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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.