Premilking teat disinfection: Is it worthwhile in pasture-grazed dairy herds?

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ABSTRACT

A controlled trial was conducted in 5 pasture-grazed commercial dairy herds in Australia in 2012 to determine whether premilking teat disinfection and drying of teats reduces clinical mastitis incidence during early lactation by at least 50%. A 50% reduction was estimated to be the minimum required to justify additional costs of labor, disinfectants, and other resources if premilking teat disinfection was implemented in a 500-cow herd averaging 8 clinical cases per 100 cow-months. A secondary aim was to determine whether this premilking teat disinfection routine reduces incidence of new udder infections. Treatment was applied in each herd for approximately 60 d (range of 59.5 to 61 d), commencing in each herd soon after the start of the herd's main or only calving period. Within each herd, cows were allocated to either the treatment (premilking disinfection) or the control (no premilking disinfection) group based on their herd identity number. During the trial period, any cow having a new case of clinical mastitis or an individual cow cell count greater than 250,000 cells/mL of milk (when preceded by individual cow cell counts of 250,000 cells/mL of milk or below) was deemed to have had a new infection. Overall, neither clinical mastitis incidence nor new infection rate differed significantly between treatment and control groups. Over the whole study period, 98 of the 1,029 cows in the premilking disinfection group and 97 of the 1,025 cows in the control group had clinical mastitis. Total cow-days at risk of clinical mastitis were similar in each group. However, clinical incidence rates were markedly lower in treatment cows in one herd (herd 3; incidence rate ratio = 0.34) and there was some evidence that new infection incidence rates were lower in treated cows in this herd (incidence rate ratio = 0.42). Rainfall during the study period was below long-term district average in all 5

study herds. Cows' teats were less dirty than in previous, wetter years for the 4 herds where no significant clinical mastitis response was detected but some teat soiling was observed in herd 3 during the study period. Routine application of premilking teat disinfection in pasture-grazed herds is unlikely to produce a worthwhile (economic) reduction in the number of clinical mastitis cases when teats are relatively clean and dry and the clinical mastitis incidence is low. However, premilking disinfection might be worthwhile during periods when teats are heavily soiled and the incidence of clinical mastitis due to environmental pathogens is high.

Key words: milking management, clinical mastitis, new infection rate

INTRODUCTION

During the past 15 yr, *Streptococcus uberis* has become the dominant pathogen isolated from clinical mastitis cases in southeastern Australian dairy herds (Charman et al., 2012). This organism is primarily an environmental pathogen; it can replicate in the environment and is found in paddocks, laneways, feeding areas, and tracks. During the past 20 to 30 yr, premilking teat preparation in typical Australian dairy herds has changed; teats are no longer washed and dried routinely before teatcups are applied. Where teats are not routinely washed before milking, mud and manure are often present on cows' teats when teatcups are applied. Because teat ends are likely to be bathed in milk contaminated with such material, this could result in udder infection with *Strep. uberis* (or other environmental pathogens).

Premilking teat disinfection has reduced clinical mastitis incidence; estimated reductions varied between 26 and 43% in studies conducted in the United States (Oliver et al., 1993a, 2001). In other trials conducted in the northern hemisphere, clinical mastitis incidences were 57 (Blowey and Collis, 1992), 33 (Hillerton et al., 1993), 26 (Oliver et al., 1993b), 28 (Oliver et al., 1994), 30 (Sérieys and Poutrel, 1996), and 23% (Ruegg and Dohoo, 1997) lower and 20% higher (Hillerton et al., 1993) among treated cows; none of these differences

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were statistically significant. Premilking teat disinfection has also reduced new infection rates. Rates of infection by major pathogens were estimated as declining by 57 (Pankey et. al., 1987), 34 (Oliver et. al., 1993a), 49 (Oliver et. al., 1993b), and 31% (Oliver et. al., 2001); for major and minor pathogens combined, declines were estimated as 40 (Hillerton et. al., 1993), 29 (Oliver et. al., 1993a), 27 (Oliver et. al., 1993b), 37 (Oliver et. al., 1994), 23 (Sérieys and Poutrel, 1996), and 38 to 41% (Oliver et. al., 2001). Rates of new infections by *Strep. uberis* were estimated as declining by 60 to 70% (Sérieys and Poutrel, 1996; Oliver et. al., 2001); estimated declines for esculin-positive streptococci were 48 to 66% (Pankey et. al., 1987; Galton et. al., 1988). In contrast, no substantial positive effects of premilking teat spraying on clinical mastitis incidence and new infection rates were evident in a small study in 3 pasture-grazed herds in New Zealand (Williamson and Lacy-Hulbert, 2013). These herds had low clinical mastitis incidences and low new infection rates during the study. In a study in 12 pasture-grazed herds in Western Australia (Depiazzi and Bell, 2002), clinical mastitis incidence was lower later in the trial in herds that used premilking teat disinfection, but not early in the trial; no statistical analyses were published. In summary, although beneficial effects of premilking teat disinfection have been demonstrated in northern hemisphere dairy herds, presumably where study cows were housed, effects in pasture-grazed herds are unclear.

The primary research objective of the current study was to assess whether, in pasture-grazed commercial dairy herds, premilking teat disinfection and drying of teats reduces the incidence of clinical mastitis in early lactation by at least 50%, relative to no premilking udder preparation. A 50% reduction was estimated to be the minimum required to justify additional costs of labor, disinfectants, and other resources if premilking disinfection were implemented. Where the purpose of premilking teat disinfection is to reduce clinical incidence, the break-even efficacy of premilking teat disinfection depends primarily on the average cost of each clinical case, the clinical mastitis incidence before implementation, and herd size. For example, at current input costs, the breakeven point for a 500-cow herd with an average of 8 cases/100 cow-months is about a 50% reduction. This assumes an average cost of \$277 for each clinical mastitis case (J. F. Penry, Countdown Downunder, Melbourne, Victoria, Australia, personal communication). At 14 cases/100 cow-months, only a 30% reduction would be required to break even, whereas at incidences of less than 8 cases, reductions greater than 50% are required to break even. Eight cases/100 cow-months is markedly higher than the Australian targets of less than 5 cases per 100 cows per month for

the first month of lactation and 2 cases per 100 cows in each subsequent month (Brightling et al., 1998). A secondary research objective was to determine whether, in pasture-grazed commercial dairy herds, this premilking teat disinfection routine reduces the incidence of new udder infections in cows in early lactation relative to no routine premilking teat disinfection or drying.

MATERIALS AND METHODS

Study Overview

A controlled trial was conducted in 2012, with cows within each of 5 pasture-based dairy herds allocated to premilking teat disinfection or no premilking teat disinfection or drying. Treatment continued in each herd for approximately 60 d (range of 59.5 to 61 d), commencing in each herd soon after the start of the herd's main or only calving period. Cows were monitored for clinical mastitis and new infections. This treatment period was selected to ensure that the trial was conducted in cows in early lactation, a period when clinical mastitis incidence was expected to be higher than later in lactation.

Herds, Cows, and Feeding Management

Herds were selected from those meeting all of the following criteria:

- (1) client of 1 of 3 veterinary practices: Maffra Veterinary Centre (Maffra, Victoria, Australia), The Veterinary Group (Timboon/Allansford, Victoria, Australia), or The University of Sydney Livestock Veterinary Teaching and Research Unit (Camden, Australia);
- (2) seasonal or split calving (all calvings occur within, respectively, 1 or 2 restricted time periods in each 12-mo period);
- (3) milked in a rotary parlor;
- (4) herd manager considered to be likely to fully implement the study protocol, and prepared to employ an additional 3 people for the study period and to have 2 of these people working in the parlor at all milkings throughout the study period;
- (5) herd manager prepared to allow some modification of the parlor: specifically, addition of Ambic Mastitis Detectors and installation of an Ambic PeraSpray unit (Ambic Equipment Ltd., Parkside, Witney, UK), a second teat-spraying unit at the cups-on position, and, where necessary, alteration of systems for hanging cups between cows to facilitate the implementation of special liner disinfection procedures before milking each treatment cow.

Preferably, herds would also have more than 550 cows in total, with over 75% of calvings in the study calving period occur in the first 6 wk, and have conducted at least 4 herd tests in the previous lactation. However, identifying herds meeting all selection criteria and preferences proved somewhat difficult, so not all herds enrolled satisfied all of these preferences.

Descriptors of study herds are shown in Table 1. Lactating cows in all herds grazed pasture and were fed supplements. In herds 1, 2, 4, and 5, lactating cows received supplementary concentrates in the parlor during milking and, in herd 1, supplementary fodder in the paddock. In herd 3, in addition to pasture, lactating cows were fed a silage and hay mix from a mixer wagon in a dedicated graveled feeding area between milkings, and a concentrate ration in the parlor during milking. The study commenced on July 2 (herd 1), August 20 (herd 2), September 3 (herd 3), August 6 (herd 4), and August 14 (herd 5), 2012. Total rainfalls from nearby weather stations combined for the 2 calendar months closest to the study midpoint dates (with long-term averages of combined totals for those 2 mo in parentheses) were 203 mm for herd 1 (211 mm), 68 mm for herd 2 (106 mm), 28 mm for herd 3 (123 mm), and 84 mm for herds 4 and 5 (97 mm). In herd 3, although rainfall during the study period was markedly less than average, the access track to the feeding area was muddy, and the entrance to the milking yard was also muddy during the study period. Both the author supervising this herd (J. Penry) and the milking staff observed some teat soiling during the study period. This is in contrast to the other 4 study herds, where teats were cleaner and drier than usual.

Allocation of Cows and Blinding

All cows that calved during the study calving periods and before the trial end date were enrolled, other than cows that calved before trial start date (herd 1) or more than 6 d before trial start date (herd 3). Cows were allocated to the premilking teat disinfection (treatment) group or no premilking teat disinfection (control) group based on odd and even cow identity numbers. This allocation method effectively resulted in blocking by herd. The data analyst (J. Morton) was blinded to treatment status of each cow until after analyses were completed. Cows in the premilking teat disinfection group were marked for the duration of the trial by paint on both hocks (plus tail tape in some herds) or by a broad paint mark on the back of their udders. Accordingly, only a very small chance existed of control cows being inadvertently treated or treatment cows failing to receive treatment due to poor identification.

Table 1. Descriptors of study herds in Australia used to determine whether premilking teat disinfection and drying reduces the incidence of clinical mastitis during early lactation in pasture-grazed herds by at least 50%

Descriptor	Herd				
	1	2	3	4	5
Region	Southwest Victoria	Macalister Irrigation Area, Victoria	Taree, New South Wales	Macalister Irrigation Area, Victoria	Macalister Irrigation Area, Victoria
Predominant breed	Holstein-Friesian/Jersey cross	Part Holstein-Friesian and part crossbred (predominantly Holstein-Friesian/Jersey)	Holstein-Friesian	Holstein-Friesian	Holstein-Friesian/Jersey/red breeds in a 3-way crossbreeding program
Calving system	Split calving	Seasonal calving	Split calving	Seasonal calving	Seasonal calving
No. of cows calved ¹	389	588	297	373	717
Milk yield ²					
Volume (L/cow per day)	19.8	26.7	33.8	25.6	23.2
Fat yield (kg/cow per day)	1.1	1.2	1.1	0.9	1.0
Protein yield (kg/cow per day)	0.7	0.9	1.1	0.8	0.8

¹During the study calving period.

²For study cows during the study period (when all study cows were in early lactation).

Treatments

Each cow received the same premilking preparation at all milkings during the trial period, depending on her allocated intervention. For treatment cows, with the operator wearing milking gloves, all teats were washed (regardless of degree of dirtiness) with low-pressure running water. Teats were sprayed before milking with a 0.1% available iodine disinfectant (DeLaval Spray & Dip RTU; DeLaval Australia Pty Ltd., Tullamarine, Victoria, Australia), with the average volume applied being about 20 mL/cow. After allowing a contact time of at least 30 s, treatment teats were dried with a disposable paper towel, 1 towel being used to dry all 4 teats. Teatcups were applied to each udder as per the normal cups-on routine for the herd.

Cows allocated to the control group and non-study lactating cows received no premilking teat disinfection or drying. Milking staff washed grossly dirty teats if they considered it necessary. Otherwise, teatcups were applied, regardless of degree of dirtiness of teat skin. This was the typical premilking procedure in all 5 study herds before the trial started.

Immediately after milking, teats of all lactating cows in each herd (i.e., treatment and control cows and non-study cows) were sprayed using either the same disinfectant as used for premilking disinfection or another registered iodophor-based teat disinfectant. Approximately 20 mL per cow was applied after each milking.

Teat Cup Flushing

Because at each milking, any treatment cow had a >50% chance of being milked with a cluster previously used to milk a control cow or a non-study cow, teatcups were flushed immediately before being applied to treatment cows, the purpose being to better simulate conditions as if all cows in the herd had received the same premilking disinfection treatment. Teatcups were flushed with 250 mg/L peracetic acid from an Ambic PeraSpray unit for 2 s per liner to achieve a minimum volume of 75 mL per cluster. Teatcups were not flushed before being applied to control cows.

Clinical Mastitis Detection

Herd managers were asked to record quarters meeting the clinical mastitis case definition (i.e., wateriness or clots that persist for more than 3 squirts of foremilk without or with heat, swelling, or pain in the udder). To increase the sensitivity of detection of clinical mastitis, an Ambic Vision Mastitis Detector (Ambic Equipment Ltd.) was installed in every long milk tube. The study design called for these filters to be checked

by the operator at the cups-off position at every milking for the duration of the trial. This person assessed each filter after each cow was milked for evidence of clots and recorded the identity of any suspect cow. Milk was stripped from each quarter of suspect cows at that time and the cups-off operator cleaned the filter with cold running water. If no milk changes were detected, this operator was asked to record the cow identity for rechecking by stripping milk from each quarter at the subsequent milking.

Individual Cow Cell Counts

For herds 1, 2, 4, and 5, individual cow cell counts (ICCC) were measured using centralized herd test SCC counting services with Bentley cell counters (Bentley Instruments Inc., Chaska, MN), with 24-h pooled milk samples collected from all cows in the herd at 2 consecutive milkings. In these herds, lactating cows were herd tested 21 to 29 d after trial start, again 21 to 26 d later, and again 13 to 21 d later (2 d before to 11 d after the 60-d trial ended). In herd 3, ICCC were obtained from SCC sensors [CellSense; Dairy Automation Ltd. (DAL), Hamilton, New Zealand]. These sensors were installed permanently in every fourth milking unit and sampled milk from individual cows milked in those units.

Milk Production Variables and Milking Characteristics

Milk production variables were measured in herds 1, 2, 4, and 5 from 24-h herd test results. In herd 3, milk production was measured using in-line volume, fat, and protein sensors (YieldSense; DAL). Results were not available for all cows at all milkings. Where results for the same cow were available for both the p.m. milking and subsequent a.m. milking, they were summed to estimate the 24-h milk production. Where results for the p.m. milking were available, the value for the subsequent a.m. milking was estimated as follows:

$$\begin{aligned} \text{a.m. milk volume} &= 1.31 \times \text{milk volume} \\ &\text{at previous p.m. milking;} \end{aligned}$$

$$\begin{aligned} \text{a.m. fat yield} &= 1.28 \times \text{fat yield} \\ &\text{at previous p.m. milking;} \end{aligned}$$

$$\begin{aligned} \text{a.m. protein yield} &= 1.30 \times \text{protein yield} \\ &\text{at previous p.m. milking.} \end{aligned}$$

These multipliers were the means of ratios of a.m.:p.m. results for the 4,231 paired data values from this herd

from August 16 to December 13, 2012, where both p.m. and the subsequent a.m. results were available from the same cow and the p.m. milk volume was more than 2 L. Where results for the a.m. milking were available, the values for the previous p.m. milking were estimated using the inverses of these ratios. Only milk production values for milkings from 11 d after trial start date to the trial end date were used in analyses. Of 2,821 cow-herd tests with milk volume that were analyzed from herd 3, 14% were based on p.m. and subsequent a.m. results, 43% were based on p.m. results, and 44% were based on a.m. results.

Average milk flow rate, milk yield, and duration of milking were measured for a single milking for a subset of cows in herd 5 using a Lactocorder (WMB AG, Balgach, Switzerland) inserted in the milk pathway between the milking cluster and milking line in one stall. Recordings were collected from all cows that were milked in the stall with the Lactocorder unit at 2 milkings (on different days).

Bacteriology

Milking staff in each herd were asked to collect 1 aseptic quarter milk sample from each quarter diagnosed with clinical mastitis. Samples were collected at the time of diagnosis, before any clinical mastitis treatment was administered. Cow identity number, date of collection, and quarter were recorded on the milk sample tube, and the sample was frozen pending routine bacteriological culture. Samples were submitted to either Gribbles Veterinary Pathology (Clayton, Victoria, Australia; herds 1, 2, 4, and 5) or the University of Sydney (Livestock Veterinary Services, The University of Sydney, Camden, New South Wales, Australia; herd 3) regularly during the study period for culture and identification of mastitis pathogens. Organisms were identified by visual examination of colonies (herds 1, 2, 4, and 5) or, for herd 3, as described by Shum et al. (2009), except that samples were plated only onto sheep blood agar.

Sample Size Calculations

Sample size calculations were based on effects on clinical mastitis incidence risk at the cow level for the first 30 d of each cow's lactation. Based on these calculations, we aimed to enroll herds so the total number of calvings in their study calving periods was about 2,500, thereby ensuring that at least 1,600 lactations (800 in each of the treated and control groups) were available for analysis of clinical mastitis at the cow level (i.e., were observed for at least the first 30 d of lactation). The statistical power of the study would then have been

almost 80% if the incidence risk of clinical mastitis in this period was 6% and the proportional reduction in clinical mastitis incidence due to premilking disinfection was 50%. Although clinical mastitis incidence rates rather than risks are reported here, these sample size calculations provided a valid basis for trial design.

Statistical Analyses

The cow was the unit of analysis, except where stated otherwise. All statistical analyses were performed using Stata software (version 12.1; StataCorp, College Station, TX).

Clinical Mastitis. Treatment cows that calved on or after the trial start date were first exposed to premilking disinfection when routine machine milking commenced (assumed to be 1 d after calving for all cows). We also assumed that any effects of premilking disinfection on new infection rate in treatment cows commenced immediately when teat disinfection was implemented, and that the typical incubation period for clinical mastitis after a new infection is 3 d (Milner et al., 1996). Accordingly, effects of premilking disinfection on clinical mastitis incidence were assessed by comparing incidences of clinical mastitis between treatment and control cows, considering cases occurring only from the fourth day after calving or, for cows calved before trial start date, from 3 d after trial start date, to the date that premilking disinfection ceased in the herd.

Incidence rates of clinical mastitis (considering only their first case for the lactation for cows having more than 1 case) were compared between treatment and control groups with Poisson regression of the binary cow-level variable: at least 1 clinical case in any quarter or no clinical cases, with treatment group, time from calving to first day at risk, and herd fitted as fixed effects, using the Stata poisson command, and after fitting herd as a random effect using the xtpoisson command. Each cow's time at risk was accounted for in these models. Times at risk described the number of days that each cow was at risk of having her first case of clinical mastitis for the lactation. For each cow, time at risk of clinical mastitis commenced on the fourth day after calving or, for cows calved before the trial start date, from 3 d after the trial start date. Cows with recorded clinical mastitis cases from 65 d before calving to the day before time at risk commenced were excluded from these analyses because the clinical duration of cases was not known and these cows may have still been clinically affected when the time at risk commenced. The time at risk ended on the date of the cow's first clinical mastitis case or, for unaffected cows, the earliest of dry-off date, date of culling or death,

or the date that premilking disinfection ceased in the herd. Robust standard errors were used when herd was fitted as a fixed effect. Interactions between treatment group and herd (as a fixed effect) were assessed using the joint Wald test. Effects of treatment group were also assessed after fitting cow age at calving to the nearest year as a categorical variable. For each regression coefficient, Euler's number (e) raised to the power of the coefficient was interpreted as an incidence rate ratio. Cumulative failure curves were generated using times to first clinical mastitis case or, for unaffected cows, times at risk, using the Stata `sts` graph command.

New Infection Rates. Individual cow cell counts in combination with clinical mastitis data were also used to identify new infections after modifying the method described by Penry and Mansell (2010). This modified method aimed to identify new infections at the cow level, considering only first infections for the lactation. New infections were considered to have occurred if cows presumed to be uninfected then had an ICC $>250,000$ cells/mL of milk or clinical mastitis.

For herds 2, 4, and 5, new infection rates were compared between treatment and control cows for 3 periods: from the last herd test in the previous lactation (or before calving for heifers) to the first herd test during the study period, from the herd's first to second herd test, and from the herd's second to third herd test. For each period, only cows presumed uninfected at the start of that period were used; cows were presumed uninfected only if they were heifers or all ICC in the previous lactation were $\leq 250,000$ cells/mL of milk, and any previous ICC in the current lactation were $\leq 250,000$ cells/mL of milk and they had no record of clinical mastitis up to 3 d after the start of the period. Herds 2, 4, and 5 had at least 4 herd tests and ICC in the previous lactation, and were used for these analyses. Herd 1 had no herd tests in the previous lactation and so was not used. Of the cows presumed uninfected at the start of the period, those whose ICC at the herd test at the end of the period was $>250,000$ cells/mL of milk or having their first clinical mastitis for the lactation during the period, or both, were classified as having become infected for the first time for that lactation during the period. As cell counts in uninfected cows are elevated early in the postpartum period (Reneau, 1986), only cows that calved more than 10 d before the first herd test were used for analyses of new infection rates between calving and first herd test. For the periods from the herd's first to second herd test, and from the herd's second to third herd test, only cows that calved more than 10 d before the period commencement date and with ICC recorded at the start of the period were included. Only cows with ICC recorded at the end of the period or clinical mastitis

during the period, or both, were included in analyses for that period. We also compared new infection rates over the total time period, using the same approach.

New infection date was assumed to be the earliest of (a) the midpoint of the interval in which ICC increased to above 250,000 cells/mL of milk and (b) 3 d before the clinical mastitis detection date. For the period from the last herd test in the previous lactation (or before calving for heifers) to the first herd test during the study period, each cow's time at risk was calculated as the time from calving date to the first herd test or, for cows becoming infected, assumed infection date. For the period from first to second herd test, each cow's time at risk was calculated as the time from first herd test to second herd test or, for cows becoming infected, assumed infection date. Each cow's time at risk for the period from second to third herd test was calculated in the corresponding way.

For herd 3, 24-h ICC [cell counts for 24-h cow-days (i.e., a p.m. milking and the a.m. milking on the following day)] were calculated as the weighted averages of the p.m. and a.m. cell counts, weighted by milk volumes at the respective milkings. Where cell counts and milk volumes were available for only the p.m. or a.m. milking, the 24-h ICC was estimated using the following regression equations:

$$\begin{aligned} 24\text{-h ICC} &= 0.9777987 \times \text{a.m. cell count} \\ &+ 8.611075 \quad (R^2 = 0.91); \end{aligned}$$

$$\begin{aligned} 24\text{-h ICC} &= 1.010917 \times \text{p.m. cell count} \\ &+ 4.346383 \quad (R^2 = 0.85). \end{aligned}$$

These equations were derived using data from 310 cow-days where cell counts and milk volumes were available for both the p.m. and subsequent a.m. milking. Geometric mean ICC were then calculated for each cow for rolling 10-d periods commencing on each day from 11 d after calving until 9 d before the trial end date, using all 24-h ICC within each 10-d period. Mean ICC were calculated only for 10-d periods when the cow had at least three 24-h ICC within the period. Each cow was classified as having become infected if either any geometric mean ICC was greater than 250,000 cells/mL of milk or if clinical mastitis was diagnosed on or between the fourth day after calving and the date of the cow's last 24-h ICC. Only study cows that calved 1 d before trial start date or later, with no clinical mastitis cases before the fourth day after calving, and with at least 1 geometric mean ICC were used for analysis of new infections. Times at risk were calculated for each cow as time from calving to the date of the last 24-h

ICCC within the trial period or, for cows that became infected, time from calving to the assumed date of infection. This was the earliest of 3 d before the cow's first clinical mastitis date for the lactation and if any geometric mean ICCC were above 250,000 cells/mL of milk, the midpoint of the earliest of such 10-d periods.

New infection rates were compared between treatment and control cows in herds 2, 4, and 5, pooled using Poisson regression with treatment group and herd fitted as fixed effects, using the Stata poisson command. Estimates were made after accounting for each cow's time at risk. Robust standard errors were used. Interactions between treatment group and herd were assessed using joint Wald tests. Effects of treatment group were also assessed after fitting cow age at calving to the nearest year as a categorical variable. New infection rates were compared within herd 3 in the same way but without fitting herd.

Individual cow cell counts were logarithmically (log base e) transformed before analyses. Linear and quadratic terms for time from calving to herd test were fitted for all ICCC analyses. Individual cow cell counts within 10 d after calving were excluded from all ICCC analyses. For herds 1, 2, 4, and 5, the cow-herd test was the unit of analysis. Logarithmically transformed ICCC were compared using linear regression, with treatment group and herd fitted as fixed effects and cow fitted as a random effect, using the Stata xtreg command. Maximum likelihood estimation was used. Interaction between treatment group and herd was assessed using the joint Wald test. The effect of treatment was also assessed after fitting cow age at calving to the nearest year as a categorical variable. Individual cow cell counts from herd 3 were analyzed separately from those from herds 1, 2, 4, and 5. For herd 3, the cow milking was the unit of analysis. The same approach as described above was used to compare ICCC between treatment and control groups, except that milking time (i.e., a.m. or p.m.) was fitted as a fixed effect, herd was not fitted, and no interactions were assessed. As ICCC were logarithmically (log base e) transformed before analyses, Euler's number raised to the power of each regression coefficient was interpreted as a ratio of geometric means. Individual cow cell count category ($\leq 250,000$ or $> 250,000$ cells/mL of milk) was analyzed similarly, using logistic regression with the Stata xtlogit command. The threshold of 250,000 cells/mL of milk was chosen based on previous research (Holdaway et. al., 1996); 250,000 cells/mL of milk is an industry standard threshold for interpreting ICCC in Australia (Brightling et. al., 2000). Interaction between treatment group and herd was assessed using the likelihood ratio test.

Milk Production. Milk production variables were compared with the cow-herd test record as the unit of analysis using linear regression with treatment group, herd, and linear and quadratic terms for time from calving fitted as fixed effects, and cow fitted as a random effect. The Stata xtreg command was used. Interactions between treatment group and herd were assessed using joint Wald tests. Effects of treatment group were also assessed after fitting age as a categorical variable. Analyses were performed with and without inclusion of herd 3.

Milking Characteristics. Average milk flow rates per cow and durations of milking were compared between treatment and control groups using linear regression. Homoscedasticity and normality of residuals were assessed by inspection of scatter plots of residuals versus fitted values and histograms of residuals, respectively.

RESULTS

Clinical Mastitis

In total, 1,110 and 1,104 cows were enrolled in treatment and control groups, respectively. Of these, 81 (7.3%) and 79 (7.2%), respectively, were lost to follow-up or excluded from assessment of effects of the premilking teat disinfection regimen on clinical mastitis incidence because they had clinical mastitis between 65 d before calving and their first day at risk; they contributed no time at risk because they calved less than 4 d before trial end date or were dried-off, culled, or died before their time at risk commenced; or because 2 calving dates were recorded. Proportions lost to follow-up or excluded by reason were similar in treatment and control groups. Thus, 1,029 and 1,025 cows from treatment and control groups, respectively, were available to assess effects of premilking teat disinfection on clinical mastitis.

Incidence rates of clinical mastitis were calculated by herd and treatment group. These incidence rates are described as the number of cows having at least 1 case of clinical mastitis per 100 cow-months of time at risk; this was equivalent to the number of cows having at least 1 case of clinical mastitis per 3,040 cow-days of time at risk, as we assumed 30.4 d per month. Individual treatment and control cows contributed between 1 and 59 cow-days; mean and median for treatment cows were 38 and 41 cow-days, and for control cows were 36 and 38 cow-days. For the treatment and control groups, 59% (22,701/38,615) and 61% (22,565/37,095) of the cow-days at risk, respectively, were from the first 30 d of the study cows' lactations. Incidence rates of clinical mastitis by herd and treatment group are shown in

Table 2. Incidence rates of clinical mastitis by herd and treatment group

Herd	Group	No. of cows contributing any time at risk	Total time at risk (cow-days) ¹	No. cows having at least 1 case of clinical mastitis ²	Incidence rate ³	Incidence rate ratio (95% CI) ⁴	P-value
1	Treatment	156	3,521	44	38.0	1.25 (0.77 to 2.02)	0.37
1	Control	170	3,995	40	30.4	Ref. group	
2	Treatment	264	10,083	22	6.6	1.00 (0.55 to 1.82)	1.000
2	Control	263	10,024	22	6.7	Ref. group	
3	Treatment	88	3,022	10	10.1	0.34 (0.15 to 0.75)	0.008
3	Control	71	1,958	19	29.5	Ref. group	
4	Treatment	171	7,053	10	4.3	1.32 (0.52 to 3.36)	0.56
4	Control	182	7,162	8	3.4	Ref. group	
5	Treatment	350	14,936	12	2.4	1.42 (0.58 to 3.50)	0.44
5	Control	339	13,956	8	1.7	Ref. group	
Pooled	Treatment	1,029	38,615	98	7.7	0.99 (0.73 to 1.35)	0.97
Pooled	Control	1,025	37,095	97	7.9	Ref. group	

¹Time at risk of clinical mastitis commenced on the fourth d after calving or, for cows calved before the trial start date, from 3 d after the trial start date. Time at risk ended on the date of the cow's first clinical mastitis case or, for unaffected cows, the earliest of dry-off date, date of culling or death, or the date that premilking disinfection ceased in the herd.

²Number of cows that had at least 1 clinical mastitis case while at risk.

³Number of cows that had at least 1 clinical mastitis case while at risk per 100 cow-months (i.e., per 3,040 cow-days) at risk.

⁴Estimated ratio of incidence rate for treatment group to that in the control group [reference (Ref.) group], with herd and time from calving to first day at risk fitted as fixed effects; ratios for individual herds were estimated after fitting treatment by herd interactions.

Table 2. Incidence rates of clinical mastitis were generally similar in treatment and control cows, except in herd 3, where a markedly lower incidence rate was observed in treatment cows. Across all herds pooled, after accounting for effects of herd and time from calving to first day at risk, the incidence rate in the treatment group was virtually identical to that in the control group (incidence rate ratio = 0.99). The lower limit of the 95% confidence interval was 0.73. Thus, this result is inconsistent with the hypothesis that, across all pasture-grazed dairy herds, this premilking disinfection reduces incidence rate of clinical mastitis by 0.50 (i.e., 50%) or more. Results were similar after fitting herd as a random effect and after adjusting for cow age (results not shown).

The *P*-value for the interaction between treatment group and herd was moderately low (0.068), providing support for the hypothesis that the effect of this premilking treatment on hazard of clinical mastitis varies by herd. In herd 3, the clinical mastitis incidence rate was markedly lower in treatment cows compared with control cows (incidence rate ratio = 0.34; 95% CI = 0.15 to 0.75; *P* = 0.008). In contrast, for herds 1, 2, 4, and 5 pooled, the incidence rate ratio was 1.20 (95% CI = 0.87 to 1.67; *P* = 0.27).

The striking difference in response to premilking treatment in herd 3 relative to herds 1, 2, 4, and 5 (pooled data) is evident in the cumulative failure curves shown in Figure 1.

Bacteriological Results from Clinical Mastitis Cases

Bacteriological results from milk samples from clinical mastitis cases collected during the study period

indicated that *Strep. uberis* was the most common mastitis pathogen isolated (pooled across all herds: 47%, ranging from 20% in herd 3 to 97% in herd 4; Table 3). Substantial proportions of samples yielded no growth (pooled across all herds: 23%). *Streptococcus agalactiae* was not isolated from any samples, and *Streptococcus dysgalactiae* was isolated from just 1 sample in 1 herd.

New Infections

Incidence rates of new infections by herd and treatment group for herds 2, 4, and 5 are shown in Table 4. These rates estimate the frequency of new infections at the cow level, considering only first infections for the lactation. Incidence rates did not differ significantly between treatment and control groups for the first period, or for all 3 periods pooled (*P*-values for interactions were 0.79 and 0.26, respectively). For each of the second and third periods, models with interaction terms were unstable due to zero incidences in some herd-group combinations. Lower limits of 95% confidence intervals for incidence rate ratios were 0.24 to 0.56. Results were similar after adjusting for cow age (results not shown).

For herd 3, new infections were analyzed using 77 treatment cows and 49 control cows. [Only study cows that calved 1 d before the trial start date or later, with no clinical mastitis cases before the fourth day after calving, and with at least 1 geometric mean ICCC were used. In total, 95 treatment cows and 72 control cows calved 1 d before trial start date or later. Of these, 3 (3%) and 10 (14%) cows, respectively, were excluded, as they had clinical mastitis before the fourth day after calving and a further 15 (16%) and 13 (18%), respec-

Figure 1. Cumulative failure curves for first cases of clinical mastitis for cows (a) in herd 3 and (b) in herds 1, 2, 4, and 5 pooled for control cows (solid line) and treatment cows (dashed line).

tively, were excluded, as they had no geometric mean ICCC.] For the included cows, incidence rates of new infections were 20.5 per 100 cow-months at risk (18/2,665 cow-days at risk) and 30.2 per 100 cow-months at risk (16/1,613 cow-days at risk) in treatment and control cows, respectively. After adjustment for cow age, some evidence existed that new infection incidence rates were lower in treated cows (incidence rate ratio for treated cows relative to control cows = 0.42; 95% CI = 0.17 to 1.08; $P = 0.072$).

ICCC

For herds 1, 2, 4, and 5 pooled, geometric mean ICCC were similar in treatment and control groups

(48,000 and 51,000 cells/mL of milk, respectively; ratio of treatment mean to control mean after adjusting for herd and calving to herd test interval = 1.00; 95% CI = 0.90 to 1.11; $P = 0.94$). The P -value for the interaction between treatment group and herd was high ($P = 0.61$). Individual cow cell counts from herd 3 were analyzed separately from those from herds 1, 2, 4, and 5. For herd 3, geometric mean ICCC were also similar in treatment and control groups (45,000 and 43,000 cells/mL of milk, respectively; ratio of treatment mean to control mean after adjusting for calving to herd test interval = 1.00; 95% CI = 0.76 to 1.31; $P > 0.99$). Estimated effects were similar when adjusted for cow age (results not shown). Results were similar for effects of treatment on ICCC category ($\leq 250,000$ or $> 250,000$ cells/mL of milk; details not shown).

Milk Production

Daily milk volume, fat or protein yield, or fat or protein concentration did not differ significantly between treatment and control groups ($P \geq 0.095$), and estimated differences were small, with relatively narrow confidence intervals. Using data from all 5 herds, estimated differences (treatment minus control) were daily milk volume (-0.2 L/cow; 95% CI = -0.6 to 0.3 L/cow), daily fat yield (-0.01 kg/cow; 95% CI = -0.03 to 0.01 kg/cow), daily protein yield (-0.01 kg/cow; 95% CI = -0.02 to 0.01 kg/cow), fat concentration (-0.01 g/100 mL of milk; 95% CI = -0.06 to 0.03 g/100 mL of milk), and protein concentration (0.00 g/100 mL of milk; 95% CI = -0.02 to 0.02 g/100 mL of milk). Results were similar after adjusting for cow age (results not shown). No evidence existed for interaction between treatment group and herd for any of the 5 milk production variables whether all 5 herds were analyzed or after excluding herd 3 (P -values for interactions were 0.23 to 0.96).

Milking Characteristics

Average milk flow rate (calculated for each cow as milk volume per milking/milking time) was 0.53 L/min greater for treatment cows compared with the control cows (95% CI = -0.02 to 1.08 L/min; $P = 0.058$), and milking time per cow was 1.06 min quicker for treatment cows (95% CI = 0.0 to 2.2 min; $P = 0.056$).

DISCUSSION

Based on these results, we conclude that under conditions such as those experienced in the pasture-grazed commercial study herds, this premilking treatment does not reduce clinical mastitis incidence, on average,

Table 3. Percentages (no.) of samples from clinical mastitis cases by bacteriological result within herd^{1,2}

Result	Herd					Pooled
	1	2	3	4	5	
Organism						
<i>Streptococcus uberis</i>	25 (2)	41 (22)	20 (11)	97 (28)	66 (19)	47 (82)
<i>Strep. uberis</i> and <i>Staphylococcus aureus</i>		2 (1)				1 (1)
<i>Strep. uberis</i> and CNS			9 (5)			3 (5)
<i>Staph. aureus</i>	13 (1)	9 (5)	2 (1)			4 (7)
CNS			9 (5)			3 (5)
CNS and environmental flora			4 (2)			1 (2)
CNS and <i>Streptococcus dysgalactiae</i>			2 (1)			1 (1)
<i>Corynebacterium bovis</i>			13 (7)			4 (7)
<i>Streptococcus</i> spp.		4 (2)				1 (2)
Environmental, skin, and enteric flora	25 (2)	15 (8)	21 (12)		7 (2)	14 (24)
No growth	38 (3)	30 (16)	21 (12)	3 (1)	28 (8)	23 (40)
Total	100 (8)	100 (54)	100 (56)	100 (29)	100 (29)	100 (176)

¹Only samples collected from clinical mastitis cases diagnosed by the milking staff during the trial period in cows enrolled in the study were included.

²Numbers of cases differed from the numbers of cows affected as reported in Table 2, as bacteriological results from cases in cows excluded from those analyses and repeat cases within the same cow were included in results reported in this table, and not all clinical mastitis cases underwent bacteriological examination.

across all herds by more than 50%, but that clinical mastitis incidence might be reduced substantially in particular circumstances. The finding that clinical mastitis incidence was markedly lower in treatment cows compared with control cows in herd 3, but not in the 4 other study herds, raises the question: “What were the important differences between herd 3 and the other 4 herds that might have contributed to such an outcome”?

In herd 3, some teat soiling was observed during the study period. This is in contrast to the other 4 study herds, where the teats were cleaner and drier than usual. Based on our observations of clean dry teats throughout the study period, we hypothesized (before results were available) that responses to this premilking treatment were unlikely in these 4 herds.

It is possible that responses to better premilking hygiene are more likely on farms where calving pads or feed pads are used, especially for high-producing herds such as herd 3. (The average yield for this herd was 34 L/cow per day, compared with 20 to 27 L/cow per day in the other 4 herds.) These conditions may be similar to those in studies in the United States where lower clinical mastitis incidences have been reported due to premilking teat disinfection (Oliver et. al., 1993a; Oliver et. al., 2001). *Streptococcus uberis* was commonly isolated in herd 3 and this herd also had the highest proportion of samples with other bacteria, including environmental organisms, skin, and enteric flora (apart from herd 1, for which very few clinical case samples were submitted for bacteriological culture), CNS, and *Corynebacterium bovis*. High prevalences of *C. bovis* are more likely when postmilking teat disinfection is not

practiced (Brooks et al., 1983), and it is possible that efficacy of the premilking treatment assessed in the current study is higher where postmilking teat disinfection is less effective.

Another, possibly important, difference in milking conditions existed for herd 3 relative to the other study herds. On the rotary milking platform in herd 3, milking clusters were prone to entangling in the cows' legs near the end of milking, possibly resulting in a higher frequency of cows kicking or treading on the cluster to aid its sudden removal. Sudden cluster removal, especially near the end of milking, is known to increase mastitis infection risk via the so-called “impact mechanism” (Mein, 2012). It seems plausible that premilking disinfection may be more effective when impacts are more common. In the absence of premilking teat disinfection, a higher concentration of potentially pathogenic organisms may be propelled into the teat canal, whereas impacts may be of lesser consequence if the teat and teat orifice had been reasonably clean at the start of milking.

These results show that not all herds with high clinical mastitis incidence will respond to this premilking treatment. Across the control cows, those in herd 3 had the highest clinical mastitis incidence, but control cows in herd 1 also had a very high incidence, yet no evidence existed of response in that herd. Further research is required to define circumstances when this premilking teat disinfection routine is economically justified under Australian conditions. Our estimate of the required reduction in the incidence of clinical mastitis in early lactation by at least 50%, relative to no premilking udder preparation, did not include ad-

Table 4. New infection incidence rates among presumed previously uninfected cows by herd and treatment group

Herd	Group	No. of cows included ¹	Total time at risk (cow-days)	No. of cows becoming infected during period ²	Incidence rate (cows newly infected per 100 cow-months at risk) ³	Incidence rate ratio ⁴ (95% CI)	P-value
Last herd test in previous lactation (or before calving for heifers) to first herd test during study period							
2	Treatment	82	1,712	8	14.2	0.83 (0.50–1.38) Ref. group	0.47
2	Control	86	1,684	11	19.9		
4	Treatment	72	2,008	9	13.6		
4	Control	71	1,804.5	11	18.5		
5	Treatment	161	4,142.5	12	8.8		
5	Control	147	3,627.5	10	8.4		
Pooled	Treatment	315	7,862.5	29	11.2	0.83 (0.50–1.38) Ref. group	0.47
Pooled	Control	304	7,116	32	13.7		
First to second herd test							
2	Treatment	82	2,132	0	0.0	0.79 (0.24–2.57) Ref. group	0.70
2	Control	94	2,418	2	2.5		
4	Treatment	67	1,463	1	2.1		
4	Control	67	1,474	0	0.0		
5	Treatment	169	3,507	4	3.5		
5	Control	153	3,171	4	3.8		
Pooled	Treatment	318	7,102	5	2.1		
Pooled	Control	314	7,063	6	2.6		
Second to third herd test							
2	Treatment	121	2,541	0	0.0	0.81 (0.34–1.94) Ref. group	0.64
2	Control	132	2,719.5	5	5.6		
4	Treatment	78	1,001	2	6.1		
4	Control	83	1,066	2	5.7		
5	Treatment	227	3,129	7	6.8		
5	Control	212	2,940	4	4.1		
Pooled	Treatment	426	6,671	9	4.1		
Pooled	Control	427	6,725.5	11	5.0		
All 3 periods pooled							
2	Treatment	137	6,385	8	3.8	0.85 (0.56–1.29) Ref. group	0.44
2	Control	151	6,821.5	18	8.0		
4	Treatment	88	4,472	12	8.2		
4	Control	95	4,344.5	13	9.1		
5	Treatment	245	10,778.5	23	6.5		
5	Control	229	9,738.5	18	5.6		
Pooled	Treatment	470	21,635.5	43	6.0		
Pooled	Control	475	20,904.5	49	7.1		

¹For each period, cows were presumed uninfected only if they were heifers or all individual cow cell counts (ICCC) in the previous lactation were $\leq 250,000$ cells/mL of milk, and any previous ICCC in the current lactation were $\leq 250,000$ cells/mL of milk and they had no record of clinical mastitis up to 3 d after the start of the period. Many cows contributed to more than 1 period so the numbers of cows used for all 3 periods pooled are less than the sums of cows from each period.

²Cows whose ICCC at the herd test at the end of the period was $> 250,000$ cells/mL of milk or having their first clinical mastitis for the lactation during the period were classified as having become infected for the first time for that lactation during the period.

³Calculated as number of cows becoming infected per 3,040 cow-days at risk.

⁴Estimated ratio of incidence rate for treatment group relative to that in the control group [reference (Ref.) group], with herd fitted as a fixed effect.

ditional benefits of reduced milking times or any reductions in new infection rates. These should be included in future considerations of the economics of premilking teat disinfection under Australian conditions. Although the assessment of effects of treatment on milking times was a minor part of our study, performed in only 1 herd, our result—that milking times are reduced—is consistent with many previous studies (e.g., Sagi et al., 1980; Merrill et al., 1987; Rasmussen et al., 1992).

As milking staff handled teats of treatment cows more than control cows, it was possible that diagnostic sensitivity of clinical mastitis detection was higher among treatment cows, thereby biasing the study results. However, any important bias for this reason was unlikely. When applying the premilking treatment, milking staff generally only touched the teats of treated cows and spent little time touching or palpating the udder. Further, to maximize the sensitivity of clinical mastitis detection, in-line filters were installed and checked by the cups-off operator at every milking for the duration of the trial. In addition, the average duration of clinical mastitis cases in the absence of treatment is several days (Chamings 1984), so the probability of detection of each case before clinical signs subside spontaneously is likely to have been high (Morton et al., 2012).

Although premilking disinfection markedly reduced clinical mastitis incidence in herd 3, the point estimate for the reduction in new infection rate was closer to 1 (i.e., closer to no effect; rate ratio 0.42 versus 0.34 for clinical mastitis incidence). Although this may reflect different effects of premilking disinfection on new infection rate and clinical mastitis incidence, it may also be due to bias toward no effect due to nondifferential misclassification because of false-positive and false-negative classification errors when identifying new infections. The sensitivity and specificity of the method used in the current study for identifying new infections in herd 3 have not been reported. However, using an ICC threshold of 250,000 cells/mL of milk, sensitivity of detection of new infections between ICC 26 to 30 d apart has been estimated as only 0.273 and specificity as 0.995 (Dohoo and Leslie 1991). At lower thresholds, sensitivity estimates were higher but specificity estimates less. Further, some apparent new infections in herd 3 may, in fact, have been infections that were present during the previous lactation or that occurred during the dry period.

CONCLUSIONS

We conclude that under conditions such as those experienced in the pasture-grazed commercial study herds, this premilking treatment does not reduce clinical mastitis incidence, on average, across all herds by more

than 50%, but that clinical mastitis incidence might be reduced substantially in particular circumstances. Routine application of this premilking treatment is unlikely to produce a worthwhile (economic) reduction in the number of clinical cases when teats are relatively clean and dry and the clinical incidence is low. However, it may be worthwhile during periods when teats are heavily soiled and the incidence of clinical mastitis due to environmental pathogens is high. From other studies and biological evidence, this premilking treatment may also be worthwhile when the incidence of clinical mastitis due to environmental pathogens is high: (a) in herds using calving or feed pads, especially high-producing herds (possibly due to changes in mastitis pathogen profile), and (b) when teat skin condition, especially teat-end condition, is poor.

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