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## Effect of dose of *Streptococcus uberis* infused into the mammary gland of lactating cows on clinical signs, bacterial count, somatic cell count and milk production

S. MCDOUGALL, K. PARKER, S. SWIFT<sup>1</sup>, S. HARCOURT<sup>2</sup> AND G. SUTHERLAND<sup>3</sup>

Animal Health Centre, PO Box 21, Morrinsville, New Zealand

### ABSTRACT

To develop a better understanding of the most common clinical mastitis pathogen in New Zealand dairy cows, *Streptococcus uberis* (SU), a challenge model is being developed. Four cows were infused with 88 and 888 colony forming units (CFU) of SU in 2 glands in each cow (i.e. all 4 glands in each cow were infused). In another 2 cows, 2 glands were infused with vehicle (Vehicle; n = 4 glands, n = 2 cows) with the other 2 glands not infused (Control; n = 4 glands, n = 2 cows). The rapid mastitis test score was elevated within 24 h post infusion (P = 0.02). *Streptococcus uberis* was isolated from all infused glands, 24 h after infusion and clinical mastitis was detected within 2 to 2.5 d of infusion. The log<sub>10</sub> bacterial count at 24 h post-infusion did not differ between the two doses of SU infused (P > 0.2). The log<sub>10</sub> somatic cell count was higher in the glands infused with 888 CFU of SU compared 88 CFU SU infused glands (3.29 ± 0.17 vs. 2.23 ± 0.17 mean ± SE log<sub>10</sub> SCC; P = 0.05). Milk production was depressed following infusion (P = 0.05). It was concluded that clinical mastitis could be induced by a single intramammary infusion of either 88 or 888 CFU of SU.

**Keywords:** *Streptococcus uberis*; clinical mastitis; SCC.

### INTRODUCTION

*Streptococcus uberis* (SU) is the most common bacterial isolate from cases of clinical mastitis in New Zealand (McDougall, 1998). Current control strategies (e.g. SAMM plan) (Woolford *et al.*, 1995) are only moderately effective in controlling mastitis due to SU (Leigh, 1999). Thus, alternative strategies are required to deal with SU. One alternate strategy is to examine the host response to SU and to select cows that have enhanced resistance to SU. Both somatic cell count (SCC) and clinical mastitis incidence are heritable, with h<sup>2</sup> estimates of 0.10 to 0.27 and 0.01 to 0.4 for SCC and clinical mastitis, respectively (Weller *et al.*, 1992; Shook and Shultz, 1994; Rupp & Boichard, 1999; Nash *et al.*, 2000).

A number of studies have investigated infusion of SU into the glands of dairy cattle (Lacy-Hulbert *et al.*, 1996; Finch *et al.*, 1997). Isolates vary in pathogenicity (Hill, 1988) and in the dose required to induce infection (Leigh *et al.*, 1990). The interval from infusion to clinical response has also been shown to be variable (Milner *et al.*, 1997). This may be related to variation among cows in immune function or among bacteria in pathogenicity.

This preliminary study examined the effect of intramammary infusion of two doses (~ 88 or ~ 888 colony forming units; CFU) of SU on clinical signs, milk production, milk bacterial count and SCC. The objective was to demonstrate that infection could be established

and to evaluate a range of clinical and laboratory tests for assessing response to infusion.

### MATERIALS AND METHODS

Six, two-year-old Jersey-Friesian cross-bred animals were selected for the study on the basis that no bacteria were isolated from duplicate milk samples collected from each gland, 8 days before infusion and on the day of infusion. Four of the six cows were randomly chosen to be infused with 88 and 888 CFU of SU (in a vehicle of 1 ml of 0.85% w/v saline and 10% v/v glycerol) in two glands each in each cow (either both front or both rear glands with each dose). The remaining two cows were infused in two glands with the SU diluent (0.85% w/v saline and 10% v/v glycerol) and the remaining two glands in these cows were left as untreated controls. Infusion occurred following placement of an 18 g x 25 mm intravenous catheter through the teat canal and syringing in the vehicle or bacterial suspension. Infusion occurred following afternoon milking (Day 0).

The rapid mastitis test (RMT) score (0, trace, 1, 2 and 3 scale) of each gland was assessed on foremilk at each milking on Days 0 to 4 by the same person. Each gland was palpated and assessed for heat (yes or no), swelling (yes or no) and pain (yes or no). Pain was diagnosed where a cow resented having a milk sample taken, i.e. the legs were lifted and/or attempts were made to kick the sampler. The presence of clots (scored as nil, minor or major), milk composition (normal, watery,

<sup>1</sup> Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

<sup>2</sup> Livestock Improvement Corporation Ltd, Private Bag 3016, Hamilton, New Zealand.

<sup>3</sup> Vialactia Biosciences, PO Box 109-185, Newmarket, Auckland, New Zealand

serous) and presence of blood in milk (yes or no) were recorded at each milking between Day 0 and Day 4. Thereafter, just the presence of clots was recorded. Detection of clots in the milk resulted in intramuscular treatment with 5 g of penethemate hydrioidide (Mamyzin, Boehringer Ingelheim, Auckland, New Zealand), repeated three times at 24 h intervals. One cow became clinical in all 4 glands again, 4 days after the end of the initial treatment period and was then treated with 3 x 12 h intramammary infusion of 1g of penicillin (Masticillin, Stockguard Animal Health, Hamilton, New Zealand).

Milk samples were collected ~24 h after infusion for bacteriology and SCC. Following aseptic preparation of the teat, milk samples (~10 ml) were collected before (pre), and two minutes after cup attachment (mid-flow) and following the end of milking (post). At mid-flow, an additional 35 ml sample was collected for subsequent SCC determination (Fossomatic, Foss Electric, Hillerød, Denmark).

Milk volume data for each milking for each cow was recorded using the in-line milk recording system (Metatron 21, WestfaliaSurge New Zealand Ltd, Hamilton) or by manual methods and the data subsequently retrieved from the database (DairyPlan C21, WestfaliaSurge New Zealand Ltd, Hamilton).

#### Bacteriology

The SU isolate infused was derived from a clinical case of mastitis from a cow in a herd near Morrinsville, New Zealand. The initial streptococcal isolate (AHCROB101) was confirmed as SU on the basis of being a Gram positive, catalase-negative, cocci that cleaved esculin, and fermented inulin, lactose, mannitol, salicin, sorbitol and trehalose but not raffinose (Quinn *et al.*, 1999). Following initial isolation, the isolate had been stored on a Dorset egg slope (Fort Richard, Auckland New Zealand) at 4°C. The isolate was recultured on horse blood agar (HBA, Fort Richard), a single colony taken and grown overnight at 37°C in Todd Hewitt Broth containing 0.5% w/v yeast extract (THBY) and mixed 1:1 with 50% v/v glycerol in water before storage at -70°C. For preparation of the challenge infusion, the isolate was cultured on HBA and a single colony taken and grown overnight on THBY. Bacteria from the overnight culture were harvested by centrifugation (7,000 x g), washed in an equal volume of 0.85% w/v saline in 10% v/v glycerol in water (SG), and serially diluted 4 ml into 36 ml of SG to give approximately 1000 and 100 CFU per ml. Aliquots (1.2 ml) of the appropriate dilutions were placed into 2 ml cryovials and snap frozen in liquid nitrogen before storage below -70°C. To verify that the snap freezing and thawing process did not affect the viability of the bacteria for infusion, three frozen aliquots were thawed and 0.1 ml plated to HBA. The cryovials were stored at -80°C until transportation on dry ice to the trial site following which the vials were thawed in a 37°C water bath for approximately 60 minutes before infusion.

For pre and post-infusion assessment of bacterial status, milk samples (10 µl) were streaked onto a 5% blood agar plate containing 0.1% esculin (Fort Richard,

Auckland, New Zealand) and incubated at 37°C for 48 h. The number of bacteria in milk were enumerated ('viable plate count') following culture onto blood agar plates of 100 µl of 10-fold serial dilutions in peptone broth (Fort Richard, Auckland).

#### Analysis

The bacterial counts and SCC were not normally distributed so were log<sub>10</sub> transformed before analysis. Bacterial count was analysed with a repeated measures ANOVA with cow, dose of bacteria and sample time (i.e. pre, mid and post milking) as the main effects. Log<sub>10</sub> SCC was analysed using a linear mixed model with dose and cow as the main effects.

The RMT scores were coded 0 to 4 (i.e. 0 = 0, trace = 1, 1 = 2, 2 = 3 and 3 = 4) for analysis. Initial inspection of the data revealed that none of the control or vehicle glands had RMT scores >0 at any time and that the glands infused with 88 and 888 CFU had similar scores across time. Thus, the glands were pooled as control (i.e. control and vehicle glands) and treated (i.e. 88 and 888 CFU infused glands) for analysis and the scores compared with  $\chi^2$  within each milking. Daily milk production (i.e. the sum of a.m. and p.m. volumes) was analysed using a repeated measures ANOVA with treatment as the main effect and the average of the daily milk production on Days -6, -4, -2 and 0 as a covariate.

All analysis was undertaken using the SPSS statistical package (version 11.5).

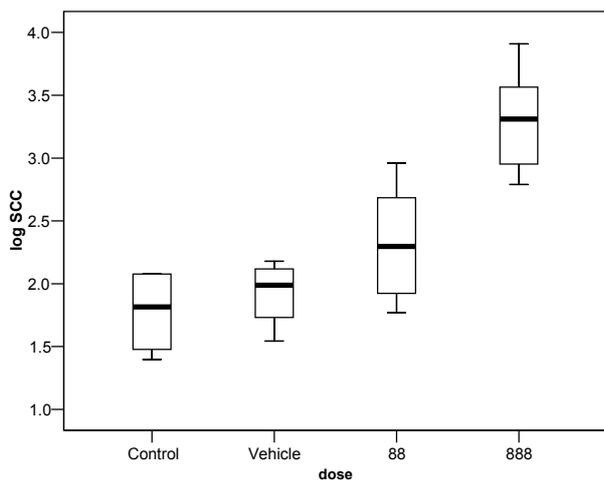
## RESULTS

*Streptococcus uberis* was isolated from each gland infused with SU, 24 h post infusion (range =  $1.6 \times 10^4$  to  $6.3 \times 10^6$  CFU/ml). The bacterial count did not differ among sample times during milking (i.e. pre, mid or post;  $P = 0.78$ ) or SU doses ( $P = 0.40$ ), while bacterial count tended ( $P = 0.08$ ) to differ among cows.

All 16 glands infused with SU were diagnosed with clinical mastitis at milking 4 or 5 post infusion. Increases in the RMT scores were first detected 2 milkings post infusion ( $P = 0.02$ ). Pain was detected in all infused glands before appearance of other clinical signs ( $2.6 \pm 0.9$  and  $2.9 \pm 1.1$  mean  $\pm$  SD milkings to detect pain in glands infused with 88 or 888 CFU of SU, respectively). Grossly evident changes in the milk were first detected at 4 milkings post infusion. The average interval from infusion to detection of clots was 5.6 milkings (SD = 1.3 milkings; range = 4 to 9 milkings). A watery appearance of milk was detected in all 8 of 8 and 6 of 8 glands infused with 88 and 888 CFU respectively, and occurred at a similar time to the appearance of clots ( $6.5 \pm 1.4$  and  $6.0 \pm 1.1$  mean  $\pm$  SD milkings for 88 and 888 CFU SU infused glands, respectively). Blood was not detected in milk at any stage. Swelling of the gland occurred in only two cows with swelling of all glands in each cow being detected at milking 7 (cow # 1216) or milking 11 (cow # 1202). No change in milk composition was detected in any gland infused with the vehicle or in any uninfused gland at any time point.

Glands infused with 888 CFU had a higher log<sub>10</sub> SCC than glands infused with 88 CFU SU and the glands infused with vehicle or not infused at all (P = 0.05)(Figure 1).

**FIGURE 1:** Boxplot of log<sub>10</sub> SCC (x 1000 cells/ml) for individual glands which were not infused (Control; n = 4 glands), infused with vehicle (Vehicle; n = 4 glands) or with 88 or 888 CFU of *Streptococcus uberis* (n = 8/group), 24 h after infusion.



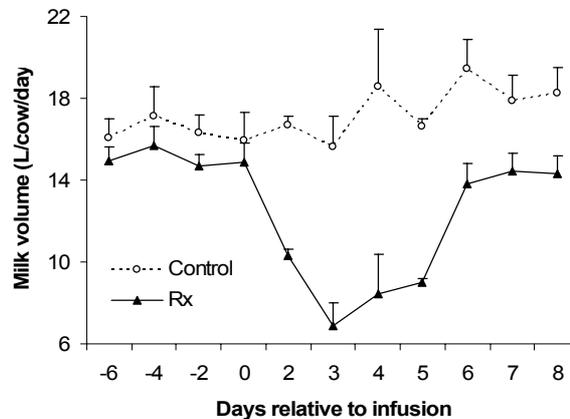
Milk production was depressed following infusion of SU (P = 0.02) (Figure 2).

### DISCUSSION

Intramammary infection and clinical mastitis occurred following infusion of either 88 or 888 CFU of a field isolate of SU. Infusion of 100 and 300 CFU of SU resulted in establishment of infection in all glands and clinical mastitis in 75% to 100% of glands in previous studies (Hill *et al.*, 1994; Finch *et al.*, 1997). However, variation in pathogenicity of SU strains has been demonstrated (Hill, 1988), thus the number of bacteria required to establish infection and induce clinical mastitis needs to be established for each individual strain.

Clinical signs of mastitis were evident within two milkings of infusion as pain was detected in some infused glands at that milking. This was manifest as resentment of handling of teats and/or glands before or during the milk sampling process. Other clinical signs such as gross changes in milk composition, for example presence of clots in milk, were evident from milking 4 onwards. The rapid mastitis test score increased at a similar time to the detection of pain but was more consistent with all infused glands having detectable elevations in RMT by milking 4 post infusion. Swelling of the mammary gland was inconsistently detected, with

**FIGURE 2:** Mean (and standard error) daily milk volume (L/cow/day) for cows infused with *Streptococcus uberis* (2 glands with 88 and 2 with 888 CFU/gland in each cow; n = 4 cows; Rx) or infused in 2 glands with vehicle and with 2 glands left untreated (Control; n = 2 cows).



only two of the four infused cows detected with this sign.

The milk bacterial count did not differ between samples collected before, during or after milking, among the two SU doses tested, and there was not a dose by time interaction. Thus, a single pre-milking sample provided a bacterial count estimate not different from milk samples collected at different stages through milking and the SU dose infused did not affect the count. Previous studies have found maximum bacterial counts in milk of between 10<sup>5</sup> and 10<sup>9</sup> bacteria/ml at milkings 2 to 14 post infusion (Hill *et al.*, 1994; Finch *et al.*, 1997). The present study only evaluated bacterial count at one time point (i.e. 24 h post-infusion) thus the maximum concentration of bacteria may have been higher if sampling had continued for longer. However, the aim of the current study was to confirm that infection had established (i.e. bacteria were recoverable following infusion), rather than to determine the maximum bacterial count.

Milk production declined by about 50%, 3 days after SU infusion. Milk production started increasing following treatment. Previous studies have demonstrated milk production depressions of between 20% and 50% within 3 days of infection that continued after treatment of the clinical infections (Hill *et al.*, 1994; Finch *et al.*, 1997). In the current study, the milk production loss was estimated only in those cows that had clinical mastitis and which were treated with antibiotics. The production loss for subclinical SU mastitis and/or where clinical SU mastitis is not detected and hence not treated, remains to be determined.

It is concluded that infection and clinical mastitis can be induced following infusion of either 88 or 888 CFU of a field strain of SU.

## ACKNOWLEDGEMENTS

Thanks to Dawn Dalley and staff at Dexcel WTARS for assistance with animal handling and sampling and to John Williamson (Dexcel), Fiona Anniss and Judith Forno (Animal Health Centre) for laboratory work. Gabrielle Stanley (LIC) provided logistics support for the study. The study was funded by Boviquest.

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