aureus* were reported for only a very few quarters that were infected. Specifically, results revealed a 50% cure of *Streptococcus agalactiae* in two infected quarters, and 100% cure of three quarters infected with *Staph. chromogenes*.[16] In a subsequent trial to document the prevalence of mastitis in heifers, and the efficacy of pre-partum administration of selected antibiotics, efficacy of intramammary tilmicosin for eliminating *Staphylococcus* species and *Streptococcus* species IMI were reported.[18] The overall cure of five infected quarters with *Streptococcus* species was 100%, and the cure rate for various species of staphylococci organisms was 95-100%.[18] The objective of this paper was to compare the efficacy of two intramammary antibiotics, one of which was tilmicosin, administered to mature dairy cows at drying-off to eliminate existing infections caused by pathogens other than *S. aureus*. The comparative efficacy of the same two antibiotics to eliminate *S. aureus*, and a detailed analysis of the risk factors associated with *S. aureus* cure, was completed as a separate objective of this trial. The results have been previously reported.[8]

Materials and Methods

Herd selection

For a period of one year, beginning in July 1999, cows in 22 commercial and two research dairy herds located in three eastern Canadian provinces participated in a formal field trial (Ontario [n=17], Quebec [n=3], Prince Edward Island [n=4]). Herds were purposely 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 three weeks, and that were one month from the time of scheduled drying-off, were eligible for enrollment. All cows within each herd that

were eligible for enrollment were randomized to a treatment schedule. The treatment schedules were established to achieve three trial objectives. The findings from the first two objectives, prevention of new infections in uninfected cows[7] and elimination of *S. aureus*[8], have been previously published.

Sampling time line and treatment randomization

A study technician collected quarter-milk samples from all eligible cows starting between 28 (day –28) and 22 (day –22) days prior to scheduled drying-off, and again between 21 (day –21) and 14 (day –14) days prior to drying-off. Drying-off date was calculated to allow cows to have a minimum 60-day dry period for residue avoidance concerns. The maximum dry period allowed was 120 days. Cows that had at least one quarter that yielded growth of a major mastitis pathogen, other than *S. aureus*, or that yielded 11 or more colony-forming units (CFU) of coagulase-negative staphylococci (CNS) in 0.01 ml of milk, on either of these first two samples, were defined as infected. These cows were sampled for a third time at drying-off (day 0). The culture result of the third sample taken on the day of drying-off was used as confirmation of infection prior to the dry period in the analysis. Each cow was randomized to receive either intramammary benzathine cloxacillin[a] or a novel intramammary formulation of 1500 mg tilmicosin phosphate[b] in all four quarters, regardless of the number of quarters infected. 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 during the first month of lactation at 3 to 9 days-in-milk (DIM). Quarters that were infected prior to the dry period were considered to be cured if they were culture-negative for the pathogen that was present prior to drying-off on this single sample.

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 dry period teat-end protection were collected. Individual cow Dairy Herd Improvement (DHI) data for the last three DHI tests prior to and including the month of drying-off, and for the first test of the subsequent lactation, were collected. The data consisted of dry-period length, somatic cell count (SCC), Linear Score (LS) and 305-day mature-equivalent milk.

Bacteriological procedures

Teats were aseptically prepared prior to collection of all samples according to the National Mastitis Council (NMC) sample collection and handling guidelines.[14] 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.[14] The microbiological procedures have been described in detail elsewhere.[7,8] For each positive quarter, the number of CFU per 0.01 ml 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 staphylococci (CNS) if greater than or equal to 11 CFU per 0.01 ml were isolated. Techniques for detecting infections caused by *Mycoplasma* species were not performed. A sample was considered contaminated if three or more colony types were present on a plate.

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). Records for 474 quarters infected with mastitis pathogens other than *S. aureus* from 272 cows on 24 farms were extracted from the database and imported into SAS version 8.01.[23] There were 68 quarters from a total 34 cows that were excluded from the analysis because either the cow received systemic antibiotics, aborted during the dry period, or had been sold. Furthermore, cows that had a dry period greater than 120 days or less than 45 days were excluded. 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. Logistic regression for cure or no cure of an existing 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 by using generalized estimation equations.[1] A compound symmetry correlation structure was used. Data were analyzed by quarter, cow and herd level, 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. Because 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 among 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.

To assess the effect that the month of drying-off had on the cure rate, a season variable was created using three-month intervals. Spring was designated as March-May, summer as June-August, fall as September-November, and winter included December-February. A parity variable was created to designate cows ending their first lactation as parity 1, cows ending their second lactation as parity 2, and all other cows to be of third or greater parity. 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. A backwards step-wise procedure was used to determine the final model. Statistical significance was declared at $P < 0.05$. The fixed effect of treatment was forced into the model. All biologically plausible two-way interactions were tested. The estimated regression parameters were converted to odds ratios.

Results

Complete data from 406 quarters, infected prior to drying-off, of 238 cows are reported. Descriptive statistics for the cows dried-off are shown in Table 1. The majority of cows were ending their first or third and greater lactation as compared to their second (43.3%, 37.8%, 18.9%, respectively). Also, the majority of cows (52.5%) identified as having a mastitis pathogen, other than *S. aureus*, were infected in only one quarter. Of the 238 cows, a total of 114 received intramammary tilmicosin, and 124 received benzathine cloxacillin. The average dry period length was 65.4 ± 10.7 days. There was no significant difference in the dry-period length of cows based on the treatment administered. From the herd survey, 21% of the 24 herds indicated that they routinely administered gram-negative core antigen vaccines during the dry period, and 29% of herds attempted to protect teat-ends after drying-off either by applying regular teat dip or a commercial teat-end barrier product.

The prevalence of specific pathogens identified prior to drying-off is shown in Table 2. The prevalence of *S. aureus* IMI and the cure of these subclinical infections has been previously reported.[8] Of the 406 quarters identified as infected, the majority (73.9%) cultured greater than 10 CFU CNS in 0.01 ml of milk. There were only three quarters (0.74%) infected with a contagious pathogen (*Streptococcus agalactiae*), whereas 24.8% were infected with environmental organisms. Of the environmental IMI, the majority were caused by environmental streptococci, with other streptococci, *Strep. uberis* and *Strep. dysgalactiae,* accounting for 16.3%, 2.9% and 1.7% of the quarter infections, respectively. There were 12 (2.9%) quarters infected with *Escherichia coli* (*E. coli*) prior to drying-off.

Table 1. Descriptive statistics of 238 cows infected with a mastitis pathogen, other than *S. aureus*, at the end of lactation.

Variable		N (%)	Mean	95 % CI
Observations		238		
Parity	1	103 (43.3)		
	2	45 (18.9)		
	3+	90 (37.8)		
No. quarters infected[1]	1	125 (52.5)		
	2	65 (27.3)		
	3	41 (17.2)		
	4	7 (3.0)		
IMM DCT received[2]	Tilmicosin	114 (47.9)		
	Cloxacillin	124 (52.1)		
Dry-period length (days)	Overall		65.4	63.9, 66.9
	Tilmicosin		64.9	63.4, 66.4
	Cloxacillin		66.0	64.5, 67.5

[1]Number of quarters infected per cow, with a pathogen other than *S. aureus,* on at least one sample prior to drying-off

[2]Randomization of cows based on intramammary dry cow antibiotic therapy administered

Table 2. Relative frequency of the isolation of specific pathogens identified as intramammary infections in 406 bacteriologically positive quarters prior to drying-off.[1]

Pathogen	N	Percent of total
Minor pathogens		
coagulase-negative staphylococci	300	73.9
Major pathogens		
Other *Streptococci* sp	66	16.3
Streptococcus uberis	12	2.9
Streptococcus dysgalactaie	7	1.7
Escherichia coli	12	2.9
Klebsiella sp	2	0.5
Streptococcus agalactiae	3	0.7
Serratia sp	2	0.5
Other pathogens		
Yeast	2	0.5
Total	406	99.9

[1]Quarters infected with *Staphylococcus aureus* not included in this dataset. Bacteriological cure and risk factors for cure of this organism during the dry period have been previously reported.

The cure rates of specific pathogens by the intramammary treatment received are detailed in Table 3. Overall, 89.2% (362/406) of quarters infected with mastitis pathogens cured following the dry period. The overall cure achieved in quarters that received tilmicosin and benzathine cloxacillin was 87.2% and 90.9%, respectively. This difference was not statistically significant ($\chi^2 = 1.44$ with 1 degree of freedom, $P = 0.2$). The cure rate of quarters infected with CNS was 87.7% overall, with a 85.2% cure in quarters that received tilmicosin, and a 90.7% cure in quarters that received benzathine cloxacillin, at drying-off. There were 12 quarters infected with *Strep. uberis* prior to drying-off, and 91.7% of these infections were cured (100% and 85.7% for tilmicosin and cloxacillin, respectively). The majority of *streptococcus* infections did not have the specific genus reported, and were considered 'other streptococci'. In total, 95.5% (63/66) of quarters infected with other streptococci were cured during the dry period (96.6% and 94.6% for tilmicosin and cloxacillin, respectively). Considering other environmental organisms, it was observed that 100% of 12 quarters infected with *E. coli* were defined as cured after the dry period, as were 50% of two quarters infected with *Klebsiella* spp, and 50% of two quarters infected with *Serratia* spp. The cure rates between the two intramammary treatments, either tilmicosin or cloxacillin, was not significantly different for any specific pathogen.

To evaluate the effect of treatment, while controlling for herd effects, parity and breed, logistic regression using generalized estimation equations was performed (Table 4). Considering all of the variables in the final model, it was concluded that only the length of the dry period and the season in which a cow was dried-off significantly influenced the probability for an individual quarter to cure an infection. As the length of the dry period increased, the probability to cure an infection decreased ($P = 0.04$). When cows were dried off in the spring months of March to May, as well as during the summer months (June to August), the probability for infected quarters to cure decreased ($P = 0.03$, $P = 0.02$, respectively) compared to the winter months. Controlling for all the variables in the model, administration of intramammary tilmicosin at drying-off, as compared to benzathine cloxacillin, did not significantly influence the probability for a quarter to cure.

Due to the limited number of individual quarters infected with a specific pathogen, generalized estimation equations could not be used to determine the influence of specific variables on the probability for quarters to cure infections caused by specific pathogens. The sparse nature of pathogen-specific data resulted in cells of the matrices computed by GENMOD to contain zero values, and thus the generalized hessian matrix was singular and computations could not be performed. However, by grouping the other streptococci, *Strep. uberis*, and *Strep. dysgalactiae*, a total of 85 quarters infected with environmental streptococci were available, and the analysis could be performed. The results of the final logistic regression model of the probability for quarters

Table 3. Relative quarter cure rate of specific pathogens by the intramammary DCT administered at drying-off.

Pathogen	IMM Tilmicosin cure N (%)		IMM Cloxacillin cure N (%)		Overall cure (%)
Minor pathogens					
Coagulase-negative staphylococci	127/149	(85.2)	136/151	(90.7)	87.7
Major pathogens					
Other *Streptococcus* sp	28/29	(96.6)	35/37	(94.6)	95.5
Streptococcus uberis	5/5	(100)	6/7	(85.7)	91.7
Streptococcus dysgalactaie	3/3	(100)	4/4	(100)	100
Escherichia coli	6/6	(100)	6/6	(100)	100
Klebsiella sp	0/1	(0.0)	1/1	(100)	50.0
Streptococcus agalactiae	1/2	(50)	1/1	(100)	66.7
Serratia sp			1/2	(50)	50.0
Other pathogens					
Yeast	1/1	(100)	1/1	(100)	100
Total	171/196	(87.2)	191/210	(90.9)	89.2

Table 4. Results of final logistic regression model for the probability of cure during the dry period in quarters infected with a mastitis pathogen other than *S. aureus*.

	β estimate	S.E.	Wald 95% Confidence Limits		p- value	Odds Ratio
Intercept	4.892	1.12	2.69	7.09	< 0.0001	
Herd[1]	-	-	-	-	-	
Breed[1]	-	-	-	-	-	
Parity[1]						
DCT[2]						
Tilmicosin	-0.474	0.38	-1.22	0.28	0.2	
Cloxacillin	-	-	-	-	-	
Dry period length (days)	-0.026	0.01	-0.05	-0.001	0.04	0.97
Season of drying-off						
Spring	-1.243	0.56	-2.35	-0.14	0.03	0.29
Summer	-1.339	0.57	-2.46	-0.22	0.02	0.26
Fall	-1.236	0.95	-3.10	0.63	0.2	
Winter[3]	-	-	-	-	-	

[1] Fixed effects forced into the model.
[2] Intramammary dry cow antibiotic infusion administered at drying-off.
[3] Estimates for the effect of spring summer and fall are relative to the referrant group of winter

infected with *Streptococcus* spp to cure is shown in Table 5. Controlling for the effects of herd, breed and parity, there was no significant effect of administering intramammary tilmicosin at drying-off, as compared to administration of cloxaciilin ($P = 0.9$). When cows were dried off during the fall months (September to November), the probability for quarters to cure infections by streptococci was significantly decreased ($P = 0.01$) compared to the winter months. When cows were administered a gram-negative core antigen as a routine management practice, the probability for quarters to cure streptococci was also significantly reduced ($P = 0.02$). The length of the dry period, and other specific management practices such as protecting teat-ends following drying-off, were tested but found not to have significant effects.

Discussion

Efficacy of intramammary tilmicosin to eliminate infections caused by *S. aureus*, both in the dry period and pre-partum in heifers, has been documented, but prior to this report, data from a large number of quarters infected with other pathogens has not been presented.[16,18] The results from this study were collected through a field trial that was conducted to evaluate the efficacy of intramammary tilmicosin to both eliminate existing *S. aureus*, and prevent new infections from occurring, during the dry period.[7,8] To facilitate producer compliance, cows with other infections were also randomized to receive treatment before entering the dry period. A total of 406 infected quarters, from 238 cows, were available for analysis to determine the efficacy of tilmicosin to cure these other mastitis pathogen infections.

Table 5. Results of final logistic regression model for the probability of quarters infected with environmental streptococcal organisms[1] to cure during the dry period.

	β estimate	S.E.	Wald 95% Confidence Limits		p- value	Odds Ratio
Intercept	-2.241	1.12	-4.43	-0.05	0.04	
Herds[2]	-	-	-	-	-	
Breed[2]	-	-	-	-	-	
Parity[2]	-	-	-	-	-	
DCT						
Tilmicosin	-0.021	0.29	-0.60	0.55	0.9	
Cloxacillin	-	-	-	-	-	
Coliform vaccine[3]	-0.935	0.41	-1.73	-0.14	0.02	0.39
Season of drying-off						
Spring	-0.178	0.36	-0.89	0.54	0.63	
Summer	-0.580	0.38	-1.32	0.16	0.13	
Fall	-2.559	1.00	-4.53	-0.59	0.01	0.08
Winter[4]	-	-	-	-	-	

[1] Environmental streptococci included *Strep. uberis*, *Strep dysgalactiae* and undifferentiated streptococci reported as 'other streptococci'.

[2] Fixed effects forced into the model.

[3] Gram-negative core antigen vaccine routinely administered as part of dry period udder health management practices.

[4] Estimates for the effect of spring, summer and fall are relative to the referrant group of winter

Cows infected with mastitis pathogens prior to drying-off were identified based on culture results of quarter-milk samples taken three times, up to and including the day of drying-off. Bacteriological cure was defined as the absence of a previously isolated pathogen in a single quarter-milk sample taken between three and nine DIM following the dry period. It would have been favorable to have duplicate milk samples both to identify and confirm infection prior to the dry period, and to identify and confirm the presence or absence of infection immediately following calving. The NMC laboratory handbook on mastitis indicates that diagnosis of IMI status based on multiple samples is more reliable than diagnosis based on a single sample.[14] Furthermore, agreement between duplicate and single quarter-milk samples has been evaluated.[10] It was shown that single samples may indeed be appropriate for determining infection status caused by contagious organisms; however, due to the high percentage of disagreement between duplicate pairs yielding environmental organisms, neither single samples, nor duplicate, would offer a high degree of accuracy in identifying these infections.[10] The results presented and discussed are based on cure being defined from a single quarter-milk sample after calving.

Among the 238 cows that were dried off in this study, a total of 42.6% of quarters were bacteriologically positive prior to drying-off. This is higher than what was reported in a survey of Ohio herds, where only 27.9% of quarters were bacteriologically positive at drying-off.[12] This difference may be due to different definitions of infections caused by minor pathogens, and the sampling strategy used to define infections. Estimates of the prevalence of quarters infected with major mastitis pathogens only have ranged from 5.0% to 23%.[11,17,21,24]

Overall, the cure of infections was substantial (89%). This cure rate is similar to what has been reported as the expected elimination rate achievable by administering DCT.[9,15] There was no significant difference in the cure of quarters that received intramammary benzathine cloxacillin and those that received intramammary tilmicosin. Similarly, the difference in cure rate between the two treatments, to cure any specific type of infection, was not significantly different. It was concluded that adminstration of intramammary tilmicosin at drying-off was as effective as benzathine cloxacillin for eliminating IMI caused by major mastitis pathogens other than *S. aureus*. Currently, there is no commercially available DCT containing tilmicosin,

and use of this antibiotic as a DCT is not approved by governmental regulatory agencies.

With very few quarters infected with either *E. coli* or *Klebsiella* spp, assessing treatment differences for these organisms was not possible. The cure rate following both tilmicosin and cloxacillin, at drying-off, was 100% for *E. coli* (6/6 for each treatment). Tilmicosin, a macrolide antibiotic, has a spectrum of activity that is generally targeted towards gram-positive organisms. Certain gram-negative bacteria, such as *Lawsonia intracellularis*, *Haemophilus* spp, *Actinobacillus* spp, *Mannheimia* spp, and *Pasteurella* spp, are susceptible to tilmicosin, but most gram-negative enteric bacteria are not.[22] It has long been considered that DCT could not play a role in the control of new dry-period IMI caused by coliform pathogens.[5,25] The results from this study should not be extrapolated to contend that DCT was effective in eliminating these infections. Rather, it is more likely that these coliform infections cured spontaneously. In studies that have followed the development and elimination of environmental IMI from prior to drying-off until the next lactation, it has been reported that the elimination of coliform IMI during the dry period by spontaneous cure may actually be 50% to 69%.[17,26]

By the same reasoning, certainly spontaneous cure of environmental streptococci can occur during the dry period. It has been demonstrated that spontaneous elimination of environmental streptococci may be anywhere between 40% and 67%.[17,26,28] However, in comparing the treatment groups, it is assumed that the risk of spontaneous cure of environmental streptococci was equal for either treatment group. Nevertheless, great care must be taken in evaluating the efficacy of treatments to reduce the rates of existing infections, especially for those caused by environmental pathogens.[26]

Variables such as parity, season and herd infection status must be controlled for in any attempt to demonstrate reductions in dry period IMI of these pathogens. These variables have been shown to significantly influence the rate at which these infections develop.[25,26,28] From studies which have studied the time of occurrence and persistency of environmental IMI through the dry period, it is still believed that any demonstrated effect of DCT to reduce environmental streptococci probably occurs in the first part of the dry period, and that management strategies such as vaccination and teat-end protection are important in reducing the risk of new IMI late in the dry period, even when DCT is used.[4,5,25,26] To date only one study has demonstrated any clinical efficacy of a DCT product to reduce gram negative-clinical mastitis in the subsequent lactation.[5]

From the final logistic regression model determining the probability of a quarter to cure during the dry period, it was observed that the length of the dry period did have a significant association with the probability to cure. As the length of the dry period increased, quarters were less likely to cure infections (OR = 0.97). Although this was statistically significant, the biological relevance of the odds ratio calculated may minimize its overall importance. Given that the effect of DCT to reduce IMI is more pronounced early in the dry period, that therapeutic levels of DCT do not extend to the prepartum period, and that DCT is generally not efficacious against gram-negative pathogens, one explanation for this finding is that quarters may have indeed cured, but were more likely to become re-infected in dry periods of greater length. Without having serial quarter samples taken throughout the dry period, or without DNA fingerprinting of bacterial isolates, this hypothesis cannot be ruled out. Since the *E. coli* cure rate was calculated to be 100%, this hypothesized occurrence of cure and re-infection would be primarily occurring in quarters that were infected with minor pathogens or the environmental streptococci. This might have been possible because it has been shown that between 27% and 36% of environmental streptococci present at calving have originated in the dry period, and that the rate of streptococcal IMI increases progressively across the dry period, even in cows that receive DCT.[25,28] The number of quarters infected with CNS has also been shown to markedly increase from cessation of milking to subsequent calving, and these organisms are skin-flora opportunists that are present in high numbers on teats in the dry period.[17,27]

The other finding from the regression model was an association between probability to cure an infection and the season when cows were dried-off. A biologically plausible reason for this finding would again be based on the underlying hypothesis that quarters were cured and then became re-infected during the these time periods. This hypothesis would be in agreement with the effect of season that others have observed.[25,28] The rate of IMI developing during the dry period is a function of exposure of the teat-end to a potential pathogen.[9] Likewise, the rate of streptococcal IMI has also been observed to be highest during the summer.[25,28] In these two previously cited studies, bedding materials and environmental conditions associated with this time period have been discussed as important sources contributing to this association.

Finally, risk factors for the cure of various specific pathogens could not be investigated. However, an analysis of the probability of quarters infected with environmental streptococci to cure during the dry period was completed by grouping data from 85 infected quarters. The season in which cows were dried off and the use of a gram-negative core antigen vaccine were significantly associated with reduced cure of quarters infected with streptococcal organisms. A documented increased risk of IMI associated with seasons has been identified. As already mentioned, the effect of season might be a re-

sult of quarters that actually cured becoming re-infected during high-risk times. However, the association between season and environmental streptococci has been observed in the summer, and not the fall months.[26,28] No biologically plausible reason to infer causality between use of gram-negative core antigen vaccines and streptococcal IMI, either cure or prevention, can be offered. It is possible that the observed effect of the use of such a vaccination strategy may be a surrogate measure of an underlying environmental mastitis problem, and less than appropriate hygiene in some herds. It is speculated that both of these significant findings, seasonality and vaccination, are not directly associated to the pharmacokinetics related to achieving bacterial cure. Rather, re-infection of quarters in the dry period, which is more likely to occur in wet seasons and poor hygienic conditions, may result in newdry-period infections at calving being mistaken for DCT failures. The finding of an association with gram-negative vaccination and reduced streptococcal cure should be the focus of further research.

Conclusions

To achieve the goal of the dry period, and have as few infected quarters as possible at the next calving, administration of DCT to all quarters of cows at the end of lactation remains the recommended practice. Indeed, novel strategies are becoming available to prevent new infections from occurring in quarters that are not infected at drying-off, and the ineffectiveness of DCT to prevent infections caused by coliform bacteria has been demonstrated. However, until such time that accurate decisions may be made on the udder health status of cows at the end of lactation, administering DCT remains an important cornerstone to udder health programs. In this regard, the current study has demonstrated the efficacy of both a commercially available dry cow product, as well as a novel intramammary treatment, to eliminate subclinical IMI during the dry-period. No commercially available preparation of tilmicosin is currently available for this use.

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Footnotes

[a]Dry-Clox®, Ayerst Laboratory, Guelph, Ontario, Canada.
[b]Provel, Division of Eli Lilly Canada, Inc, Guelph, Ontario, Canada.

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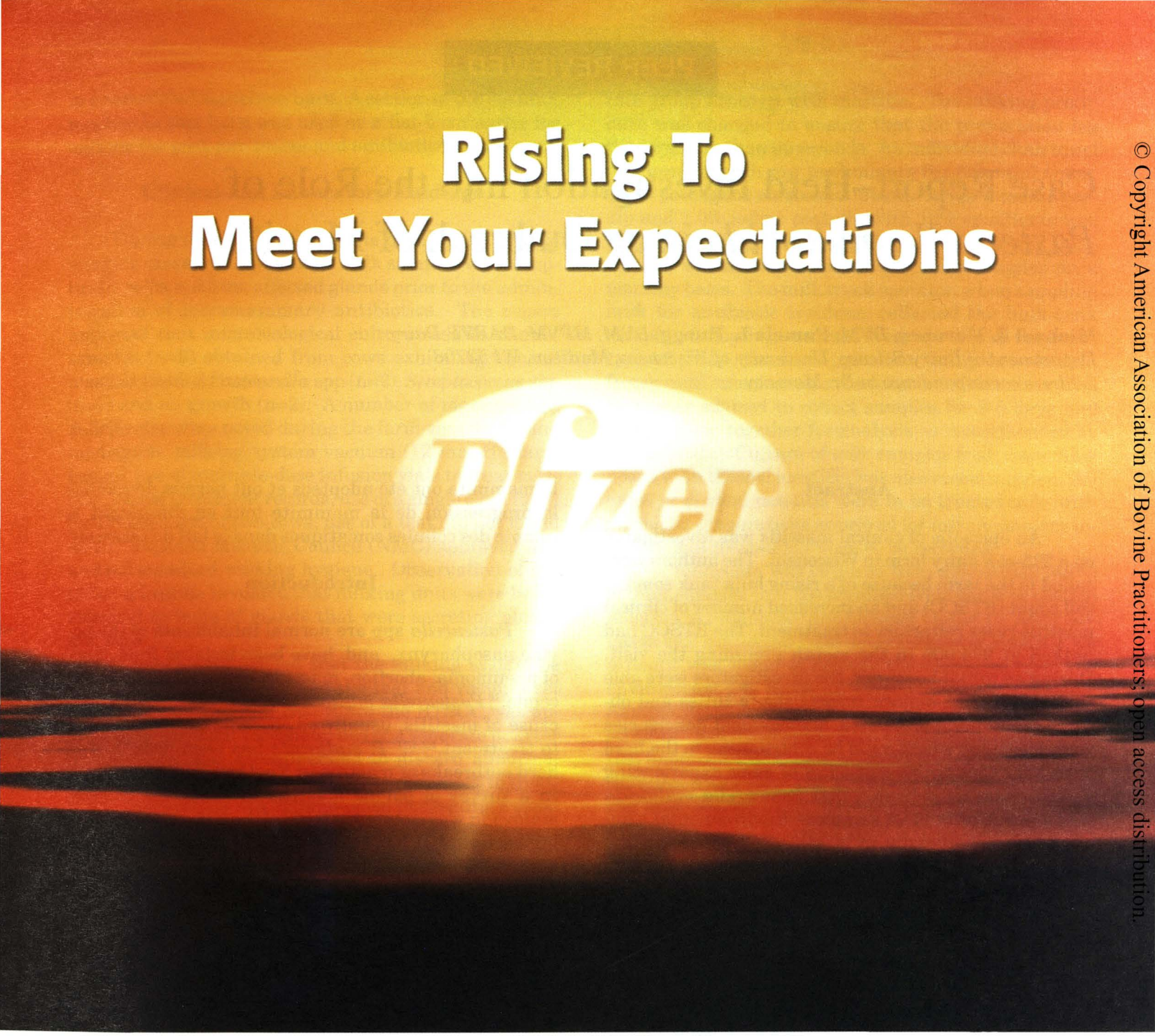

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