The use of an internal teat sealant in combination with cloxacillin dry cow therapy for the prevention of clinical and subclinical mastitis in seasonal calving dairy cows

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ABSTRACT

Cows ($n = 2,053$) from 6 seasonally calving dairy herds were enrolled in a trial to compare the efficacy of 2 dry cow treatments. Cows received either a combination dry cow therapy of 600 mg of cloxacillin (CL) followed by an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate in all 4 quarters immediately following their final milking for the season, or only an intramammary infusion of 600 mg of CL. All cases of clinical mastitis were recorded and cultured during the first 150 d of lactation in each herd, and cow somatic cell count (SCC) was measured between 7 and 50 d postcalving. A large difference was found between treatment groups in the rate at which cows were diagnosed with clinical mastitis over the first 21 d of lactation, after which time the rate at which cows were diagnosed with clinical mastitis was similar between treatment groups. Analysis of the relative proportions of cows with clinical mastitis was performed at both the gland and cow levels. The relative risk (RR) of clinical mastitis diagnosed within 21, 30, and 100 d of calving in a gland treated with the ITS-CL combination was, respectively, 0.30 [95% confidence interval (CI) = 0.21–0.44], 0.39 (0.28–0.53), and 0.58 (0.46–0.75) that of the CL group. An interaction between treatment and previous SCC was found when clinical mastitis was analyzed at the cow level. In a subset of cows that had low SCC in their previous lactation, the RR of mastitis in cows with the ITS-CL combination within 21, 30, and 100 d of calving was, respectively, 0.54 (95% CI = 0.33–0.87), 0.57 (0.37–0.88), and 0.69 (0.50–0.99) that of cows that received only CL at drying off. In the subset of cows that had at least 1 high SCC in the previous lactation, the RR of mastitis in the ITS-CL combination group within 21, 30, and 100 d of calving was, respectively, 0.26 (95% CI = 0.16–0.44), 0.37 (0.24–0.57), and 0.72 (0.55–0.96) that of the CL-only group. The ITS-CL

combination of dry cow treatments was associated with a reduction in subclinical mastitis [SCC $\geq 250,000$ cells/mL; RR = 0.80 (95% CI = 0.65–0.98)] when compared with treatment with CL alone. The use of an ITS in combination with CL dry cow treatment was associated with significantly lower clinical and subclinical mastitis in the following lactation, with a greater difference found in cows that had a history of subclinical mastitis in the previous lactation.

Key words: mastitis, dry cow, internal teat sealant, combination therapy

INTRODUCTION

Mastitis is an economically important disease of dairy cattle. There are costs associated with production losses, culling, veterinarian intervention, antibiotics and other therapies, discarded milk, milk quality penalties, and labor (Stevenson and Lean, 1998; Huijps and Hogeveen, 2007; Bar et al., 2008). The proportions of different pathogens isolated from milk samples collected within the Macalister irrigation district (Victoria, Australia) from clinical cases of mastitis have changed over the last 15 yr, with the environmental pathogen *Streptococcus uberis* becoming more common in milk samples submitted for culture. In a collection of 339 milk samples taken from clinical cases of mastitis in 1995 and 1996, 26.6% of the cultures yielded *Strep. uberis*. In a collection of 507 milk samples taken from clinical cases of mastitis in 2007 and 2008, 60.4% of the cultures yielded *Strep. uberis*. A similar change has been reported in the literature of the United Kingdom, with the 5-point mastitis control plan the likely cause of the reduction in mastitis cases caused by contagious pathogens. Despite these controls, however, an overall increase in *Strep. uberis* mastitis has been reported (Bradley, 2002).

Infection with environmental bacteria is more likely during the dry period than at any other time during the lactation cycle, particularly in the early and late stages of the dry period (Bradley and Green, 2004). This susceptibility of the udder to infection is likely

Received November 29, 2009.

Accepted June 14, 2010.

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attributable to either failure of formation of the keratin plug in the teat canal in the early dry period or the loss of the keratin plug in the late dry period (Williamson et al., 1995; Bradley and Green, 2004; Paulrud, 2005). Infections that occur over the dry period may not manifest as clinical cases until well after calving, with 50% of the cases of environmental mastitis diagnosed within 100 d of calving being attributed to dry period infection (Woolford et al., 1998; Bradley, 2000; Bradley and Green, 2004).

Administration of dry cow antibiotic therapy to cure existing IMI and prevent new mastitis infections over the dry period has been well documented and forms part of the 5-point mastitis control plan (Berry et al., 2004). Further, Williamson et al. (1995) demonstrated that the administration of a long-acting dry cow antibiotic to uninfected quarters at drying off reduced the overall incidence of new infections with *Strep. uberis* from 12.3% for untreated quarters to 1.2% of quarters ($P < 0.01$). The use of an internal teat sealant (ITS), which provides a physical barrier to infection of the teat canal, has similarly been documented to prevent new infections over the dry period, either alone or in combination with antibiotic dry cow therapy (Woolford et al., 1998; Berry and Hillerton, 2002; Berry et al., 2004; Laven and Lawrence, 2008; Newton et al., 2008).

The objective of the current trial was to compare clinical and subclinical mastitis incidence following calving in herds in which all of the cows were treated at drying off with either an intramammary infusion of 600 mg of cloxacillin (CL; Orbenin Enduro, Pfizer Animal Health, New York, NY) or with a combination of intramammary infusions of 600 mg of CL and an ITS containing 2.6 g of bismuth subnitrate (Teatseal, Pfizer Animal Health). The intramammary infusion of 600 mg of CL is the most commonly used dry cow therapy program in the Macalister irrigation district.

MATERIALS AND METHODS

In May 2008, cows ($n = 2,053$) from 6 seasonally calving dairy herds within the Macalister irrigation district were enrolled in the trial to compare the efficacy of 2 dry cow treatments. The owners of the herds selected to participate in the study were clients of the Maffra Veterinary Centre (Maffra, Victoria, Australia), had cooperated with the practice in several previous studies, and were diligent record keepers. Further, the herds selected had herd test records from at least 5 herd tests in the 2007 to 2008 lactation. Cows were classified as subclinically infected if they had at least 1 SCC of $\geq 250,000$ cells/mL in the 2007 to 2008 lactation (Victorian Mastitis Research Group, 1992; Brightling et al., 2000). A sample size of 2,000 cows was required

based on the assumption that there may have been a difference of 5% incidence of clinical mastitis between the 2 treatment groups. Analysis of the proportions of cows that had clinical mastitis in the previous lactation revealed that almost 20% of cows had clinical mastitis within 1 mo of calving. A 5-percentage-point reduction in clinical cases (15 vs. 20%) required 1,000 cows per group [95% CI ($\alpha = 0.05$), with a power of 80% ($\beta = 0.2$); Abramson, 2004].

Participants agreed to record all cases of clinical mastitis in a standard format for the first 150 d of lactation, to collect milk samples from as many cases of clinical mastitis as possible, and to subject their herd to a herd test every month for at least the first 3 mo of lactation. This herd testing interval was selected in an attempt to ensure that every cow would have at least 1 herd test within 7 to 50 d of calving to analyze milk production and SCC. The participants could then revert to their normal herd testing frequency for the remainder of the season. Cows were dried off between May and July 2008. Cows that were identified with an even number received an intramammary infusion of 600 mg of CL followed by an intramammary infusion of an ITS containing 2.6 g of bismuth subnitrate in all 4 quarters immediately following their final milking for the season. Those cows with odd numbers received only an intramammary infusion of 600 mg of CL. The odd and even number method of treatment allocation was used because of the difficulty in applying any other type of random treatment allocation in a commercial setting. In the seasonal calving herds used, large numbers of cows were dried off as part of normal farm management practices. It was felt that supplying numbers generated randomly to farm operators may cause confusion and errors at drying off. Also, milking personnel needed to be aware of the cows in which the ITS was used so as not to confuse the teat sealant with mastitis clots. The results of this trial need to be interpreted with the consideration that some bias may be reported by farmers identifying clinical mastitis cases with the knowledge that odd and even cows received different dry cow therapies. All intramammary infusions were performed by farm staff who were instructed in the appropriate techniques for aseptic insertion of intramammary infusions, which included thoroughly cleaning teat ends with cotton balls soaked in 70% ethanol solution. For those cows that received both CL and ITS infusions, each teat was cleaned a second time between treatments.

Following calving in the spring of 2008, all clinical cases of mastitis were recorded (the record included the date, cow identification, the quarter affected, and the treatment used) and samples from these cases were collected and stored on farm in the freezer compartment of

a commercial refrigerator. The case definition of clinical mastitis was the presence of clotted or discolored milk beyond the first 3 squirts of milk when the suspect quarter was hand stripped, or a hot or swollen quarter, or both. In cases where glands had more than 1 case of clinical mastitis, the first case of mastitis was recorded as the mastitis date and the milk was cultured. Milk samples were collected into sterile sample tubes after the teat end was disinfected using 70% ethanol solution. The frozen milk samples were collected approximately every 2 wk and forwarded to the Royal Melbourne Institute of Technology (Biotechnology, RMIT University, Bundoora, Australia) for milk culture.

Bacteriology

The microbiologists performing the bacteriology were blinded as to treatment groups. Frozen samples were packed tightly and sent in batches by overnight delivery to the laboratory. Most milk samples were only partly thawed on arrival at the laboratory. They were thawed completely, mixed thoroughly, and examined macroscopically. Then, 50- μ L aliquots were spread evenly over the surface of 1 Edward's agar plate (Oxoid, Adelaide, Australia) and 1 horse blood agar plate (prepared from Oxoid ingredients). After incubation for 48 h at 37°C, suspected pathogens were identified on the basis of typical colonial morphology. A small number of cultures showed heavy mixed growth, usually including *Bacillus* spp., several strains of gram-negative bacteria, and a variety of other species. These are typical of bacteria derived from the environment. These cultures were impossible to evaluate because if any pathogens were present, they would have been overgrown by *Bacillus* spp. (large colonies) and other species. Colonies morphologically resembling potential pathogens were identified by conventional biochemical methods. For example, gram-positive cocci were identified as *Strep. uberis* if they were catalase-negative and hydrolyzed hippurate and esculin or *Staphylococcus aureus* if they were catalase and coagulase-positive.

Data Handling

Clinical mastitis data were collected from each herd at regular intervals and entered into an Excel (Microsoft Corporation, Redmond, WA) spreadsheet. Herd records, which included dry-off date, calving date, breed, and herd test details (which included the SCC for each cow at every herd test), were downloaded from Herd Improvement Co-operative Australia Ltd. (Maffra, Victoria, Australia) and imported into the program Dairy Data (Warrnambool Veterinary Clinic, Warrnambool, Victoria, Australia). Cow identity, age, breed, calving

date, dry-off dates, production, and SCC figures for each cow in the herd were then exported from Dairy Data to Excel files. Excel was used to calculate variables for analysis within the statistical software programs STATA/IC 10.1 for Windows and PASW Statistics 17.0 for Windows (SPSS Inc., 2009; StataCorp, 2009).

Of the 2,053 cows (8,212 glands) that received dry cow therapy, 2,013 cows (8,052 glands) calved with a minimum dry period of 35 d. Of these cows, 1,923 cows were herd tested between 7 and 50 d after calving.

Data Analysis

Analysis of clinical mastitis data was initially performed at both the gland and cow level because the actions of the dry cow treatments (prevention of clinical mastitis) were at the gland level (McDermott et al., 1994; Parker et al., 2008). Treatments were allocated at the cow level and an interaction between treatment and SCC history was assessed at the cow level. Analysis of subclinical mastitis (an SCC of $\geq 250,000$ cells/mL at the first herd test after calving) at first herd test was performed at the cow level because herd testing used combined 4 quarter milk samples from each cow.

Dependent binary variables included incidence of clinical mastitis at 21, 30, and 100 d postcalving and subclinical mastitis (SCC $\geq 250,000$ cells/mL) at first herd test. The time periods 30 and 100 d postcalving were used because the incidence of mastitis within the first month of lactation is commonly assessed and benchmarked in seasonal calving dairy herds, and approximately half of the cows had data for the first 100 d of lactation, so residual dry cow effect later into lactation was able to be assessed (Brightling et al., 2000). The study discussed in this paper showed that the rate at which clinical mastitis was diagnosed between the 2 groups was very different up to approximately 21 d after calving, so further analysis of the time period 21 d into lactation was undertaken. An analysis of the prevalence of postcalving clinical mastitis in which *S. uberis*, or *Escherichia coli*, or both, was isolated was also carried out. For analysis of subclinical mastitis at first herd test, those cows with a case of clinical mastitis before their herd test were excluded.

Dependent continuous variables included milk production (liters and TS) and linear score (LS) at first herd test. The LS was defined as the natural logarithm of the cow's SCC and was used because the log transformation of SCC resulted in normalization of residuals (Ali and Shook, 1980).

Independent continuous variables (production, treatment to calving interval, age) were treated as continuous if the relationship to the outcome of interest was linear. As such, age was recoded as a categorical variable as 3,

Table 1. Number of cows and mean ($\pm$ SD) variables for each treatment group^{1,2}

Variable	ITS-CL	CL
No. treated	1,031	1,022
No. calved with dry period ≥ 35 d	1,010	1,003
No. herd tested	967	956
Age (yr)	5.3 $\pm$ 2.1	5.3 $\pm$ 2.1
Dry-off date	June 27, 2008 $\pm$ 12.2 d	June 26, 2008 $\pm$ 13.0 d
Calving date	August 30, 2008 $\pm$ 16.7 d	August 30, 2008 $\pm$ 16.8 d
Dry period (d)	63.6 $\pm$ 15.7	64.4 $\pm$ 16.8
Cow SCC ($\times$ 1,000 cells/mL)	303 $\pm$ 796	404 $\pm$ 855
Production (L)	30.6 $\pm$ 7.6	30.2 $\pm$ 7.5
Production (kg of milk solids)	2.29 $\pm$ 0.50	2.28 $\pm$ 0.49
DIM at end of study period (d)	98.6 $\pm$ 18.8	98.5 $\pm$ 19.0

¹Glands within cows were infused with either 600 mg of cloxacillin (CL) or with a combination of intramammary infusions of 600 mg of CL and an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate at drying off at least 35 d before calving.

²Herd test performed between 7 and 50 d after calving.

4, 5, and ≥ 6 yr old. Treatment was coded as CL only or CL and ITS. Breed data obtained from herd records was incomplete and not able to be analyzed.

Multiple logistic regression was used to analyze variables with dichotomous outcomes (diagnosis of clinical or subclinical mastitis) with herd as a fixed effect in all models (Hosmer and Lemeshow, 2000). Factors tested in models included herd, age, length of dry period, milk production, interval from calving to first herd test, treatment group, and history of previous high cell count.

All variables that were associated with an outcome of interest ($P < 0.2$) were included in a full model, and a reverse stepwise model building process using a likelihood-ratio test statistic was used to arrive at a final model, with threshold P -values being <0.05 for inclusion and >0.10 for exclusion. Treatment and herd were forced to remain in each model to calculate the final odds ratio and 95% confidence interval of the effect of treatment and method of examination on diagnosis of mastitis. Biologically plausible interactions were investigated. Goodness of fit was examined using the Hosmer-Lemeshow test, and the ability of each model to discriminate between those cows that experienced the outcome of interest versus those who did not was estimated by calculating the receiver operating characteristic value for each model. The relative risk and 95% confidence interval were calculated using a modified Poisson regression with the variables obtained from the final logistic regression models, and only the relative risks have been reported because they are considered to be a better estimate of the magnitude of the treatment effect than odds ratios (Dohoo et al., 2003; McNutt et al., 2003; Zou, 2004). The log-binomial model was not used because convergence could not be achieved in all models (McNutt et al., 2003).

Interactions involving treatment and length of dry period, previous SCC history, and production were assessed. An interaction between treatment group and previous lactation SCC (evidence of subclinical mastitis compared with cows with no evidence of subclinical mastitis) was found. Subset analysis was used to numerically describe the order of magnitude difference in clinical mastitis incidence within cows depending on their previous SCC history (Pocock et al., 1987; Oxman and Guyatt, 1992).

The intraclass correlation of gland within cow and cow within herd were calculated using 1-way ANOVA for each independent variable because the glands within cows are not biologically independent (McDermott et al., 1994; Barkema et al., 1997). The calculated intraclass correlations were found to be greater than 0.2 for many variables, so in the final Poisson models reported for gland-level analysis, cow was added as a random effect statement to account for correlation within cows.

Kaplan-Meier survival analysis in which treatment was the main effect was used to graphically represent the cumulative proportion of quarters and cows infected for each treatment group. Treatment to clinical mastitis interval was defined as the date a quarter was first diagnosed minus the calving date plus 1, such that a cow diagnosed on the day of calving had a calving to mastitis interval of 1.

RESULTS

Of the 2,013 cows that calved with a minimum dry period of 35 d, 1,010 received the ITS-CL combination and the remaining 1,003 received CL alone at drying off. Table 1 shows the distribution of variables and numbers of cows within each treatment group.

Table 2. Number and percentage¹ of bacteria species isolated from clinical mastitis at different times after calving within treatment groups²

Bacteria isolated	≤30 d after calving				≤100 d after calving				All cases of clinical mastitis			
	ITS-CL	CL	Total	%	ITS-CL	CL	Total	%	ITS-CL	CL	Total	%
CNS	2	4	6	3.3	4	6	10	3.1	4	6	10	2.9
Coliform ³	2	13	15	8.3	3	19	22	6.7	3	19	22	6.5
<i>Corynebacterium bovis</i>	1	1	2	1.1	3	2	5	1.5	3	3	6	1.8
No pathogen isolated ⁴	5	17	22	12.2	18	30	48	14.7	20	30	50	14.7
<i>Staphylococcus aureus</i>	3	2	5	2.8	7	6	13	4.0	7	6	13	3.8
<i>Streptococcus</i> spp. ⁵	0	7	7	3.9	6	7	13	4.0	6	9	15	4.4
<i>Streptococcus uberis</i>	34	89	123	68.3	76	140	216	66.1	81	143	224	65.9
No culture results available ⁶	20	43	63	—	44	76	120	—	45	77	122	—
Total	67	176	243		161	286	447		169	293	462	

¹Percentage of cases in which a culture was performed.

²Glands within cows were infused with either 600 mg of cloxacillin (CL) or with a combination of intramammary infusions of 600 mg of CL and an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate at drying off at least 35 d before calving.

³Includes *Escherichia coli* and other coliform bacteria.

⁴No pathogenic bacteria were isolated (included contaminated samples).

⁵Includes all *Streptococcus* spp. other than *Streptococcus uberis*.

⁶No milk sample was collected.

During the study period (the first 150 d of lactation for each herd) there were 462 cases of clinical mastitis; of those, 243 and 447 cases occurred within 30 and 100 d of calving, respectively. During the study period of 150 d, all cows had calved for at least 53 d (mean = 98.6 d; SD = 18.9; range = 53–150 d). Table 2 shows the distribution of bacterial isolates from cases of clinical mastitis.

Effect of Treatment on Clinical Mastitis

Cases of clinical mastitis were recorded for up to 150 d following calving start date for each of the 6 herds. The cumulative proportion of quarters (Figure 1) and cows (Figure 2) with clinical mastitis was graphically

represented to visualize any effect of treatment so that indices (proportions of cases of clinical mastitis at varying time intervals postcalving) could be determined. Both figures show that there was a large difference between treatment groups in the rate at which cows were diagnosed with clinical mastitis over the first 21 d of lactation, after which time the rate at which cows were diagnosed with clinical mastitis was similar between treatment groups but still higher in the groups that did not receive ITS. The overall incidence of clinical mastitis after 100 DIM was still very different between treatment groups.

The proportions of clinical mastitis at gland level within each treatment group 21, 30, and 100 d after calving is presented in Table 3. The table shows a rela-

Table 3. Proportions of glands diagnosed with clinical mastitis and the proportion of glands in which an environmental pathogen was isolated at milk culture within 21, 30, and 100 d of calving for each treatment group

Item	Treatment group ¹				RR ²	95% CI		<i>P</i> -value
	ITS-CL		CL					
	%	Proportion	%	Proportion				
All clinical cases								
≤21 d	1.20	49/4,040	3.90	158/4,012	0.30	0.21	0.44	<0.0001
≤30 d	1.68	68/4,040	4.44	178/4,012	0.39	0.28	0.53	<0.0001
≤100 d	7.66	162/2,114	12.90	287/2,224	0.58	0.46	0.75	<0.0001
Environmental pathogen ³								
≤21 d	0.65	26/4,017	2.39	95/3,963	0.25	0.14	0.43	<0.0001
≤30 d	0.93	37/3,995	2.39	100/3,935	0.34	0.21	0.55	<0.0001
≤100 d	3.82	79/2,069	7.26	156/2,147	0.49	0.34	0.72	<0.0001

¹Glands within cows were infused with either 600 mg of cloxacillin (CL) or with a combination of intramammary infusions of 600 mg of CL and an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate at drying off at least 35 d before calving.

²Relative risks (RR) were calculated using a generalized estimating equation using Poisson distribution and log link, accounting for fixed effect of herd, previous SCC status, and treatment group and adjusted for clustering on cow.

³Cases of clinical mastitis that occurred within 21, 30, or 100 d of calving and from which *Streptococcus uberis*, *Escherichia coli*, or other coliform bacteria were isolated. Those cases of clinical mastitis from which no culture results were available have been excluded from analysis.

Figure 1. Cumulative proportion of glands diagnosed with clinical mastitis relative to time of calving for each of 2 treatment groups (cloxacillin only and internal teat sealant/cloxacillin combination) from Kaplan-Meier analysis. Log rank statistic 37.34, $P < 0.0001$.

Figure 2. Cumulative proportion of cows diagnosed with clinical mastitis relative to time of calving for each of 2 treatment groups (cloxacillin only and internal teat sealant/cloxacillin combination) and stratified by previous SCC history (previous low SCC: all SCC in previous lactation $< 250,000$ cells/mL, previous high SCC: at least one SCC in previous lactation $\geq 250,000$ cells/mL) from Kaplan-Meier analysis. Log rank statistic pooled across strata 21.25, $P < 0.0001$.

Table 4. Cow-level relative risk (OR) and 95% CI of clinical mastitis within 21 d for each treatment group,¹ analyzed using multiple logistic regression (n = 2,013)

Variable	Coefficient	SE	OR	95% CI		P-value
				Lower	Upper	
ITS-CL	-0.66	0.25	0.52	0.32	0.85	0.009
Previous high SCC ²	0.73	0.21	2.07	1.38	3.01	<0.001
Teat sealant × previous low SCC interaction	-0.82	0.38	0.44	0.21	0.92	0.028
Intercept	-2.17					

¹Glands within cows were infused with either 600 mg of cloxacillin (CL) or with a combination of intramammary infusions of 600 mg of CL and an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate at drying off at least 35 d before calving.

²At least 1 SCC in previous lactation was $\geq 250,000$ cells/mL.

tive risk (**RR**) of clinical mastitis diagnosed within 21, 30, and 100 d of calving in the ITS-CL combination group to be, respectively, 0.30 (95% CI = 0.21–0.44), 0.39 (0.28–0.53), and 0.58 (0.46–0.75) that of the CL group. Proportions and relative risks of mastitis from which an environmental pathogen was isolated were also presented.

An interaction between treatment and previous SCC status was found when clinical mastitis proportions were analyzed at the cow level. The interaction was graphically represented in Figure 2. Table 4 shows the coefficients, standard errors, and *P*-values from cow-level logistic regression analysis of clinical mastitis within 21 d of calving.

A subset analysis was performed on the 2 groups of cows, those with a previous history of high SCC (n = 886) and those cows in which the SCC was low for all herd tests in the previous lactation (n = 1,127). The relative risk of a clinical mastitis diagnosis within 21, 30, and 100 d of lactation is presented in Table 5.

Effect of Treatment on Subclinical Mastitis

The variable “subclinical mastitis” included cows that had not had a case of clinical mastitis diagnosed before their first herd test but had an SCC $\geq 250,000$ cells/mL at their first herd test, which was conducted 7 to 50 d after calving. Of the 1,812 cows with herd test data, 1,666 cows (865 treated with the ITS-CL combination, 801 treated with with CL only) had not been diagnosed with a case of clinical mastitis at their first herd test (Table 6).

The LS of each treatment group was compared using linear regression, which showed that there was a significant difference between treatment groups in a model accounting for herd, age, production (liters), and SCC status in previous lactation. The LS for the cows treated with the ITS-CL combination was 4.41 ± 0.05 compared with 4.72 ± 0.05 for the cows treated with CL. The difference in LS was 0.31 (95% CI = 0.19–0.43; *P* < 0.0001).

Table 5. Proportions of cows diagnosed with clinical mastitis grouped based on their SCC count history within 21, 30, and 100 d of calving for each treatment group

Item	Treatment group ¹				RR ²	95% CI		<i>P</i> -value
	ITS-CL		CL					
	%	Proportion	%	Proportion				
Previous high SCC ³								
≤21 d	4.07	18/442	15.09	67/444	0.26	0.16	0.44	<0.0001
≤30 d	6.56	29/442	17.34	77/444	0.37	0.24	0.57	<0.0001
≤100 d	30.57	81/265	43.14	129/299	0.72	0.55	0.96	0.024
Previous low SCC ⁴								
≤21 d	4.58	26/568	8.59	48/559	0.54	0.33	0.87	0.011
≤30 d	5.63	32/568	10.02	56/559	0.57	0.37	0.88	0.010
≤100 d	17.31	54/312	24.77	82/331	0.69	0.50	0.99	0.041

¹Glands within cows were infused with either 600 mg of cloxacillin (CL) or with a combination of intramammary infusions of 600 mg of CL and an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate at drying off at least 35 d before calving.

²Relative risks (RR) were calculated using a generalized estimating equation using Poisson distribution and log link, accounting for fixed effect of herd and treatment group.

³Cows that had at least 1 high ($\geq 250,000$ cells/mL) SCC in their previous lactation.

⁴Cows that had no high SCC in their previous lactation.

Table 6. Relative risk (RR) and 95% CI of subclinical mastitis (SCC $\geq$ 250,000 cells/mL) at first herd test 7 to 50 d after calving for cows in each treatment group¹ that did not have a case of clinical mastitis recorded before first herd test (n = 1,666)

Variable	Coefficient	SE	RR	95% CI		P-value
				Lower	Upper	
ITS-CL	-0.22	0.11	0.80	0.65	0.98	0.035
Previous low SCC ²	-0.44	0.12	0.64	0.51	0.82	<0.0001
Age (yr)						
2	Reference					
3	0.65	0.21	1.92	1.27	2.89	0.002
4	0.73	0.23	2.07	1.33	3.23	0.001
>4	1.27	0.19	3.57	2.45	5.19	<0.0001
Production (L)	-0.05	0.01	0.95	0.94	0.96	<0.0001
Intercept	-0.59					

¹Glands within cows were infused with either 600 mg of cloxacillin (CL) or with a combination of intramammary infusions of 600 mg of CL and an internal teat sealant (ITS) containing 2.6 g of bismuth subnitrate at drying off at least 35 d before calving.

²All SCC in previous lactation were <250,000 cells/mL.

DISCUSSION

The results of this trial must be interpreted with the consideration that it was not possible to blind the operators as to which cows were treated with ITS. This has the possibility of causing bias in the diagnosis of mastitis in cows after calving given that the herd operators were aware of which cows received ITS.

The ITS acts by providing a physical barrier to infection of the udder by sealing the teat canal. Once a cow calved and was either sucked by a calf or milked out by milking machine, the ITS would not have played a further role in preventing new IMI. The rate at which clinical mastitis cases were recorded for each treatment group was different until approximately 3 wk into lactation (Figures 1 and 2). This provides further evidence that many cases of clinical mastitis diagnosed after calving are actually the result of infection that occurred before calving and ITS removal. This is consistent with the findings of Bradley (2000), who found that enterobacterial infections acquired during the dry period were responsible for 52.6% of all infections diagnosed during the first 100 d of lactation.

The relative proportions of clinical mastitis in each treatment group were analyzed at 14 and 21, 30, and 100 d postcalving. Parker et al. (2008) used a 14-d analysis in a trial that investigated the use of ITS in heifers. Laven and Lawrence (2008) reported the incidence of mastitis in the first 11 wk of calving, Godden et al. (2003) reported a 60-d mastitis proportion, and Berry and Hillerton (2007) and Newton et al. (2008) reported 100-d mastitis proportions in other studies that have compared blanket antibiotic dry cow therapy with a combination of antibiotic dry cow therapy and ITS.

Within these other studies, Laven and Lawrence (2008) found an RR of cow-level clinical mastitis of

0.63 in the cows treated with ITS and CL compared with cows treated only with CL within the first 11 wk of lactation in one herd. The Godden et al. (2003) trial demonstrated a difference in quarter clinical mastitis percentage of 2.1% (5.9 vs. 8.0%) at 60 d postcalving for a combination of ITS and 500 mg of CL versus 500 mg of CL only. Berry and Hillerton (2007) compared a combination of cefalonium and ITS with cefalonium only and found that the difference in new infections at calving was 3.7% for a combination of ITS and antibiotic versus 7.3% for antibiotic alone. In that trial, however, the difference was greatest for cows with a long dry period, implying that the antibiotic cover provided by cefalonium may be almost as good as combination therapy for cows with a short dry period. They also found that clinical mastitis proportions at 100 d postcalving were lower in the combination group (RR = 0.26) regardless of dry period length. Newton et al. (2008) compared the 100-d quarter incidence of mastitis between the ITS-CL combination and CL only and found an odds ratio of clinical mastitis to be 0.47. Therefore, there is some good evidence across 4 other trials that there is benefit in using ITS in combination with antibiotics compared with antibiotic dry cow therapy alone in reducing postcalving clinical mastitis. In contrast, earlier work described by Woolford et al. (1998) found that the use of combination antibiotic with ITS provided no greater benefit in terms of mastitis prevention than the use of either ITS or antibiotic treatment alone in cows with low SCC.

The difference in clinical mastitis rates in the first 3 wk of lactation is likely to be of economic significance in the herds studied. The economic benefit is dependent on the price of ITS and costs associated with administration of the product, milk price, and cost of replacement cattle. In spring of 2008, the cost of a single case

of clinical mastitis was estimated at AU\$229 based on costs and time of treatment, discarded milk, reduced milk production, and mortality and culling risk (Dairy-Australia, 2008). Assuming that the use of ITS cost AU\$1,200 per 100 cows, and the RR of clinical mastitis in a cow treated with ITS compared with a cow that was not treated with ITS was 0.30, the break-even incidence of clinical mastitis above which treatment with ITS was of benefit was estimated to be about 6% within the first 21 d of lactation. The incidence of clinical mastitis in early lactation varies from farm to farm and from season to season; therefore, recommendations for the use of ITS in combination with CL at dry off would require individual recommendations for different herds. The estimated cost benefit described does not account for further gains expected from lowering of herd bulk milk cell count and reduction in the incidence of subclinical mastitis cases.

The interaction between the use of ITS-CL combination and previous SCC history was an interesting finding in this study. It would appear that the effect of ITS-CL combination use was greatest in cows that had a high SCC in the previous lactation. The graphical representation of this interaction is presented in Figure 2. Those cows with a history of high SCC had a higher incidence of clinical mastitis than those that did not have a history of high SCC. However, until 3 wk after calving, the cows with a previous high SCC that were treated with the ITS-CL combination had a similar incidence of clinical mastitis as those cows treated with ITS-CL combination that did not have a previous high SCC. A possible cause of this interaction is that there is an anatomical or physiological reason that some cows are more prone to developing mastitis, such as a wide or short teat canal, inferior teat canal keratin production, or inferior immune function (Klein et al., 2005; Paulrud, 2005). It would appear that the use of the ITS-CL combination helped to reduce the incidence of clinical mastitis in these cows by blocking entry of bacteria via the teat canal. However, once they calved and the ITS was removed, the protection was no longer afforded to them and they succumbed to clinical mastitis at a higher rate than those cows that did not have a high SCC in the previous lactation. Within the subset of cows that had low SCC in the previous lactation, the use of the ITS-CL combination was still associated with a reduced incidence of clinical mastitis, but the difference was not as great as those cows with a high SCC. Another conclusion to be made from this finding is that selecting those cows with a history of low SCC is another means of reducing the incidence of clinical mastitis within a herd.

In this study the use of the ITS-CL combination at drying off was associated with a reduction in the inci-

dence of subclinical mastitis as assessed by SCC at the first herd test 7 to 50 d after calving. Apart from Godden et al. (2003), we have not found other studies that have reported significant differences in SCC between treatment groups. In the study reported by Godden et al. (2003), the SCC was measured within 8 d of calving and showed a significant difference in LS between quarters treated with a preparation of both ITS and 500 mg of CL compared with quarters treated only with CL. In the current study, the authors have found a significant reduction in the proportion of cows with high SCC ($\geq 250,000$ cells/mL) at the first herd test as well as a significantly lower LS in those cows treated with ITS. The LS model is used because the log transformation of SCC enables the use of linear regression to compare SCC between treatment groups; however, the practical implications that can be derived from such analysis are limited to demonstrating a statistically significant difference between 2 treatment groups and modeling factors associated with such a difference.

One of the trial objectives was to determine whether there was a difference in the use of ITS-CL combination in preventing mastitis caused by different bacteria. However, there was such a low incidence of bacteria other than *Strep. uberis* that a comparison within the subset of *Staph. aureus* group or other contagious pathogens was not possible.

CONCLUSIONS

The use of a combination dry cow therapy of 600 mg of CL followed by an ITS containing 2.6 g of bismuth subnitrate was compared with an intramammary infusion of 600 mg of CL alone for the effect of each treatment on clinical and subclinical mastitis outcomes. The use of combination treatment at drying off was shown to be of large benefit in reducing postcalving clinical mastitis for the first 3 wk of lactation and had an effect on reducing subclinical mastitis. The use of combination dry cow therapy is likely to be of benefit in herds that have a mastitis incidence of 6% or more in the first 3 wk of lactation.

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