THE USE OF A BISMUTH-BASED TEAT SEAL AND THE BACTERIOCIN LACTICIN 3147 TO PREVENT DRY PERIOD MASTITIS IN DAIRY COWS

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SUMMARY

Mastitis in dry cows has traditionally been controlled with long-acting intramammary antibiotic formulations. However, the widespread use of antibiotics, particularly for prophylactic application, is likely to be restricted in the future. As a consequence, there is now a growing requirement for effective alternatives to prevent mastitis. A bismuth-based, intramammary teat seal has recently been shown to be as effective as “dry cow” antibiotic therapy for the prevention of new dry period infections. A new prototype formulation containing the bacteriocin, lacticin 3147 and teat seal has also been shown to be effective in controlling Staphylococcus dysgalactiae and Staphylococcus aureus using experimental infection models both in non-lactating and lactating dairy cows.

INTRODUCTION

Dairy cows are particularly susceptible to mastitis during the dry period and Staphylococcus aureus, Streptococcus dysgalactiae and Streptococcus uberis are still the dominant pathogens associated with dry period infections in Irish herds. During the last three decades long-acting intramammary antibiotics have been used routinely as a means of curing existing infections in mastitic cows and also for preventing new infections in previously uninfected cows (2). While dry cow antibiotic therapy has helped to reduce the incidence of mastitis in the past, there will be a greater emphasis on reducing antibiotic inputs in the future due to the perceived connection between the over-use of antibiotics and the emergence of antibiotic resistant organisms. The debate on “to treat or not to treat” uninfected cows at drying-off has been ongoing for many years and some researchers have recommended that dry cow antibiotic therapy should be reserved for infected cows and not applied as a routine prophylactic measure (4,5). On the other hand, it has been shown that the incidence of new intramammary infections increases when uninfected cows are left untreated, particularly in the early part of the dry period. Studies in New Zealand (20) showed that 16% of a sample of 528 untreated quarters, uninfected at drying off, developed a new infection during the dry period. Berry (1) also showed that between 30% and 50% of untreated cows at drying off developed new infections during the dry period compared to a new infection rate of between 0% and 15% in cows treated with antibiotics.
The continued use of antibiotics in the dry period for either therapeutic or prophylactic purposes has some disadvantages, including the perceived connection to the emergence of antibiotic-resistant human pathogens particularly with the increased incidence of organisms such as methicillin-resistant *S. aureus*, which is prevalent in nosocomial infections in humans (6). Such concerns have prompted the World Health Organisation to issue recommendations on global programmes to try to reduce the use of antibiotic therapies for both human and animal applications in the future (19).

Sealing the teats of uninfected cows at the end of lactation may provide an acceptable alternative to blanket treatment with antibiotics. External applications of sealers reported by Oliver *et al.*, (10) failed to achieve satisfactory control 48 hours after administration. Farnsworth *et al.*, (7) also reported on the use of an acrylic teat seal applied externally by dipping teats after milking. This formulation was effective in reducing the incidence of sub-clinical mastitis. The authors also recommended its use particularly for the control of coliform infections. The use of an internal sealer comprising of bismuth subnitrate and acriflavine was also considered as an alternative to antibiotic therapy in uninfected cows at the end of lactation (9). This product was successful in preventing new dry period infection in studies using artificial bacterial challenge, but further work was required to improve the persistence of the seal for more extended dry periods.

Recent developments in non-antibiotic internal teat sealing technology have proved to be very effective in preventing new cases of mastitis during the dry period when infused into the teats of uninfected cows at drying-off. The teat seal formulation contains a heavy inorganic bismuth salt in a mineral oil base and is infused from a plastic syringe which is of a similar type to those used for infusion of intramammary antibiotic. In the New Zealand study reported by Woolford *et al.*, (20), Teatseal™ [Bimeda (NZ) Ltd, Auckland, New Zealand] was as effective as a long-acting antibiotic containing 250 mg of cephalonium in preventing naturally occurring infections in cows which were classified as uninfected at drying-off. In that study *Str. uberis* was the principal pathogen causing mastitis in the dry period.

Bismuth-based teat seals are biologically inert and do not have an associated anti-microbial activity. Attractive non-antibiotic additives to enhance teat sealing formulations might include bacteriocins such as nisin (3, 12) or lacticin 3147 (13). Bacteriocins are proteins produced by some bacteria that have the ability to kill other organisms. Both nisin and lacticin 3147 are natural foodgrade anti-microbials with a broad-spectrum of inhibition against Gram-positive bacteria. Taylor *et al.* (17) showed that a single intramammary infusion of nisin was effective in treating both streptococcal and staphylococcal infections in bovines. In these experiments, however, the nisin preparation produced an adverse cellular response in the udder. More recent interest in nisin was reported by Sears *et al.* (16) who showed that nisin in combination with lysostaphin cured 66% of
S. aureus, 95% of Streptococcus agalactiae and 100% of Str. uberis infections. Nisin has since found application in two commercial teat hygiene products that are currently used for the prevention of mastitis.

In recent years a number of new bacteriocins have been isolated and characterised in a collaborative study between Moorepark Research Centre and University College Cork. One of these bacteriocins, designated lacticin 3147, is produced by Lactococcus lactis ssp. lactis DPC3147 and was first isolated from Irish kefir-like grains used for bread-making (11). Lacticin 3147 has been shown to be effective against all Gram-positive bacteria tested to date including mastitis-causing pathogens (14).

This paper describes a series of experiments in the development of a teat seal and lacticin 3147 formulation for the prevention of mastitis in non-lactating cows.

**EXPERIMENTAL STUDIES**

**Teat seal/lacticin 3147 formulation**

The teat seal formulation used in the studies was similar to the seal used in the New Zealand study (20). However, the surfactant Tween 80 was also added to facilitate the release of lacticin 3147 from the sealing material. A liquid preparation of lacticin 3147 was prepared as described previously (15, 18) and blended with teat seal. The blended formulation was filled into plastic syringes (4 g per fill). The efficacy of the teat seal containing lacticin 3147 was assessed in vivo by artificial challenge using both non-lactating and lactating cow models. The impact of the bacterial challenge was manipulated by increasing the number of colony forming units inoculated or by increasing the depth to which the bacteria were introduced into the teats.

**Streptococcus dysgalactiae efficacy study in non-lactating cows**

A study in non-lactating cows was designed to ensure that the Str. dysgalactiae challenge loading was sufficient to ensure the partial failure of the teat seal alone. The expected impact of this technique was to increase the infection level and provide a high level of challenge against the seal plus lacticin 3147 combination. Sixty-eight uninfected udder quarters were selected in 18 dairy cows. After the last milking of the lactation, thirty-three teats were infused with teat seal and 35 with teat seal combined with 20,000 AU (arbitrary units) of lacticin 3147. Within cow treatment comparisons were made using treatment pairs selected at random to either right front and right hind quarters or left front and left hind quarters. Three days after infusion, 68 treated teats were inoculated via the streak canal to a depth of 17 mm with 100 µl of antibiotic-free skim milk containing $1.5 \times 10^4$ cfu/ml of Str. dysgalactiae. The challenge organism was classified as Str. dysgalactiae ssp. dysgalactiae by SDS-PAGE total protein profiling (BCCM Culture Collection; Laboratorium voor Microbiologie, Universiteit Ghent, Belgium) and was previously isolated from a case of clinical mastitis in the research herd attached to Moorepark Research Centre.
Following challenge, the cows were observed twice daily for signs of clinical mastitis. Udder quarters were allowed to develop definite clinical signs of mastitis before a sample of secretion was taken for bacteriological analysis. The trial was ended 8 days after inoculation when samples of secretion were collected from all remaining non-clinical quarters.

Str. dysgalactiae isolates were typed by RAPD fingerprinting using a random primer. Genomic DNA was isolated from the challenge strain, Str. dysgalactiae and from teat isolates (after challenge) by a modification of the method by Hoffman and Winston described by Gardiner et al., (8). This procedure was used to ensure that the bacteria recovered from clinically affected quarters and from the quarters harbouring Str. dysgalactiae could be confirmed as the challenge strain.

Results of Streptococcus dysgalactiae challenge study

Fourteen (42%) clinical Str. dysgalactiae infections developed in the sealed teats and 2 (6%) in the teats treated with teat seal and lacticin 3147. On the last day of the trial, 6 additional quarters in the sealed group were shedding the challenge strain while there were no recoveries from the other teats infused with seal plus lacticin 3147 (Table 1). All of the clinical infections caused by the challenge strain in the sealed teats developed in the first four days after challenge compared with one in the teats infused with seal plus lacticin 3147 (Figure 1). The difference in incidence of new infections caused by the challenge strain was significant (p<0.001). Time to survival distribution analysis between the rate of occurrence of each clinical event also showed that the difference between the treatments was significant (p<0.001).

Table 1. Clinical mastitis and bacterial recoveries after challenge with Streptococcus dysgalactiae in quarters treated with teat seal and teat seal plus lacticin 3147

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Quarters (n)</th>
<th>Clinical infections</th>
<th>Non-clinical recoveries</th>
<th>Clinical infections and non-clinical recoveries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seal</td>
<td>33</td>
<td>14 (42.0%)</td>
<td>6</td>
<td>20 (61.0%)</td>
</tr>
<tr>
<td>Seal / lacticin</td>
<td>35</td>
<td>2 (6.0%)</td>
<td>0</td>
<td>2 (6.0%)</td>
</tr>
</tbody>
</table>
Figure 1. Daily incidence rate of new clinical infections caused by the challenge strain of *Streptococcus dysgalactiae* in quarters treated with teat seal and teat seal + lacticin 3147

**Staphylococcus aureus** survival study

Sixteen uninfected lactating cows were selected and within those, 58 quarters were used for the experiment. After the morning milking, 29 quarters were infused with teat seal combined with 32,768 AU of lacticin 3147 and 29 served as untreated controls. One hour after infusion, both treated and untreated teats were inoculated *via* the streak canal to a depth of 17 mm with 100 µl of antibiotic-free skim milk containing $1.7 \times 10^3$ cfu of *S. aureus* DPC5246.

The evening milking was omitted. At the next morning milking, 18 hours later, teat seals were removed from the treated teats and milk samples were collected from all quarters for bacteriological analysis. *S. aureus* isolates were identified and enumerated in the foremilk samples to assess differences in recoveries between treatments.

**Results of Staphylococcus aureus** survival study

*S. aureus* survived in 19 of the 29 control quarters (66%) and in 4 (14%) of the quarters treated with teat seal and lacticin 3147. This difference was significant (P<0.001), (Table 2). Recovery bacterial counts were also made on the milks containing surviving *S. aureus* to assess differences between treatment and control. Overall, the presence of teat seal plus lacticin 3147 reduced the *S. aureus* recovery counts (Figure 2) and the difference in log-transformed recovery data was significant (P<0.001). These data indicated that, in addition to reducing the number of teats shedding viable *S. aureus,*
the teat seal plus lacticin 3147 also reduced the number of challenge organisms in those teats from which \textit{S. aureus} were recovered.

**Table 2.** The effectiveness of teat seal plus lacticin 3147 in eliminating \textit{Staphylococcus aureus} in artificially infected teats of lactating cows compared with untreated controls.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Teats (n)</th>
<th>Teats shedding \textit{S. aureus} (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>29</td>
<td>19 (66%)a</td>
</tr>
<tr>
<td>Seal + lacticin 3147</td>
<td>29</td>
<td>4 (14%)b</td>
</tr>
</tbody>
</table>

a,bValues with different superscripts are significant P<0.001

**Figure 2.** Staphylococcal recoveries from untreated teats and from teats infused with teat seal plus lacticin 3147 after they had been inoculated with \( \sim 1.7 \times 10^3 \) cfu per teat of \textit{Staphylococcus aureus} DPC5246

**DISCUSSION**

The development of non-antibiotic formulations for the prevention of mastitis in cows has the potential to reduce the dependence on antibiotics for prophylactic therapies in the future. The trials reported here form part of a more comprehensive data set on a non-antibiotic approach to mastitis prevention.

These trials have shown that the teat seal and lacticin 3147 combination in the absence of conventional antibiotic therapy was effective in controlling \textit{Str. dysgalactiae} infections in the dry period under conditions of experimental challenge. Moreover, the challenge strain of \textit{Str. dysgalactiae}
could not be isolated from the remaining non-clinical udder quarters after eight days of exposure to the challenge strain. The presence of lacticin 3147 was also associated with a delayed onset of mastitis in the two quarters in which infection occurred. For example, the first clinical case of mastitis in an udder quarter treated with teat seal plus lacticin 3147 occurred 4 days after inoculation, at a point where 13 quarters containing teat seal alone had already become clinically infected.

In the lactating cow model using *S. aureus* as the challenge pathogen, teat seal and lacticin 3147 significantly reduced the numbers of teats shedding the challenge organism. In addition, the population of viable bacteria in teats shedding *S. aureus* was significantly reduced relative to the control udder quarters. This decrease in bacteria numbers occurred during a relatively short exposure period of 18 hours. The survival of some of the *S. aureus* may have been due to an insufficient concentration of lacticin 3147, or a lack of physical contact between the bacteria and the teat seal formulation. Insufficient release of lacticin 3147 from the teat seals or too short an exposure period may have also contributed to the survival of some *S. aureus* in this lactating cow model.

A significant outcome of our trials was the *in vivo* evidence of the ability of lacticin 3147 to retain activity against *Str. dysgalactiae* in the teat and prevent the onset of clinical mastitis during the 8-day trial period. This trial provided convincing evidence that the bismuth-based teat seal and lacticin 3147 combination offered very effective protection against a significant challenge with an important mastitis-causing pathogen.

While all *S. aureus* were not eliminated in the lactating cow model, there was a significant reduction in staphylococcal recoveries from the teats infused with the teat seal plus lacticin 3147 combination. Since the results of this trial demonstrate that teat seal plus lacticin 3147 reduces the number of teats shedding *S. aureus* in a lactating cow, then it may be speculated that the risk of infection should be considerably reduced also in a dry cow model with a similar product.

The choice of teat seal as a delivery vehicle for lacticin 3147 has a number of advantages. The teat seal alone has already been shown to provide an effective barrier against new infection in a large animal trial in New Zealand (20). That study showed that teat seal was as effective as a long-acting antibiotic containing the cephalonium (250 mg) in preventing naturally occurring infections in 528 dairy cows which had been selected as non-infected at drying-off. In the New Zealand study, however, the principal pathogen causing new infections was *Str. uberis* and the incidence of other mastitis-causing pathogens was too low to make valid comparisons. One of the main advantages in combining the seal with a broad-spectrum bacteriocin, therefore, is that in addition to the physical barrier effect of the teat seal, the seal also localises the anti-microbial inhibitor in the teat sinus. Since anti-microbial activity is not required throughout the complete mammary gland for mastitis prevention using the teat sealing system, then
the amount of bacteriocin required per seal treatment will be small relative
to antibiotic usage with conventional dry cow therapy. However, the
optimum concentration of lacticin 3147 to be incorporated into teat seal to
increase the efficacy still remains to be established.

Currently in New Zealand, the teat seal product without the addition of
lacticin 3147 is recommended for use in cows with a milk cell count of
<150,000 cells/ml at drying off. The seal is applied routinely and without
associated antibiotic treatment. The incorporation of a non-antibiotic anti-
microbial such as lacticin 3147 should provide an additional barrier against
Gram-positive pathogens, which may be introduced inadvertently into the
teat by the herdsman at the time of infusion or may enter naturally during
the dry period. The principal advantage is that neither of the components
contain antibiotics and, therefore, should not compromise current
biomedical applications.

REFERENCES
Shepton Mallet, pp 37-43.
Science: Mastitis Control – progress and prospects. J. Dairy Sci. 51:
481-512.
inhibits several Gram-positive mastitis-causing pathogens. J. Dairy Sci.
72: 3342-3345.
4. Browning, J.W., Mein, G.A., Barton, M., Nicholls, T. J. and Brightling,
P. (1990) Effects of antibiotic therapy at drying-off on mastitis in the
infections including the role of the microbiology laboratory. Clin
sealer for prevention of intramammary infection in lactating cows.
J.A.V.M.A. 177: 441-444.
(1998) Development of a probiotic Cheddar cheese containing human-
derived Lactococcus paracasei strains. Appl. Environ. Microbiol. 64:
2192-2199.
9. Meaney, W.J. (1977) Effect of a dry period teat seal on bovine udder
infection. Irish J. Agric. Res. 16: 293-299.
10. Oliver, J., Dodd, F.H. and Neave, F.K. (1956d) The importance of the
33-41.


