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Efficacy of a novel internal dry period teat sealant containing 0.5% chlorhexidine against experimental challenge with *Streptococcus uberis* in dairy cattle

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ABSTRACT

The incidence of clinical mastitis and infection status at calving was assessed in quarters treated with 1 of 2 internal teat sealants at the time of dry off. Two contralateral quarters per cow (n = 63 cows) were treated with a sealant that contained 0.5% chlorhexidine; the other quarters were treated with a commercial teat sealant. Ten cows were untreated (controls). On d 2, 4, and 16 after dry off, cows were challenged with *Streptococcus uberis S210* strain. Cows were examined daily for 34 d after drying off and cases of clinical mastitis were recorded. Milk samples were collected for culture from any quarters that developed clinical mastitis during the first 34 d after drying-off and from all quarters on d −5 and 0 relative to treatment and at the first and twentieth milking after calving. The incidence of clinical mastitis during the examination period was lower in treated quarters (n = 7/252; 1.5%; lower incidence for those treated with chlorhexidine-containing teat sealant n = 3/126; 1.2%) than in untreated quarters (n = 13/40; 26.8%). The protection against intramammary infection after calving, adjusted for the effect of cow, was higher in quarters treated with the novel teat sealant (89/105; 15.2%; 95% CI = 9.6–23.4) than in those treated with the commercial teat sealant (71/104; 31.7%; 95% CI = 23.5–41.3) and untreated controls (6/28; 78.6%; 95% CI = 59.8–90.0), respectively. Quarters treated with teat sealants were less likely to have an intramammary infection after calving and had a lower incidence of clinical mastitis during the early dry period than did untreated controls in this challenge study.

Key words: internal teat sealant, challenge, dry period, *Streptococcus uberis*

INTRODUCTION

Principles that prevent IMI during the dry period are minimizing bacterial challenge from the environment and maximizing and supplementing the defense mechanisms of the mammary gland (Bradley and Green, 2004). Antibiotic dry cow therapy (**DCT**) is a means of preventing new infections during the dry period and of eliminating existing subclinical infections. Treatment with antimicrobials at drying off risks the development of resistant strains of bacteria and violative antibacterial residues in milk after calving. To avoid these risks, artificial teat sealants were developed to prevent new IMI (Meaney, 1977; Woolford et al., 1998). Teats which become closed by the keratin plug or an artificial seal after drying off are less likely to become infected in the dry period (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002). The barrier formed by a sealant occurs faster than is so without treatment, thus decreasing the entry of mastitis-causing organisms into the gland while a keratin plug forms.

New Zealand's pasture-based seasonal dairy system is associated with some specific problems for the management of the dry period. The length of the dry period is variable and in many cases cows are dried off as dictated by pasture growth and feed availability. The rate of new IMI is related to the length of the dry period. Longer dry periods have been associated with an increase in the incidence of new IMI (Natzke et al., 1975; Rindsig et al., 1978; Bradley and Green, 2004; Berry and Hillerton, 2007; Laven, 2008). This may relate to the duration of action of the DCT as the concentration of antibiotic falls and the protective role against new infection challenge is diminished (Bradley and Green, 2000; Sanford et al., 2006; Berry and Hillerton, 2007). The efficacy of internal teat sealants appears unaffected by the length of the dry period when used alone or in combination with DCT (Woolford et al., 1998; Huxley et al., 2002; Berry and Hillerton, 2007). Because the prediction of calving date in New Zealand is often not reliable and the infection status of cows is unknown,

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the best mastitis protection is expected from a combined use of DCT and internal teat sealant (Bradley and Green, 2004). For known uninfected quarters the use of internal teat sealant alone has been advocated (Woolford et al., 1998; Bradley and Green, 2004).

The use of internal teat sealants presents the risk of introducing new IMI during their administration. This risk could potentially be decreased if an antimicrobial compound were incorporated into the sealant (Ryan et al., 1998; Crispie et al., 2004a) if it possesses a suitable spectrum of activity.

This study compared the efficacy of a teat sealant containing chlorhexidine with a commercial teat sealant not containing an antimicrobial agent and with untreated controls. Treatments were administered at drying off to healthy dairy cows, which were subsequently challenged with a known strain of *Streptococcus uberis*. Chlorhexidine was used because of its activity against most gram-positive bacteria of importance in New Zealand and other infectious organisms, including some gram-negative bacteria when it is at higher concentrations (Heit and Riviere, 2009). The null hypothesis tested was that chlorhexidine-containing teat sealant would not affect the incidence of clinical mastitis in the dry period or the prevalence of IMI after calving.

MATERIALS AND METHODS

This study was approved by the Kaiawhina Animal Ethics Committee (AEC 005/09).

Animals

Seventy-three cows less than 8 yr old from Massey University Agricultural Farm Services Dairy Number 4 (Palmerston North, New Zealand) with negative California Mastitis Test (CMT) and <200,000 cells/mL 9 d before drying off were used in the present study. The experimental unit was the quarter. Sixty-three cows were allocated as treatment cows (treated group) and 10 were untreated controls (untreated group). Treated cows had a front and a contralateral rear quarter treated with the novel chlorhexidine-containing teat sealant and the remaining 2 quarters treated with a commercial teat sealant. The treatment was alternated between the cows. Cows were randomized on SCC using the block randomization seed option of GenStat software (version 9.1; VSN International, Hemel Hempstead, UK). Five cows failed to complete the study due to abortion (1; untreated), traumatic injury resulting in death (1; treated), clinical milk fever resulting in death (1; treated), and being culled as nonpregnant (2; treated) leaving data from 68 cows for analysis of IMI status at calving.

Treatment Products and Treatment Administration

Two treatment products were used in this study: Bomac ATS, containing bismuth subnitrate 65% and chlorhexidine 0.5% (Bomac Laboratories Ltd., Auckland, New Zealand), referred to as chlorhexidine-containing teat sealant; and Teatseal, containing 65% bismuth subnitrate (Pfizer Animal Health, Auckland) as a positive control, referred to as commercial teat sealant.

Treatments were administered within 2 h after the last milking for the 2008–2009 season using the partial insertion technique. Before treatment, teats of all cows (including untreated controls) were cleaned and disinfected with alcohol-based teat wipes (Bomac Teat wipes, Bomac Laboratories Ltd.). No massage of the teats was performed after treatment administration but the teats of all cows (including untreated controls) were sprayed with an iodine-based teat spray (TeatGuard Plus, Ecolab Ltd., Hamilton, New Zealand) following label recommendations.

Procedures

Duplicate quarter milk samples were collected aseptically 5 d before drying off, on the day of drying off and at the first and twentieth milking after calving. All milk samples were cultured following the National Mastitis Council Guidelines (Hogan et al., 1999) at the Microbiology Laboratory of the Institute of Veterinary, Animal and Biomedical Sciences (IVABS), Massey University (Palmerston North, New Zealand).

All cows were challenged by dipping the teat barrel in the challenge broth for 1 to 2 s by a single person blinded to treatment. The concentration in the broth of colony-forming units of a *Strep. uberis* S210 strain on d 2, 4, and 16 after treatment is shown in Table 1. Challenges were performed in different facilities from the normal milking shed to avoid the milk let-down reflex. Separate containers were used to dip the left front and right rear quarters from those used for the other 2 quarters. In this way, no possibility of cross-contamination existed between different products and the blinding of the trial was not compromised. Each cow was dipped with 2 new challenge broths. A new broth was prepared for each day of the challenge.

The challenge broth was prepared by thawing the isolate, streaking onto blood agar plates (Fort Richard Laboratories Ltd., Auckland, New Zealand), incubation at 37 ± 2°C under CO_2-enriched conditions, harvesting colonies from the plates using cotton swabs (Fort Richard Laboratories Ltd.), and suspending them in normal saline (0.9% wt/vol NaCl). The turbidity was adjusted to a McFarland turbidity standard of 0.5 (Remel, Lenexa, KS) by adding normal saline.

Table 1. The concentration of colony-forming units of a *Streptococcs uberis* S210 strain per milliliter in the challenge broth at different challenge days

After treatment (d)	Concentration (cfu/mL)
2	7.7×10^8
4	5.4×10^7
16	2.3×10^7

Udders were visually examined and palpated daily until 34 d after drying off, with the exception of d 1 and 3, by a person blinded to treatment allocation. This was the defined palpation period. Quarters were observed and palpated for the presence of clinical signs consistent with mastitis. Each quarter was subjectively judged as mastitic (score $\geq$3) or nonmastitic (score $\leq$2) according to standardized criteria (Table 2). All examinations were performed by a single veterinarian blinded to treatment.

Quarters affected by clinical mastitis were sampled for microbial culture before treatment was administered to them. Microbial culture was performed by spreading 10 μL of milk from a single quarter onto a quarter of a 5% sheep blood agar plate, which was incubated for up to 72 h at 35 to 37°C in aerobic conditions (Hogan et al., 1999). Any mastitic quarters were then treated according to the clinical presentation. For the treatment of affected quarters, Ubro Yellow (Boehringer Ingelheim NZ, Auckland, New Zealand; containing penethamathe hydroiodide, dihydrostreptomycin, framicetin, and prednisolone) was administered once daily for 3 d after complete milking-out of the affected quarters. This treatment regimen was used when up to 3 quarters in the same cow were affected. When 4 quarters were affected in the same cow, Mamyzin (Boehringer Ingelheim NZ; containing penethamathe hydroiodide) was administered i.m. once daily with 10 g on the first day and 5 g on the 2 subsequent days. Thereafter, quarters were observed daily and treated as required, but any subsequent episode of mastitis in the same quarter was not included in the statistical analysis.

The incidence of clinical mastitis during the palpation period was calculated as the proportion of quarters affected by clinical mastitis from the total number of quarters in a group. The incidence of clinical mastitis caused by the challenge organism during the palpation period was calculated as the percentage of quarters affected by clinical mastitis caused by *Strep. uberis* from the total number of quarters in a group. Whenever *Strep. uberis* was isolated, it was assumed it was the challenge strain. Previous work by this group using a highly discriminative method (pulsed-field gel electrophoresis) confirmed that all clinical cases during the dry period caused by *Strep. uberis* were identical to the challenge strain (data on file).

Results from culture were assessed according to guidelines from the National Mastitis Council (Hogan et al., 1999): (a) Growth of 3 or more colony types on a quadrant was reported as a contaminated sample, (b) 1 or 2 colonies growing on the quadrant was reported as an uninfected sample, and (c) more than 2 colonies, but less than 3 colony types growing on the quadrant was reported as an infected sample and the predominant colony type was isolated and identified.

Identification of the cultured isolates was performed by an assessment of colony morphology, Gram stain reaction, and several biochemical tests. Bacilli were classified as gram-positive or gram-negative bacilli, with no further testing. *Corynebacterium bovis* isolates were identified based on their cultural characteristics and colony morphology. Gram-positive, catalase-positive organisms were categorized as either coagulase-positive staphylococci or CNS, based on the results of a tube rabbit plasma coagulase test (Remel). Gram-positive and catalase-negative organisms were further tested for their ability to hydrolyze esculin (Fort Dodge, Auckland, New Zealand). The esculin-negative organisms were subjected to the Christie–Atkins–Munch-Petersen (**CAMP**; Fort Dodge) reaction and categorized as either CAMP-positive or CAMP-negative bacteria. Nonhemolytic, esculin-positive, gram-positive cocci were further tested for their ability to grow in buffered azide glucose glycerol broth (BAGG; Fort Dodge) and

Table 2. Quarter and teat examination scores and description

Score	Description
0	No evidence of irritation, soreness, redness, or swelling of the quarter. No stripping of quarters.
1	No or virtually no evidence of irritation, soreness, redness, or very slight swelling of the quarter. No stripping of quarters.
2	Evidence of irritation or soreness of a minor intensity or slight redness or swelling, likely to originate from residual milk in the quarter. No stripping of quarters.
3	Evidence of irritation or soreness of a moderate intensity or moderate redness or swelling of the quarter. Secretion contains small clots and flecks.
4	Evidence of irritation or soreness of a severe intensity or severe redness (beet redness) or severe swelling of the quarter. The secretion contains large clots and flecks.
5	Evidence of severe irritation or soreness, severe swelling or redness, associated with a generally sick animal. The secretion contains large clots and flecks.

fermented inulin (Fort Dodge). The BAGG-positive inulin fermenters were identified as *Strep. uberis*. All of the other gram-positive, catalase-negative cocci were identified as *Streptococcus* spp.

The presence of an IMI in any quarter after calving was determined based on an assessment of the culture results. A gland was defined as being infected if growth of ≥3 colony forming units per quadrant of any of the major mastitis-causing organisms (i.e. coagulase-positive staphylococci, streptococci, and gram-negative rods) was present at a sampling and if a minor or uncommon or any combination of mastitis-causing organisms was isolated at both samplings after calving. The prevalence of infection after calving was evaluated independently of the infection status pre-drying off and the incidence of clinical mastitis during the palpation period. Any quarters with contaminated samples or missing samples after calving were excluded from analysis unless they were a sample containing a major mastitis-causing organism.

Statistical Analysis

All analyses were undertaken using SAS version 9.1 (SAS Institute, 2003).

Statistical differences between the cumulative percentages of quarters becoming infected in the treatment groups (chlorhexidine-containing teat sealant, commercial teat sealant, and untreated controls) were analyzed using survival analysis using the SAS LIFETEST procedure.

Udder palpation scores for each quarter were analyzed as categorical data using the Fisher exact test with respect to treatment (chlorhexidine-containing teat sealant, commercial teat sealant, and untreated controls).

Records of clinical mastitis for each quarter during the 34-d palpation period were analyzed using the SAS GLIMMIX procedure with a logistic regression model that included the fixed effect of treatment (chlorhexidine-containing teat sealant, commercial teat sealant, and untreated controls) and the random effect of a cow. The variable had a binomial distribution and analyses were performed after the logit-transformation. Least squares means of incidence of clinical mastitis and their 95% confidence intervals were obtained and back-transformed to the binomial scale. The same procedure was applied to compare the incidence of clinical mastitis in the treated versus untreated quarters during the palpation period.

The success in preventing IMI measured as presence or absence of infection after calving was analyzed with the GLIMMIX procedure. The logistic regression model included the fixed effect of treatment (chlorhexidine-containing teat sealant, commercial teat sealant, and untreated controls) and random effect of a cow. Least squares means and 95% confidence intervals for IMI after calving for each treatment were back-transformed and are presented as means and 95% confidence intervals. The effect of the length of the dry period on the success of the prevention of IMI after calving was not significant (Table 3). Hence, it was not included in the final model for estimation of the success of the prevention of IMI after calving. The prevalence of infected (positive on culture) quarters per group was also analyzed using the GLIMMIX procedure, including the fixed effects of treatment (chlorhexidine-containing teat sealant, commercial teat sealant, and untreated controls), sampling point, and their interaction. Least squares means and standard errors of the prevalence of infected or noninfected quarters at each sampling point were back-transformed and are presented as means and their standard errors. The prevalence of contaminated quarters was not different between the groups and they were excluded from the analysis.

The least squares means of lengths of dry periods and their 95% confidence intervals and differences for the treatment groups were estimated using the SAS MIXED procedure with a linear model that included the fixed effect of treatment (chlorhexidine-containing teat sealant, commercial teat sealant, and untreated controls).

RESULTS

Dry Period

The lengths of the dry periods were similar between the treated (103.1 d; 95% CI = 98.0–108.3) and untreated (96.1 d; 95% CI = 83.0–109.3; $P = 0.334$) cows.

Udder Palpation Scores

Palpation results were available for all 73 cows (Table 4) for the 34 d after drying off, with the exception of 1 cow from the untreated control group that had udder palpation performed until d 17; then the cow aborted and was excluded from the study. Four further cows (all treated) missed palpations on a total of 6 occasions. The effect of treatment on the frequency of palpation scores was significant ($P < 0.001$).

The frequency of palpation scores ≥3 was highest on d 10, 16, and 19 for the chlorhexidine-containing teat sealant-treated quarters and on d 6, 16, and 19 for commercial teat sealant-treated quarters (maximum value of 3/126 quarters on d 19). The frequency of palpation scores ≥3 in untreated quarters was highest on d 9, 10, and 14 (4/40 quarters on each occasion).

Table 3. Prevalence of infected or noninfected quarters in percent (standard errors in parentheses) among groups[1]

Status	d −5			d 0			Milking 1			Milking 20		
	ATS	TS	UC	ATS	TS	UC	ATS	TS	UC	ATS	TS	UC
Noninfected	31.40 (0.04)	33.90 (0.04)	28.21 (0.12)	32.80 (0.04)	35.48 (0.03)	27.50 (0.12)	3.48 (0.24)[b]	22.12 (0.05)[a]	23.33 (0.18)[a]	12.96 (0.08)[b]	20.00 (0.06)[b]	54.54 (0.12)[a]
Infected	68.60 (0.04)	66.10 (0.04)	71.79 (0.12)	67.20 (0.04)	64.52 (0.03)	72.50 (0.12)	96.52 (0.24)[a]	77.88 (0.05)b	76.67 (0.18)b	87.04 (0.08)[a]	80.00 (0.06)[a]	45.46 (0.12)[b]

[a–b]Values within rows and sampling points after calving having different superscript letters differ ($P < 0.05$).

[1]d −5 and 0 are days relative to drying off; milking 1 and milking 20 are the number of milkings after calving. ATS = treatment with a teat sealant containing bismuth subnitrate 65% and chlorhexidine 0.5% (Bomac Laboratories Ltd., Auckland, New Zealand); TS = treatment with Teatseal, containing 65% bismuth subnitrate (Pfizer Animal Health, Auckland), a positive control commercial teat sealant; UC = untreated controls.

Clinical Mastitis During the Palpation Period

During the palpation period, 20 quarters from 9 cows developed clinical mastitis. Fourteen of these mastitis cases were caused by *Strep. uberis* (Table 5). Quarters treated with chlorhexidine-containing teat sealant had 1.2% infected (95% CI = 0.3–5.4) versus 26.8% in untreated quarters (95% CI 7.6–62.0; $P < 0.001$).

The highest risk for the incidence of clinical mastitis during the palpation period was between 6 and 19 d after drying off (Figure 1).

Milk Culture Results

Thirteen contaminated samples, and 13 and 5 missing samples from the chlorhexidine-containing commercial teat sealant and untreated controls, respectively, were not available for analysis. The culture results are presented in Table 6. The distribution of mastitis-causing organisms was similar in the groups before drying off, but differences were evident after calving. A higher prevalence of major mastitis-causing organisms was detected in the untreated quarters and quarters treated with the commercial teat sealant compared with quarters treated with the chlorhexidine-containing teat sealant. This was largely an effect of coagulase-positive staphylococci rather than the challenge organism. The prevalence of minor mastitis-causing organisms was 2.5 and 16.9% at the first milking and 10.2 and 13.6% at the twentieth milking in quarters treated with the chlorhexidine-containing teat sealant and those treated with the commercial teat sealant, respectively. Quarters affected by clinical mastitis in the first 34 d after drying off were treated at the time of diagnosis and signs of clinical mastitis subsided. Despite this, *Strep. uberis* was isolated from 7 quarters after calving: 5 from the untreated group and 1 of each treated with commercial or chlorhexidine-containing teat sealant.

The prevalence of IMI after calving was significantly different between the groups (Table 7). It was highest in untreated quarters, followed by quarters treated with the commercial teat sealant. The lowest prevalence of IMI after calving was in quarters treated with the chlorhexidine-containing teat sealant.

DISCUSSION

This study demonstrates that administration of an internal teat sealant containing chlorhexidine at the last milking of the lactation resulted in the lowest prevalence of IMI with any pathogen observed at calving compared with treatment with a conventional teat sealant or no treatment. The incidence of clinical mastitis during the first 34 d of the dry period was

Table 4. Effect of treatment on palpation scores in the first 34 d after drying off

	Chlorhexidine-containing teat sealant		Commercial teat sealant		Untreated		Total	
Score	n	%	n	%	n	%	n	%
0	3,930	92	3,898	91.3	1,078	83.4	8,906	90.5
1	291	6.8	299	7	137	10.6	727	7.4
2	48	1.1	70	1.6	58	4.5	176	1.8
3	3	0.1	5	0.1	15	1.2	23	0.2
4	0	0	0	0	4	0.3	4	0
5	0	0	0	0	0	0	0	0

significantly lower in quarters treated with chlorhexidine-containing or the commercial teat sealant than in untreated quarters. The incidence of clinical mastitis caused by the challenge organism during this period was not significantly different between groups treated with either teat sealant but was significantly lower in them than in the untreated group. The lower prevalence of IMI after calving in the quarters treated with the chlorhexidine-containing teat sealant should result in a lower prevalence of mastitis throughout lactation, as noninfected cows at calving are less likely to develop mastitis (Barkema et al., 1998; Woolford et al., 1998). The modest decrease of IMI after calving was because of bacteria different from the challenge organisms. This suggests that the risk of infection by other organisms was decreased. However, as the prevalence of other organisms was not a subject of interest, this en passent observation is not a definite conclusion.

It would be expected that microbial challenge under natural conditions would be less intense than that in the present study. The challenge broth contained a concentration of bacteria that would not be expected to occur under natural conditions. Moreover, quarters were exposed to challenge with a single strain of *Strep. uberis*. This differs from the situation in natural conditions, in which animals would be expected to encounter a diverse microbial challenging flora. Despite these caveats, the challenge model used in the present study was valid, because both the model and the strain used for the present study have been used previously with success. This model was shown to be highly effective in causing IMI in untreated quarters in the early dry period (Fernandez, 2007; K. Petrovski, N. B. Williamson, A. Grinberg, N. Lopez-Villalobos, T. J. Parkinson, and I. G. Tucker, unpublished data). Hence, the results are applicable to the external population. Only cows with <200,000 somatic cells/mL and no history of clinical mastitis in the previous lactation were included in the present study. Cows from a single farm were used in the current study to prevent inter-farm variability (Barkema et al., 1999; Godden et al., 2003; Newton et al., 2008). As the effect of the individual cow on the results of challenge experiments is generally significant (Huxley et al., 2002; Newton et al., 2008), this was included in the modeling. The split study design, in which 2 quarters per cow were treated with the novel sealant and the other 2 quarters with a conventional sealant, which was used in the present study may actually underestimate the true efficacy of the prevention capabilities of the test items at the cow level (Berry et al., 2003). Regardless, any such effect would affect both products used in the study equally and not bias the results toward either of the treatments.

The number of cows in the present study was adequate to allow rejection of the null hypothesis (power analysis not shown). Although relatively few cows were enrolled in the negative control group, this approach was taken to limit the unnecessary suffering of animals, given that the challenge procedure is highly effective. The treated and untreated groups had similar infection status before drying off, as only 2 glands (1 in the chlorhexidine-containing teat sealant-treated and 1 in

Table 5. Distribution of cases of clinical mastitis (CM), the probability of a quarter being affected by CM (probability total), and the probability of a quarter of being affected with CM caused by the challenge organism (probability challenge) in the first 34 d after drying off

		Quarters affected by CM		Quarters with positive isolation			
Group	Treatment group	n	%	n	%	Probability total (% and 95% CI)	Probability challenge (% and 95% CI)
1	Chlorhexidine-containing teat sealant	3	2.4	1	0.8	1.2 (0.3–5.4)	0.7 (0.1–5.1)
1	Commercial teat sealant	4	3.2	1	0.8	1.8 (0.5–6.6)	0.7 (0.1–5.1)
2	Untreated	13	32.5	12	30.0	26.8 (7.6–62.0)	25.3 (8.6–55.0)

Table 6. Summary of the culture results in percent (and numbers) among groups[1]

	Chlorhexidine-containing teat sealant				Commercial teat sealant				Untreated			
Item[2]	d −5	d 0	M1	M20	d −5	d 0	M1	M20	d −5	d 0	M1	M20
Organism												
Coagulase-positive staphylococci	0.8 (1)	0.0 (0)	0.0 (0)	1.7 (2)	0.0 (0)	0.0 (0)	3.4 (4)	4.2 (5)	0.0 (0)	0.0 (0)	0.0 (0)	8.3 (3)
Streptococcus uberis	0.0 (0)	0.0 (0)	0.8 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.8 (1)	0.0 (0)	2.5 (1)	0.0 (0)	15.6 (5)	0.0 (0)
Major total	0.8 (1)	0.0 (0)	0.8 (1)	1.7 (2)	0.0 (0)	0.0 (0)	4.2 (5)	4.2 (5)	2.5 (1)	0.0 (0)	15.6 (5)	8.3 (3)
Bacillus spp.	0.0 (0)	0.0 (0)	0.8 (1)	0.0 (0)	0.0 (0)	0.0 (0)	1.7 (2)	0.0 (0)	2.5 (1)	0.0 (0)	0.0 (0)	0.0 (0)
CNS	23.4 (29)	32.5 (41)	0.0 (0)	7.6 (9)	26.6 (33)	34.9 (44)	10.2 (12)	11.0 (13)	22.5 (9)	25.0 (10)	0.0 (0)	25.0 (9)
Corynebacterium bovis	6.5 (8)	0.0 (0)	0.0 (0)	2.5 (3)	5.6 (7)	0.0 (0)	1.7 (2)	2.5 (3)	0.0 (0)	2.5 (1)	3.1 (1)	13.9 (5)
Gram-negative rods	0.0 (0)	0.0 (0)	1.7 (2)	0.0 (0)	0.0 (0)	0.0 (0)	3.4 (4)	0.0 (0)	0.0 (0)	0.0 (0)	3.1 (1)	2.8 (1)
Minor total	29.8 (37)	32.5 (41)	2.5 (3)	10.2 (12)	32.3 (40)	34.9 (44)	16.9 (20)	13.6 (16)	25.0 (10)	27.5 (11)	6.3 (2)	41.7 (15)
Contaminated	2.4 (3)	0.8 (1)	2.5 (3)	8.5 (10)	4.8 (6)	1.6 (2)	4.2 (5)	11 (13)	2.5 (1)	0.0 (0)	6.3 (2)	8.3 (3)
No growth	66.9 (83)	66.7 (84)	94.1 (111)	79.7 (94)	62.9 (78)	63.5 (80)	74.6 (88)	71.2 (84)	70.0 (28)	72.5 (29)	71.9 (23)	41.7 (15)
Sampled quarters (n)	124	126	118	118	124	126	118	118	40	40	32	36

[1]d −5 and 0 are days relative to drying off; M1 and M20 are the number of milkings after calving.

[2]Major total = coagulase-positive staphylococci and *Streptococcus uberis* together; minor total = *Bacillus*, CNS, *Corynebacterium bovis*, and gram-negative rods together.

the untreated group) were infected with a major pathogen at drying off (Table 6). The study design did not allow for detailed analysis of microbial results and, hence, no lengthy discussion on this will be attempted. The overall effect of treatment on the prevalence of IMI was significant. The study did not attempt to estimate the effect of the teat sealants on existing IMI. As chlorhexidine was incorporated in the novel teat sealant solely for its local activity in the teat canal and teat cistern, it was assumed that cows with pre-existing infection that cured did so as a result of self-cure rather than the effect of the introduced chlorhexidine in the teat sealant. This could be the subject of further research. Additionally, the lengths of the dry periods were also similar (103 and 96 d for the treated and untreated cows, respectively). Hence, differences in the length of the dry period cannot explain the observed differences in quarter IMI at calving. Regardless, a higher probability of infection at calving would be expected in treated quarters because of their longer dry period (Rindsig et al., 1978; Berry and Hillerton, 2007).

Six quarters that developed clinical mastitis during the first 34 d after treatment and were treated for clinical mastitis according to the study protocol yielded *Strep. uberis* after calving. Previous work by this group has confirmed by pulsed-field gel electrophoresis that all clinical cases during the dry period and most subclinical infections after calving were caused by *Strep. uberis* identical to the challenge strain (data on file). The in vitro tests performed on the challenge strain have shown a high susceptibility to all β-lactam antibiotics. The reason for the finding of these infections after calving is not clear. They may have resulted from treatment failure or reinfection.

Since their development in the 1970s, internal teat sealants containing bismuth subnitrate have been evaluated in Canada, Ireland, New Zealand, the United Kingdom, and the United States. Studies have demonstrated that application of teat sealant to uninfected mammary glands is at least as effective as a long-acting DCT, if not better, in decreasing the rate of new IMI during the dry period (Meaney, 1977; Woolford et al., 1998; Berry and Hillerton, 2002; Godden et al., 2003; Crispie et al., 2004a,b; Cook et al., 2005). The chlorhexidine-containing teat sealant used in the present study demonstrated superior protection to that of the conventional teat sealant when measured as the prevalence of IMI with all pathogens after calving. The reason for this benefit, compared with the lack of advantage of combining teat sealant and DCT (Woolford et al., 1998), is not clear. However, it appears to support the hypothesis of the study; namely, that it is a result of the local activity of chlorhexidine destroying organisms in the teat cavity, either those which invaded before the sealant formed

Table 7. Means and 95% confidence intervals of the quarter level infection rate after calving

Treatment	Mean	95% CI	Difference from chlorhexidine-containing teat sealant
Chlorhexidine-containing teat sealant	15.24	9.55–23.44	NA
Commercial teat sealant	31.73	23.52–41.26	0.009
Untreated	78.57	59.79–90.04	0.002

a perfect plug or those introduced with the treatment. On the other hand, antimicrobial concentration from the DCT around the time of calving may be lower than the minimal inhibitory concentrations, leading to increased susceptibility to new infections. Moreover, the teat sealants form a persistent barrier, which, in the case of the chlorhexidine-containing teat sealant may protect against organisms invading teats that have the sealant plug loosened from the teat cistern wall, such as leaky teats.

Currently, available teat sealants are not ideal for preventing new IMI during the dry period, inasmuch as a failure to protect 2.0 to 42.5% of quarters has been reported (Woolford et al., 1998; Huxley et al., 2002; Bradley et al., 2010). The most likely reason for a failure of prevention could be the risk of contaminating quarters during treatment, because conventional teat sealants lack constituents with antimicrobial properties (Bradley and Green, 2004). Aseptic technique during administration is paramount for the infusion of any intramammary product, but it is known that farming practice varies widely, possibly based on the assumption that it is less important when infusing antimicrobial formulations (Woolford et al., 1998). Prophylactic administration of DCT to uninfected quarters could predispose cows to new IMI because of disruption of the epithelial integrity of the teat canal; accidental introduction of mastitis-causing organisms from around the teat end, particularly when they are resistant to the antimicrobial used; or disruption of the normal microflora (Williamson et al., 1995; Huxley and Bradley, 2002; Godden et al., 2003; Crispie et al., 2004b). The effect of new IMI introduced by this procedure is difficult to quantify: they may persist until the next lactation but also could result in acute onset of clinical mastitis before the active involution of the gland is finished (Smith et al., 1985; Bradley and Green, 2004). The coagulase-positive staphylococci present at calving and absent at drying off in quarters treated with commercial teat sealant may have been introduced during treatment or have invaded the teat canal in the early dry period from micro skin lesions surrounding the teat canal. This is supported by the lower prevalence of minor mastitis-causing organisms in quarters treated with

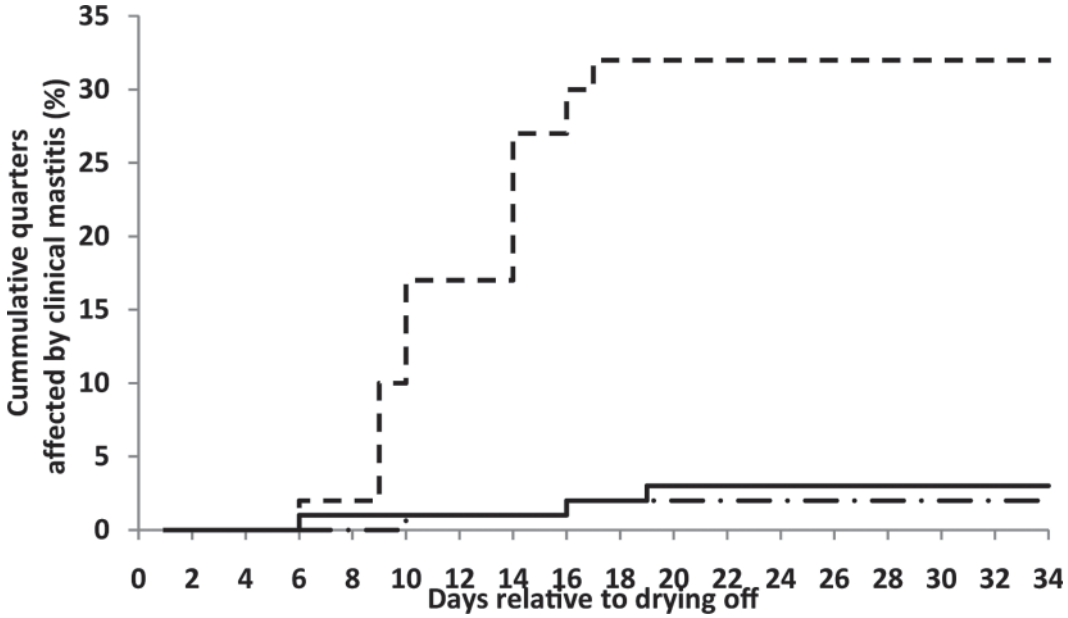

Figure 1. Survival analysis from treatment to incidence of clinical mastitis (chlorhexidine-containing teat sealant – · –, commercial teat sealant ——, untreated – – –) during the first 34 d after drying-off.

chlorhexidine-containing teat sealant when compared with quarters treated with commercial teat sealant and untreated quarters (Table 6). Therefore, to overcome this shortcoming of currently-available teat sealants, the addition of an appropriate antimicrobial agent, such as in the novel internal teat sealant, appears to be a worthwhile approach. The confidence of farmers in sealant may increase with the presence of antimicrobial compounds in the formulations. The sealant provides a barrier throughout the dry period against many microbial species that gain entry into the gland through the teat canal. Furthermore, an effective antimicrobial will inhibit or kill mastitis-causing organisms that evade the teat sealant plug (Ryan et al., 1998; Godden et al., 2003). This possibly explains the observed lower number of infected quarters at calving that was found in the present study in quarters treated with the novel internal teat sealant when compared with untreated quarters and those treated with the commercial teat sealant. The results support a view that chlorhexidine-containing teat sealant decreased the ability of major and minor mastitis-causing organisms to penetrate the teat canal and establish IMI during the dry period.

CONCLUSIONS

This study demonstrates positive effects from the use of an existing and a novel internal teat sealant containing chlorhexidine in cows with low SCC and no history of clinical mastitis during the previous lactation on the prevalence of new IMI after calving and on the incidence of clinical mastitis in the nonlactating period.

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