



## Effects of antibiotic dry-cow therapy and internal teat sealant on milk somatic cell counts and clinical and subclinical mastitis in early lactation

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### ABSTRACT

The objective of this study was to determine the efficacy of an internal teat sealant (TS; Teatseal; Zoetis Australia, Silverwater, NSW, Australia), when used in combination with antibiotic dry-cow therapy (ADCT) administered at dry-off, on milk individual somatic cell count (ISCC), milk production and components, and the incidence of clinical and subclinical mastitis in dairy cows up to 60 d after calving, when compared with ADCT only. Multiparous Holstein, Jersey, or Holstein cross cows ( $n = 2,200$ ) from 8 farms in southern and eastern Australia were randomly assigned to treatment of all 4 quarters with ADCT alone or with ADCT plus TS (ADCT + TS) at dry-off in this randomized, multisite clinical trial. Individual milk yield, fat and protein percentages, and ISCC were measured at intervals of  $14 \pm 3$  d after calving for the first 60 d of lactation. The first measurement occurred between 10 and 24 d after calving. Clinical mastitis and health events were recorded from dry-off to 60 d of lactation. Milk samples were collected from first cases of clinical mastitis and subjected to bacteriology. Treatment and the interaction of treatment by time did not affect milk yield, ISCC weighted by milk yield, or fat and protein percentages. Treatment with ADCT + TS decreased geometric mean ISCC compared with treatment with ADCT alone over the first 60 d of lactation. Geometric mean ISCC ( $\times 10^3$  cells/mL) was 32.0 [95% confidence interval (CI): 26.8 to 38.3] and 43.5 (95% CI: 36.2 to 52.1) for ADCT + TS and ADCT alone, respectively. The odds of at least 1 case of subclinical mastitis (ISCC  $\geq 250,000$  cells/mL) were 1.9 times higher (95% CI: 1.4 to 2.6) with ADCT alone in the first 60 d of lactation compared with ADCT + TS. Use of ADCT + TS reduced the estimated incidence of at least 1 case of subclinical mastitis on all 8 farms, compared with use of ADCT alone. Only 4 cows that calved 40 to 100 d

after dry-off had a first case of clinical mastitis in the dry period. Five percent of cows (76 cases from 1,528 cows included in this analysis) that calved 40 to 100 d after dry-off had a first case of clinical mastitis between 0 and 60 d in milk. Of these first cases of clinical mastitis, 43 cases (5.7% of 761 cows) occurred in the ADCT group and 33 (4.3% of 767 cows) in the ADCT + TS group, but this was not significantly different. Proportional hazards estimates of survival showed no difference in the number of days postcalving to detection of first cases of clinical mastitis between the ADCT and ADCT + TS groups over the first 60 d postpartum. The estimated hazard ratio for clinical mastitis over this period in the ADCT + TS cows (relative to ADCT alone) was 0.70 (95% CI: 0.43 to 1.14). The combination of ADCT and TS provides benefits over ADCT use alone through improved prevention of subclinical mastitis and reduced ISCC in the first 60 d of lactation. **Key words:** dry-cow therapy, individual somatic cell count, intramammary infection, mastitis, teat sealant

### INTRODUCTION

Interventions that provide a means to reduce the risk of infection of clinical or subclinical mastitis and the sequelae to infection, such as elevated individual cow SCC (ISCC), independent of causal organisms, are of great potential benefit to the dairy industry (Rabiee and Lean, 2013). Antibiotic dry-cow therapy (ADCT) is widely used at the end of lactation. It is designed to cure existing IMI and prevent new IMI during the dry period (Smith et al., 1966). Despite the use of ADCT, clinical mastitis caused by environmental pathogens remains common. Bradley and Green (2000) showed that 52% of clinical coliform mastitis cases in the first 100 d of lactation occurred in quarters that were infected during the dry period. Most antibiotic formulations persist during the early to middle dry period but do not cover the entire dry period (Gruet et al., 2001).

Part of the cow's natural defense mechanisms against IMI after dry-off is the formation of a keratin plug in the teat canal that acts as a physical barrier to mastitis-

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causing pathogens. Failure or delay in formation of the keratin plug during the dry period is an important risk factor for new IMI. Dingwell et al. (2003) reported that quarters with “open” teat-ends and quarters that had cracked teat-ends were both 1.7 times more likely to develop new IMI during the dry period, compared with quarters that “closed” and that were not cracked. Williamson et al. (1995) showed that 45 to 55% of quarters had open teat-ends 7 d after dry-off, and 97% of the clinical infections in the 21 d after dry-off occurred in these open quarters. A combination of the benefits of ADCT and the use of an internal teat sealant (TS) to mimic the protective effects of the keratin plug to provide protection during the entire dry period is used in Australia, North America, Europe, and New Zealand. One such TS is Teatseal (Zoetis Australia, Silverwater, NSW, Australia), a nonantibiotic viscous formulation that is primarily composed of 65% bismuth subnitrate (inert salt) and liquid paraffin administered in a syringe similar to that of ADCT. The TS acts as a physical barrier to invasion of the teat canal by mastitis-causing pathogens for up to 100 d (Woolford et al., 1998).

A meta-analysis from Rabiee and Lean (2013) showed that bismuth subnitrate-based TS (Teatseal or Orbesal; Pfizer Animal Health, West Ryde, Australia) in the presence of ADCT reduced the risk of clinical mastitis after calving in lactating cows by 48% [risk ratio (RR) = 0.52; 95% CI: 0.37 to 0.75].

The few studies on the effects of the combination of ADCT and TS on the estimated linear somatic cell score (LS) of milk ISCC after calving had differing responses. Godden et al. (2003) and Runciman et al. (2010) showed a reduced LS in cows treated with ADCT and TS compared with ADCT alone. Only a trend toward a reduction in LS was reported by Baillargeon and LeBlanc (2010) at the second herd test, but no treatment effects were found at the first or third herd tests. Mütze et al. (2012) found no difference in ISCC between ADCT and ADCT + TS treated cattle over the first 3 mo of lactation. Rabiee and Lean (2013) pooled raw data from Cook et al. (2005), Sanford et al. (2006), and Baillargeon and LeBlanc (2010), and showed that the estimated LS of cows treated with a combination of ADCT and TS was not significantly different from that of those treated with ADCT only; however, Rabiee and Lean (2013) concluded that further studies were needed in this area.

Studies on the effects of the combination of ADCT and TS on subclinical mastitis are scarce, but Runciman et al. (2010) found that the RR of subclinical mastitis, defined as ISCC  $\geq 250,000$  cells/mL at the first herd test (conducted 7 to 50 d after calving) in ADCT + TS cows compared with ADCT only was 0.80 (95%

CI: 0.65 to 0.98;  $P = 0.035$ ), indicating a lower risk of subclinical mastitis in ADCT + TS cows.

The objective of this study was to determine the efficacy of a TS product (Teatseal; Zoetis Australia) when used in combination with ADCT administered at dry-off, on milk ISCC, milk production and components, and the incidence of clinical and subclinical mastitis of multiparous dairy cows up to 60 d after calving compared with ADCT only.

## MATERIALS AND METHODS

### Experimental Design

Multiparous cows from 8 commercial pasture-based dairy herds in southern and eastern Australia were enrolled in this multicenter, randomized controlled trial with blocking between May 2014 and June 2015. Each cow, provided she met the required study inclusion criteria, was enrolled from dry-off until 60 DIM. All experimental procedures were approved by the *Scibus* Animal Ethics Committee (*Scibus* 0414-1215) and complied with Australian Animal Welfare Regulations.

### Herd Selection

Herds were selected on (1) location, (2) willingness to comply with the study protocol, (3) number of cows, (4) good biographical records of cows, (5) absence of *Mycoplasma bovis* on a bulk-vat PCR test (RtMastitis major-4, Dairy Technical Services Ltd., Kensington, VIC, Australia), and (6) eligibility of cows. Herds were not selected based on previous mastitis incidence or ISCC.

The target enrollment was 1,000 cows per treatment group. Sample size was determined based on a reduction in LS of 0.5 between an anticipated LS of 4.7 (approximately 30,000 in ISCC) with statistical power 0.9 at  $\alpha = 0.05$ , based on a single prior ISCC and 3 repeated samples using Stata (version 12.0; StataCorp LP., College Station, TX). The estimates used for the sample size calculation were derived from previous studies on TS and ISCC (Rabiee and Lean, 2013).

### Cow Selection

Cows were eligible for enrollment if they (1) had no clinical signs of disease, (2) had a predicted dry period between 40 and 80 d, (3) were entering their second or greater lactation, (4) had 4 functioning quarters free from teat abnormalities with teat-end scores of  $\leq 3$  on a scale of 1 to 5 (Britt and Farnsworth, 2011), (5) lameness score  $\leq 2$  on a scale of 1 to 5 (Sprecher et al.,

1997), (6) had not received antibiotic or antiinflammatory treatment during the previous 30 d, (7) had a BCS of 2 to 4 on a 5-point scale (Edmondson et al., 1989), and (8) had unique identification.

### **Treatment Assignment and Administration**

Cows were assigned to 1 of the 2 following treatment groups: ADCT (Orbenin Enduro Dry Cow, Zoetis Australia) or ADCT + TS (Teatseal, Zoetis Australia) based on the following blocking factors: anticipated calving date, milk production, and ISCC from previous herd test using statistical software (SAS for Windows, version 9.3; SAS Institute Inc., Cary, NC). Separate allocation lists were generated for each herd and day of treatment. For each herd and day of treatment, eligible cows were sorted into groups of at least 6 cows with similar expected calving date (e.g., within 2 wk) and milk production (e.g., within 5 L). Within each of these groups, cows were sorted by ISCC into blocks, with a target block size of 6. The precise block size varied slightly depending on the herd size and combinations of blocking factors. Within each block, cows were randomly allocated to treatment groups using a 50:50 ratio.

The ADCT contained dynamilled (i.e., superfine and equally ground) cloxacillin at 600 mg/syringe (syringe weight = 3.6 g) and the TS contained bismuth subnitrate at 650 mg/g (syringe weight = 4 g). Study personnel who administered the treatments were not masked to treatments. All other study, farm, and laboratory personnel were masked to treatment allocation. The statistical analysis was not conducted by a person masked from the treatment allocation.

All dry-off treatments were administered by trained personnel wearing plastic gloves. Before treatment, cows were milked out and teat-ends were scored (Britt and Farnsworth, 2011). Each teat-end was scrubbed with teatwipes that contained 70% isopropanol (Prepare Teatwipes, Zoetis Australia, or Livwipes, Livingston International Pty. Ltd., Rosebery, NSW, Australia) until no manure or mud was visible on the wipe. At that point, a new wipe or sterile surface of the wipe was applied to the teat-end. Each quarter from every cow was infused with 1 syringe of ADCT in the teat canal, and ADCT was massaged up the teat canal. The nozzle of the ADCT syringe was only partially inserted up the teat canal. For cows in the ADCT + TS group, teat-ends were scrubbed with teatwipes and sterilized again between ADCT and TS infusion. One syringe of TS was infused for each quarter, and the nozzle of the syringe was fully inserted in the teat canal for infusion. Following infusion, all cows were manually sprayed with diluted iodine.

### **Herd Management**

In all 8 herds, enrolled cows were kept with the main herd throughout the study and subjected to the routine management practices of the respective farm, including treatment protocols for clinical mastitis. All herds fed a transition ration during the 3 wk before calving, fed grain during twice-a-day milking, and practiced post-milking teat disinfection.

During the study, an acute mycotoxicosis was diagnosed in cows from farm 5. Clinical cows were treated intramuscularly with up to three 20-mL doses of an antiinflammatory (ketoprofen, Ilium Veterinary Laboratories, Smithfield, NSW, Australia) given daily on consecutive days and monitored daily. Cows that were severely affected were dried off and removed from the study. Changes in individual cow BW and milk yield before and after the mycotoxicosis, herd test data, clinical signs, and treatment status were used to determine if data were suspected to be compromised. Data from 11 cows were removed from the herd test immediately following the mycotoxicosis, and all herd test data recorded after the mycotoxicosis were removed from 28 cows. These assessments of the effect of mycotoxicosis and exclusion of data were done by individuals masked to the group allocation and before any statistical analyses.

### **Clinical Mastitis Monitoring and Milk Sampling**

First cases of clinical mastitis were monitored for the first 60 DIM for each enrolled cow that calved with a dry period of 40 to 100 d. Herd personnel were trained in the identification, grading, and aseptic sampling of clinical mastitis cases. The dates of detection and treatment and quarter affected were recorded. Clinical mastitis was identified in quarters based on changes in the cow, udder, or milk. Mastitis severity was graded according to the following definitions: mild—minor alterations in milk (i.e., clots or flakes); acute—cow may or may not have been sick (i.e., fever and lack of appetite), udder was hot, swollen, painful, and hard, and milk was abnormal (i.e., discolored and contained clots and/or blood); and severe—cow was extremely ill and depressed, udder may have been gangrenous, and milk was abnormal (Dairy Australia, 2014). Milk samples were aseptically collected from quarters suspected of first or new cases of clinical mastitis before treatment administration by scrubbing the teat-ends with a teatwipe, stripping the foremilk 3 times, and collecting a minimum of 1 mL of foremilk into sterile 15-mL collection tubes by holding the tube at a 45° angle. Milk samples were frozen at -20°C until bacteriology.



## Bacteriology

Frozen milk samples were cultured according to the technique described by Shum et al. (2009), which is based on the *Laboratory Handbook of Bovine Mastitis* (National Mastitis Council, 1999), with modifications (Sears and McCarthy, 2003). Briefly, samples were mixed via inversion and plated onto a triplate containing sheep blood agar (SBA) no. 2, MacConkey's agar no. 3, and Edward's modified medium (EDM; Oxoid Ltd., Basingstoke, UK) using a sterile swab. Culture plates were incubated aerobically at 36°C for a total of 48 h and examined for growth at 24- and 48-h intervals. Milk samples were not cultured for *Mycoplasma* during this study because samples from the bulk vat were screened for *Mycoplasma* before the study, and clinical signs consistent with *Mycoplasma* mastitis or *Mycoplasma* infections were not observed on any of the farms during the course of the study.

The preliminary classification of bacterial isolates was based on the growth characteristics of bacteria on the respective media. *Streptococcus*, *Aerococcus*, and *Enterococcus* spp. were identified by their growth on SBA and EDM, and *Staphylococcus* spp. by growth on SBA alone. Isolation of >2 bacteria from a sample was considered to reflect contamination and the result was considered nondiagnostic.

Bacterial isolates were further characterized by Gram staining and testing with potassium hydroxide, catalase, coagulase, bile esculin, Christie, Atkins, Munch-Peterson (CAMP), and alkaline phosphatase tests, plus identification on Enterococcosel Agar (Becton Dickinson, Sparks, MD) and modified Rambach agar (Colman and Ball, 1984; Fertally and Facklam, 1987; Watts et al., 1993; Zadoks et al., 2005).

## Milk Testing

Milk samples were collected every  $14 \pm 3$  d after the first eligible enrolled cow at each farm had calved. Milk was analyzed for milk yield, milk fat percentage, milk protein percentage, and ISCC by local herd recording agencies according to their standard procedures.

## Additional Data Collection

All calving dates, treatments, treatment dates, deaths, sale, or culling throughout the dry period and the first 60 DIM were recorded for all cows.

## Statistical Analysis

A biometrics representative from the Zoetis VMRD Biometrics Group (Parkville, VIC, Australia) was re-

sponsible for all data summaries and analyses. All statistical tests were 2-sided at the 5% level of significance and performed in SAS for Windows (version 9.3). The experimental unit was the individual animal.

Data were included in this analysis if a cow (1) calved within a post-dry-off interval of 40 to 100 d (d 0; enrollment); (2) had her first herd test on or between d 10 and 24 postcalving; and (3) had at least one subsequent herd test, either 11 to 17 d after the first herd test (second herd test) or 22 to 34 d after her first herd test (analyzed as data for third herd test). This provided 3 time points, with actual days postcalving as follows, each with an approximately 14-d interval with minimal overlap: (1) 10 to 24 d postcalving, mean 17 d; (2) 23 to 40 d postcalving, mean 31 d; and (3) 36 to 54 d postcalving, mean 45 d.

No cows were included in the analysis if they had only one herd test result, and data were not included from fourth herd tests that occurred before 60 d postpartum. Data from individual herd tests were excluded if initiation of antibiotic treatment occurred within 7 d of a herd test or if the data were not a composite of both morning and afternoon sample measurements for all of the following variables: milk yield, fat and protein percentages, and ISCC.

For cows included in the analysis, the number of cows, milk yield, and ISCC data at enrollment were summarized descriptively for each treatment group from each farm (Table 1). The primary outcome was ISCC at 3 herd tests during the first 60 d postpartum (the first being 10 to 24 d postpartum for each cow). Each ISCC value was log-transformed [ $\ln(\text{ISCC} + 1)$ ]. The ISCC was also analyzed as a secondary outcome weighted by milk yield to provide an indication of bulk milk SCC. Milk yield (L) was used as a weighting variable for each untransformed ISCC observation. Log-transformed ISCC and ISCC weighted by milk yield, milk yield, fat percentage, and protein percentage were each analyzed using a general linear mixed model for repeated measures, with terms including the fixed effects of treatment group, time point, and the interaction of these effects, the random effects of farm, block, and animal, and the interaction terms for treatment group and farm, and treatment group, time point (herd test), and farm. The *P*-values of main effects of treatment group and time point and their interaction are presented for each milk variable in Table 2, along with overall least squares means and 95% CI for treatment group. Least squares means and 95% CI for log-transformed ISCC were presented following back-transformation to the original scale (geometric means; Table 2). Least squares means and 95% CI for each treatment group at each time point were produced (data not shown). As the main effect

of treatment group was significant at the 5% level for log-transformed ISCC, pairwise comparisons between treatment groups were made at each time point. Least squares means differences between the treatment groups, standard errors, 95% CI, and *P*-values were presented for each time point.

The presence or absence of clinical mastitis was summarized for each treatment group for the dry period and postcalving period separately. Dry period mastitis was summarized for all enrolled animals that calved 40 to 100 d after dry-off (enrollment). Treatment groups were not compared in terms of dry cow mastitis as the total number of such cases was too low for meaningful statistical analysis. Postcalving mastitis was summarized for animals with mastitis from 0 to 60 d postcalving; animals without mastitis were included in this analysis provided they were included in the primary analysis of herd test data. The binary outcome variable (presence or absence of clinical mastitis postcalving) was analyzed using a generalized linear mixed model with terms including the fixed effect of treatment group, the random effects of farm and block, and the interaction term for treatment group and farm. The outcome was changed from 0 to 0.001 for nonmastitis cases to facilitate convergence. Data on the number of cows treated for first cases of clinical mastitis, quarters affected, and mastitis-causing bacteria are summarized in Table 3 (but were not analyzed).

Time to mastitis (days postcalving) was analyzed using survival analysis methods for censored data, with nonmastitis cases censored at the time of the last herd test up to 60 d postcalving. The trends over time were illustrated using a Kaplan-Meier analysis and the treatment groups were compared using a semiparametric Cox proportional hazards model (proc phreg) with the fixed effects of treatment group, strata effects of farm and block, and the random effect of the interaction term for treatment group and farm. Estimated hazard ratios (**HR**) for comparing the treatment groups were presented, along with 95% CI.

The presence or absence of subclinical mastitis postcalving (defined as at least 1 ISCC of  $\geq 250,000$  cells/mL from d 10 to 60 postcalving) was summarized for each treatment group for the postcalving period, using data from animals included in the primary analysis of herd test data (Table 4). This binary outcome variable was analyzed using a generalized linear mixed model with terms including the fixed effect of treatment group, the random effects of farm and block, and the interaction term for treatment group and farm. Means and differences between treatment groups were presented using back-transformed odds and odds ratios, with corresponding 95% CI.

**Table 1.** Summary of number of cows, region, breed, milk yield (mean  $\pm$  SD), and Ln individual SCC (ISCC; mean  $\pm$  SD) of cows based on the last herd test before dry-off included in the final analysis for treatment groups from 8 farms in southern and eastern Australia<sup>1</sup>

Item	Farm								Total
	1	2	3	4	5	6	7	8	
Region	Western districts	South coast NSW	Finley	Finley	Derwent Valley	South coast NSW	Finley	Finley	
Breed <sup>2</sup>	J	HF	HF $\times$ J	HF	HF $\times$ J	HF	HF $\times$ J	HF	
No. of cows	52	50	64	21	53	50	419	30	739
Milk yield (L)	15.1 $\pm$ 4.76	15.3 $\pm$ 4.40	11.3 $\pm$ 2.25	17.8 $\pm$ 7.75	11.8 $\pm$ 3.19	18.1 $\pm$ 5.12	9.9 $\pm$ 2.80	18.1 $\pm$ 5.40	12.0 $\pm$ 4.68
Ln ISCC	5.23 $\pm$ 0.89	5.13 $\pm$ 1.05	5.37 $\pm$ 0.82	3.85 $\pm$ 1.72	4.87 $\pm$ 0.84	4.74 $\pm$ 1.11	4.49 $\pm$ 0.94	4.69 $\pm$ 1.11	4.70 $\pm$ 1.03
ADCT + TS									
No. of cows	53	48	62	27	59	67	409	24	749
Milk yield (L)	13.7 $\pm$ 4.84	15.1 $\pm$ 4.63	11.7 $\pm$ 2.13	17.4 $\pm$ 4.81	12.8 $\pm$ 3.43	18.6 $\pm$ 4.30	9.9 $\pm$ 2.68	18.6 $\pm$ 5.00	12.2 $\pm$ 4.59
Ln ISCC	5.23 $\pm$ 0.73	5.03 $\pm$ 0.91	5.36 $\pm$ 0.76	3.46 $\pm$ 1.29	4.96 $\pm$ 0.63	4.60 $\pm$ 1.00	4.53 $\pm$ 0.94	4.55 $\pm$ 1.09	4.68 $\pm$ 0.99

<sup>1</sup>Treatment groups: ADCT = antibiotic dry-cow therapy (Orbenin Enduro Dry Cow, Zoetis Australia, Silverwater, NSW, Australia); ADCT + TS = ADCT and teat sealant (Teatseal, Zoetis Australia).

<sup>2</sup>J = Jersey; HF = Holstein Friesian; HF  $\times$  J = crossbreed.

**Table 2.** Least squares means and lower and upper 95% CI for treatment groups and *P*-values for the main effect of treatment group and time and their interaction for milk measures<sup>1,2</sup>

Variable	Mean (lower and upper 95% CI)		<i>P</i> -value		
	ADCT	ADCT + TS	Group (G)	Time (T)	G × T
Milk yield (L)	30.4 (26.6, 34.1)	30.3 (26.6, 34.0)	0.918	<0.001	0.804
Fat (%)	4.04 (3.78, 4.30)	4.04 (3.78, 4.30)	0.977	<0.001	0.945
Protein (%)	3.37 (3.13, 3.61)	3.35 (3.11, 3.59)	0.367	<0.001	0.718
Geometric mean ISCC <sup>3</sup> (×10 <sup>3</sup> cells/mL)	43.5 (36.2, 52.1) <sup>4</sup>	32.0 (26.8, 38.3) <sup>4</sup>	0.021	<0.001	0.748
ISCC <sup>3</sup> weighted by milk yield	149.6 (91.4, 207.7)	105.0 (47.1, 162.9)	0.129	0.110	0.566

<sup>1</sup>Data are from 1,488 cows that met the inclusion criteria of calving with a dry period between 40 to 100 d, had a herd test within 10 to 24 DIM, and had a total of at least 2 complete herd tests from both a morning and afternoon milking within 60 DIM. Data from cows treated with antibiotics within 7 d of a herd test were removed.

<sup>2</sup>Treatment groups: ADCT = antibiotic dry-cow therapy (Orbenin Enduro Dry Cow, Zoetis Australia, Silverwater, NSW, Australia); ADCT + TS = ADCT and teat sealant (Teatseal, Zoetis Australia).

<sup>3</sup>ISCC = individual SCC.

<sup>4</sup>Geometric mean and CI were back-transformed from a natural log.

## RESULTS

### Study Animals

In total, 2,200 cows were allocated to treatment groups and 2,080 of these cows met the enrollment criteria. Of the 120 cows that did not meet the enrollment criteria, 57 were from the ADCT group and 63 were from the ADCT + TS group. Numbers and reasons are as follows: <4 functioning quarters (41 cows), not present at enrollment (22 cows), lameness score ≥3

(14 cows), still in milk (8 cows), clinical illness or had received antibiotics in the 30 d before enrollment (8 cows), not pregnant (8 cows), teat score ≥3 (5 cows), BCS <2 or >4 (5 cows), aborted (4 cows), received wrong treatment (3 cows), and to be culled (2 cows).

Of the enrolled cows, 1,044 received ADCT and 1,036 received ADCT + TS. The number and proportion of total study cows enrolled on each farm ranged from 62 to 944 and from 3.0 to 45.4%, respectively. Of these 2,080 enrolled cows, 1,844 (88.7%) calved within the eligible dry-period window of 40 to 100 d: 929 cows

**Table 3.** Summary of number of cows diagnosed and treated for clinical mastitis during the first 60 d postpartum, quarters affected, and bacteriology

Item	Group <sup>1</sup>		Total
	ADCT	ADCT + TS	
Total no. of cows diagnosed	43	33	76
Total no. of cows treated	40	29	69
Total no. of cows not treated	2	3	5
Total no. of cows unknown if treated	1	1	2
Quarter(s) affected <sup>2</sup>			
Back right	11	12	23
Back left	15	9	24
Front right	8	9	17
Front left	9	5	14
Unrecorded quarter	7	3	10
Bacteria cultured <sup>3</sup>			
No growth	13	11	24
<i>Streptococcus uberis</i>	7	5	12
Coliforms	2	9	11
<i>Streptococcus dysgalactiae</i>	7	4	11
<i>Staphylococcus aureus</i>	4	3	7
CNS	3	0	3
<i>Streptococcus</i> spp.	1	1	2
<i>Aerococcus</i> spp.	0	1	1
<i>Enterococcus faecalis</i>	1	0	1
Mixed growth	0	1	1

<sup>1</sup>Treatment groups: ADCT = antibiotic dry-cow therapy (Orbenin Enduro Dry Cow, Zoetis Australia, Silverwater, NSW, Australia); ADCT + TS = ADCT and teat sealant (Teatseal, Zoetis Australia).

<sup>2</sup>Some cows had more than one quarter affected.

<sup>3</sup>In total, 25% of the clinical mastitis cases did not have milk samples collected.

(89.0%) and 915 cows (88.3%) from the ADCT and ADCT + TS groups, respectively.

In total, 1,488 cows (71.5% of enrolled cows) met the inclusion criteria for analysis of herd test data: 739 from the ADCT group and 749 from the ADCT + TS group. Reasons for exclusion from analysis included death, disease, culling, abortion, first herd test not between 10 and 24 d postpartum, and <2 herd tests containing both morning and afternoon herd test measurements for all of the following variables: milk yield, fat and protein percentages, and ISCC within the first 60 d postpartum. Some cows were ineligible for more than one of these reasons.

Data on the number of cows included in the analysis and milk yield and ISCC at the last herd recording before dry-off are summarized for each farm in Table 1. Mean milk yield and ISCC were well balanced between the ADCT and ADCT + TS groups as a result of the method of allocation. The mean  $\pm$  SD days' dry for these cows was  $58.1 \pm 11.9$  and  $58.3 \pm 11.4$  from the ADCT and ADCT + TS groups, respectively.

### Milk Profile

*P*-Values for the main effects of treatment group and time and their interaction for milk measures collected from 2 or 3 herd tests up to 60 DIM with the first herd test occurring between 10 to 24 DIM are presented in Table 2. We detected no significant interaction between treatment group and time for milk yield, fat and protein percentage, Ln ISCC (values presented as geometric mean ISCC in Table 2), or ISCC weighted by milk yield. The ADCT + TS cows had a reduced

geometric mean ISCC of 11,500 cells/mL, compared with the ADCT cows ( $P = 0.021$ ; Table 2). Figure 1 shows the geometric mean ISCC for the ADCT and ADCT + TS groups at herd tests 1 to 3. The least squares means difference ( $\pm$ SE) between ADCT and ADCT + TS for Ln ISCC overall was  $0.297 \pm 0.112$  (95% CI: 0.052 to 0.541). The back-transformation of this overall difference in Ln ISCC has an estimated ratio of 1.35 (95% CI: 1.05 to 1.72). The least squares means difference ( $\pm$ SE) between the ADCT and ADCT + TS groups for Ln ISCC was greatest at the second herd test ( $0.328 \pm 0.126$ , 95% CI: 0.064 to 0.591;  $P = 0.018$ ; Figure 1), compared with at the first and third herd tests ( $0.312 \pm 0.128$ , 95% CI: 0.045 to 0.579;  $P = 0.025$ , and  $0.251 \pm 0.130$ , 95% CI: -0.019 to 0.521;  $P = 0.067$ , respectively).

Milk yield, fat and protein percentages, and ISCC weighted by milk yield were not significantly different between treatment groups. Milk yield, fat and protein percentages, and Ln ISCC were influenced by time, whereas ISCC weighted by milk yield was not influenced by time (Table 2).

### Clinical Mastitis

Four cows (0.2%) of the 1,844 cows that calved 40 to 100 d after dry-off were reported to have a first case of clinical mastitis in the dry period. All cows were from the ADCT + TS group and all were from different farms. These cases occurred at 1, 4, 20, and 53 d before calving. A milk sample was collected from only 1 of the 4 cases, and *Streptococcus uberis* was identified from this sample.

Five percent of cows that had calved 40 to 100 d after dry-off had a first case of clinical mastitis between 0 and 60 DIM. The number of cases was 43 (5.7%) in the ADCT group and 33 (4.3%) for the ADCT + TS group out of 1,528 cows included in this analysis but this was not significantly different ( $P = 0.194$ ). A summary of the number of cows treated for first cases of clinical mastitis and the quarters affected is presented in Table 3. Of the cases of clinical mastitis, 69 cases were treated and more cases occurred in the back 2 quarters (data not analyzed).

A proportional hazards model of the survival data showed no significant difference in the number of d after calving to detection of first cases of clinical mastitis between the ADCT and ADCT + TS groups ( $P = 0.153$ ). The estimated HR for ADCT + TS cows (relative to ADCT alone) was 0.70 (95% CI: 0.43, 1.14); that is, the estimated hazard of clinical mastitis for the ADCT + TS cows was 30% less than for the ADCT-only cows over the first 60 d of lactation. A Kaplan-Meier survival curve showing the percentage of clinical mastitis cases

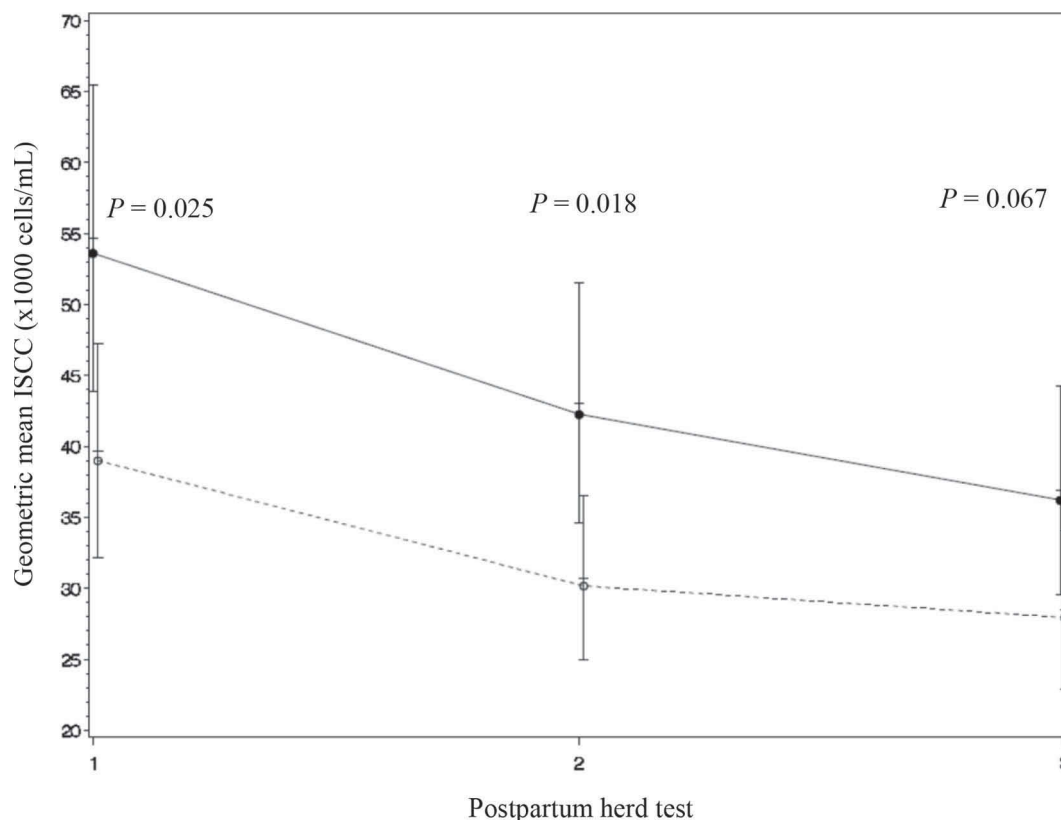
**Table 4.** Number and percentage of cows that had subclinical mastitis (at least 1 individual SCC  $\geq 250,000$  cells/mL during the first 60 d postpartum) from each of the 8 farms<sup>1</sup>

Farm	Group <sup>2</sup>				Total	
	ADCT		ADCT + TS			
	No.	%	No.	%	No.	%
1	12	23.1	9	17.0	21	20.0
2	17	34.0	8	16.7	25	25.5
3	20	31.3	9	14.5	29	23.0
4	7	33.3	4	14.8	11	22.9
5	7	13.2	3	5.1	10	8.9
6	10	20.0	8	11.9	18	15.4
7	51	12.2	34	8.3	85	10.3
8	9	30.0	3	12.5	12	22.2
Total	133	18.0	78	10.4	211	14.2

<sup>1</sup>Only data from cows included in the analysis of herd test data in Table 2 have been used.

<sup>2</sup>Treatment groups: ADCT = antibiotic dry-cow therapy (Orbenin Enduro Dry Cow, Zoetis Australia, Silverwater, NSW, Australia); ADCT + TS = ADCT and teat sealant (Teatseal, Zoetis Australia).





**Figure 1.** Geometric mean individual SCC (ISCC; with 95% CI) back-transformed from a natural log for cows administered with antibiotic dry-cow therapy (ADCT; solid line, ●) or ADCT plus internal teat sealant (ADCT + TS; dashed line, ○) at 1 to 3 herd tests during the first 60 d postpartum. Herd test 1 occurred between 10 and 24 DIM, herd test 2 between 11 and 17 d after the first herd test, and herd test 3 between 11 and 17 d after the second herd test or 22 to 34 d after the first herd test if the second herd test was missed. *P*-values indicate least squares means difference between ADCT and ADCT + TS at each herd test.

between the ADCT and ADCT + TS groups over the first 60 d postpartum is provided in Figure 2.

### Bacteriology

Milk samples were collected from 57 (75%) of the cows that were included in the herd test analysis with a first case of clinical mastitis from at least one quarter occurring between 0 to 60 DIM. Bacteriology of these samples for cows in the ADCT and ADCT + TS groups is presented in Table 3. Twenty-four milk samples produced no growth (13 from the ADCT and 11 from the ADCT + TS group). *Streptococcus uberis*, coliforms, and *Streptococcus dysgalactiae* were the most numerous groups detected, with 12, 11, and 11 cases reported in total, respectively. More coliforms were detected in samples from the ADCT + TS cows compared with those from the ADCT cows (9 vs. 2, respectively but this data were not analyzed). *Staphylococcus aureus* was detected in 7 samples, CNS in 3 cases, and *Streptococcus* spp. in 2 cases. Singular cases of *Aerococcus* spp., *Enterococcus faecalis*, and mixed growth occurred.

### Subclinical Mastitis

Overall, the ADCT group had 1.9 greater odds (95% CI: 1.4 to 2.6;  $P < 0.001$ ) of at least 1 case of subclinical mastitis defined as  $\geq 250,000$  cells/mL, compared with the ADCT + TS group. In the ADCT group, 18% of cows had at least 1 case of subclinical mastitis up to 60 DIM and only 10.4% of cows in the ADCT + TS group. Cows in the ADCT + TS group from each farm had a lower number and percentage of cows that had subclinical mastitis in the first 60 DIM (Table 4). The percentage of subclinical mastitis ranged from 8.9 to 25.5% of cows from each farm (Table 4).

### Other Health Events

The main health event reported during the first 0 to 60 DIM for cows included in the herd test analysis was milk fever, with a total of 24 cases reported (13 for ADCT and 11 for ADCT + TS; data not analyzed). Five cases of foot-related lameness were reported (3 for ADCT and 2 for ADCT + TS) and for cows in the



ADCT + TS, single cases of retained placenta, metritis, and photosensitization were reported. Underreporting of health events is suspected on some farms.

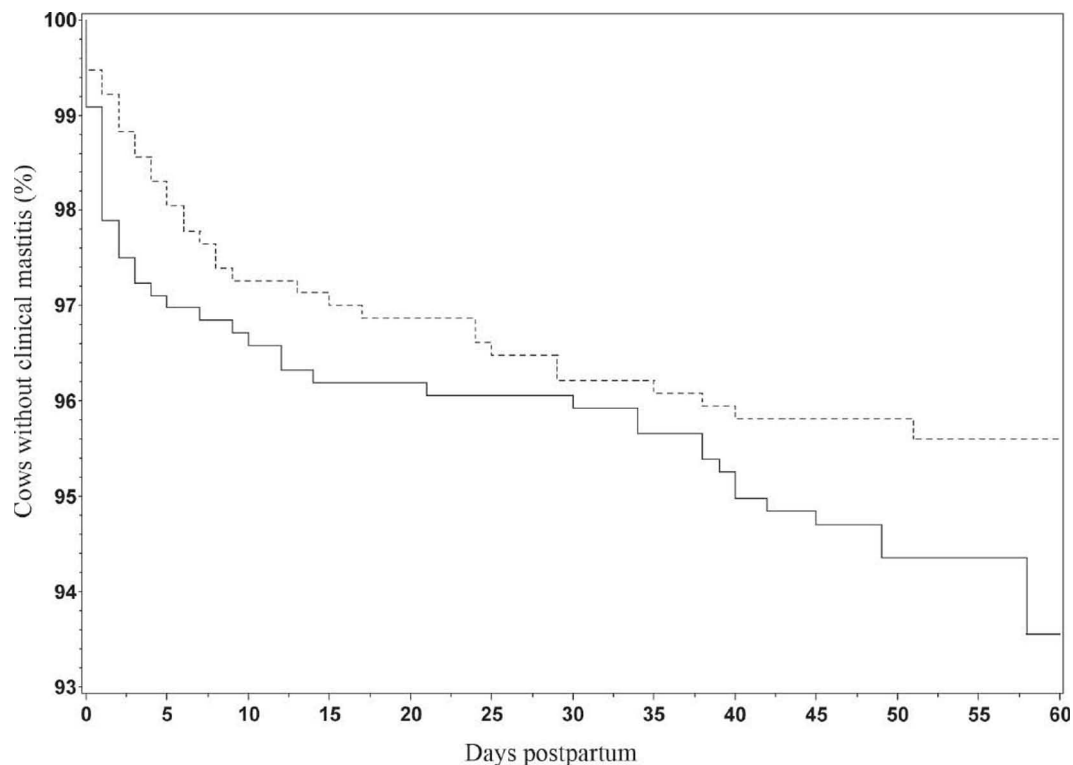
## DISCUSSION

This study is one of the first to focus on the efficacy of the combination of ADCT plus TS on ISCC. Our findings demonstrated that TS in the presence of ADCT decreased ISCC in the first 60 d of lactation, with a higher (by 11,500) geometric mean ISCC occurring in cows treated with ADCT only compared with those treated with ADCT + TS at dry-off. The combination of ADCT and TS also reduced the odds of at least 1 case of subclinical mastitis (ISCC  $\geq 250,000$  cells/mL) in early lactation; the odds ratio for ADCT alone (relative to ADCT + TS) was 1.9 (95% CI: 1.4 to 2.6).

The decrease in geometric mean ISCC in early lactation in cows treated with the combination of ADCT and TS compared with ADCT alone is likely to be the result of a combination of good hygiene at dry-off, the positive effects of the long-acting antibiotics in the ADCT, and the TS mimicking the physical barrier of the keratin plug during the dry period. It is important to note, however, that the ISCC analysis was performed on the log scale and the resulting least squares means

were back-transformed for presentation of geometric means. The highest ISCC values are highly influential when calculating raw means but have a smaller effect on the calculated geometric means. Hence, raw ISCC and ISCC weighted by milk yield group means will be higher than geometric mean ISCC values. The group means for the ISCC weighted by milk yield measure, which was based on untransformed ISCC data, provide an indication of bulk milk cell count values.

In a similar Australian study, Runciman et al. (2010) used the same ADCT and TS products as in the current study and showed that the mean LS for the cows treated with ADCT and TS was significantly lower compared with cows treated with ADCT only ( $P < 0.001$ ) after accounting for herd, age, milk production, and ISCC status in previous lactation. Godden et al. (2003) demonstrated that quarters treated with ADCT and TS (cloxacillin 500 mg, Schering-Plough Corp., Kenilworth, NJ; and Orbeaseal, Pfizer Animal Health, New York, NY) had a significantly lower mean LS at both 1 to 3 DIM and 6 to 8 DIM than quarters treated with ADCT only ( $P < 0.001$ ). However, Baillargeon and LeBlanc (2010) found that LS only tended to be lower in the ADCT and TS (penicillin and novobiocin, NovoDry, Pfizer Animal Health, Kirkland, Canada; or 300 mg of cephapirin, CefaDri, Wyeth Animal Health,



**Figure 2.** Kaplan-Meier survival curve of the percentage of cows diagnosed with clinical mastitis over the first 60 d postpartum that were treated with antibiotic dry-cow therapy (ADCT; solid line) or ADCT plus internal teat sealant (ADCT + TS; dashed line) at dry-off.

Guelph, Canada), compared with ADCT group at their second herd test ( $P = 0.07$ ). No significant reduction in LS was observed by those same authors between ADCT plus TS and ADCT only groups at their first and third herd tests ( $P = 0.11$  and  $0.62$ , respectively). Mütze et al. (2012) showed that the ISCC over the first 3 mo of lactation was not different between ADCT and ADCT + TS treated cows ( $P = 0.37$ ).

Interestingly, Rabiee and Lean (2013) demonstrated that the estimated LS of pooled raw data of 3 studies (Cook et al., 2005; Sanford et al., 2006; Baillargeon and LeBlanc, 2010) from 32 herds, before and after controlling for LS at dry-off, was not significantly different between cows treated with ADCT and TS and those treated with ADCT only. Data provided to Rabiee and Lean (2013) from Cook et al. (2005) and Sanford et al. (2006) were not published in the original papers.

The higher risk of subclinical mastitis for both groups at the first herd test after calving, compared with the second and third herd tests postcalving in this current study (as indicated by ISCC) and greatest effect of treatment at the second herd test postcalving (as indicated in Figure 2) are consistent with findings by Baillargeon and LeBlanc (2010). These findings may indicate that the combination of ADCT and TS provides the greatest benefit in ISCC reduction during this period. Godden et al. (2003) and Sanford et al. (2006) had observation periods the same as the current study (60 d postcalving), whereas Baillargeon and LeBlanc (2010), Berry and Hillerton (2002), and Cook et al. (2005) had observation periods of 105 d after calving.

The lack of a significant effect of ADCT + TS on the incidence of clinical mastitis in this study is not consistent with results from a meta-analysis by Rabiee and Lean (2013) that showed the combination of ADCT and TS reduced the risk of clinical mastitis after calving in lactating cows by 48% (RR = 0.52; 95% CI: 0.37 to 0.75). However, the reduction in risk of 30% over the first 60 d is broadly consistent with many of the studies included in the meta-analysis of Rabiee and Lean (2013) and highlights the need for large numbers of cattle to provide adequate study power in health studies. Godden et al. (2003) produced a survival analysis using the Cox proportional hazards regression model with a very similar estimate for the reduction in risk of failure in treated quarters by 60 DIM (HR = 0.67;  $P < 0.05$ ).

Baillargeon and LeBlanc (2010) reported a daily probability of mastitis 24% lower in cows administered with ADCT + TS (HR = 0.76, 95% CI: 0.59 to 0.97;  $P = 0.03$ ) than cows in the ADCT group during the first 105 d postcalving. Runciman et al. (2010) reported a RR of clinical mastitis diagnosed within 21, 30, and 100 d of calving in ADCT + TS treated cows as 0.30 (95%

CI: 0.2 to 0.44), 0.39 (0.28 to 0.53), and 0.58 (0.46 to 0.75) compared with that of ADCT cows, respectively.

Despite no formal statistical analysis of the number of mastitis-causing pathogens cultured between each treatment group, the higher number of coliforms cultured in the ADCT + TS cows was not expected. The higher number of clinical mastitis cases in the back, as opposed to front, quarters is consistent with findings by Adkinson et al. (1993) and Barkema et al. (1997) that the risk of clinical mastitis is higher in these quarters. However, Berry et al. (2003) found no statistical differences in infection status at calving between back and front or right and left quarters when cows were treated with ADCT only or TS only.

The combination of ADCT and TS reduced subclinical mastitis overall and on each of the 8 farms. Baillargeon and LeBlanc (2010) and Runciman et al. (2010) appear to be the only authors that examined the incidence of subclinical mastitis between ADCT and ADCT + TS treated cows based on ISCC. Runciman et al. (2010) showed that the RR of subclinical mastitis, defined as ISCC  $\geq 250,000$  cells/mL at their first herd test (conducted 7 to 50 d after calving), in ADCT + TS treated cows compared with ADCT only was 0.80 (95% CI: 0.65 to 0.98;  $P = 0.035$ ). Baillargeon and LeBlanc (2010) found that 22% of cows that received ADCT + TS had an ISCC  $> 200,000$  cells/mL, compared with 27% of cows in the ADCT group at their first herd test (Chi-squared  $P = 0.03$ ). As  $\geq 250,000$  cells/mL were used as the definition of subclinical mastitis in the current study, the findings by Baillargeon and LeBlanc (2010) support those of our study but are not directly comparable.

A benefit in milk yield could be hypothesized from the decrease in ISCC in cows that received the combination of ADCT and TS compared with ADCT only because ISCC can influence milk yield; however, no treatment effect on milk yield or ISCC weighted by milk yield was observed in this study. The lack of effect on milk protein and fat percentages is consistent with the lack of effect on milk yield.

## CONCLUSIONS

The combination of ADCT and TS decreased ISCC over the first 60 d of lactation under Australian field conditions. The cows treated with ADCT alone had a higher geometric mean ISCC than those treated with ADCT + TS at dry-off. Further, the odds of at least 1 case of subclinical mastitis (ISCC  $\geq 250,000$  cells/mL) were 1.9 times higher (95% CI: 1.4 to 2.6) with ADCT alone in the first 60 d of lactation compared with the use of ADCT and TS in combination. A reduction in the incidence of clinical mastitis was not demonstrated

by the combination of ADCT and TS. The prevention of mastitis depends on many factors in addition to dry-cow therapy, including aseptic dry-off technique, environmental and milking hygiene, and monitoring and management of cows, particularly during high-risk periods. Adoption of the use of the combination of ADCT and TS may be beneficial for reduction of ISCC.

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