

Evaluation of the Efficacy of an Internal Teat Sealer During the Dry Period

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ABSTRACT

The efficacy of an internal dry period teat sealer containing bismuth subnitrate (Product A; Teatseal, Cross Vetpharm Group Ltd, Ireland) was compared with a long-acting antibiotic preparation containing cephalonium (Product B; Cepravin Dry Cow, Schering-Plough Ltd, UK), by assessing the number of new intramammary infections (IMI) acquired during the dry period and the number of cases of clinical mastitis during the first 100 d of lactation. Selection of study animals was based on historical data. No cases of clinical mastitis and all routine cow level somatic cell counts $\leq 200,000$ cells/ml during the previous lactation were used to select cows likely to be uninfected with a major pathogen at drying off. Compared with the antibiotic tube, quarters that received the teat sealer acquired significantly fewer new IMI caused by *Escherichia coli*, all *Enterobacteriaceae*, and all major pathogens combined. There was no significant differences in the number of IMI caused by any other major pathogen. There was no significant difference in the severity or number of quarter or cow cases of clinical mastitis between product groups. Sixty quarters (3.2%) were infected with major pathogens at drying off, 27 (2.9%) in teat sealer and 33 (3.5%) in antibiotic tube cows. The dry period cure rate was not significantly different (63% product A, 70% product B). This is the first controlled study to demonstrate the efficacy of an internal bismuth teat sealer in protecting quarters against new dry period IMI caused by major mastitis pathogens, particularly environmental organisms, under UK field conditions.

(Key words: dry cow therapy, teat sealer, intramammary infection, mastitis)

Abbreviation key: BMSCC = bulk milk SCC, LRS = likelihood ratio statistic.

INTRODUCTION

Antibiotic dry cow therapy was developed in the 1940s to control summer mastitis caused by *Arcanobacterium pyogenes* (Pearson, 1950, 1951). Its use was adopted in the UK during the 1960s as part of the “Five Point Plan” to control contagious mastitis (Smith et al., 1967). It has two objectives: to cure existing IMI and prevent the acquisition of new IMI during the dry period (Smith et al., 1966).

The pressure to control contagious mastitis has increased in the UK since the implementation of European Union Milk Hygiene Directive 92/46/EEC—which deemed milk with a 3-mo geometric mean bulk milk SCC (BMSCC) of greater than 400,000 cells/ml as unfit for human consumption—and the introduction of milk payment bonuses for BMSCC of below 150,000 cells/ml.

The reduction in the UK national herd SCC indicates that the proportion of cows with subclinical IMI, due to a major mastitis pathogen, has fallen substantially; this fall has reduced the need for antibiotic therapy to cure preexisting infections at drying off. Internal and external teat sealants that provide physical protection against new IMI during the dry period are an alternative to antibiotic dry cow therapy in uninfected cows. External seals can reduce the levels of new dry period IMI compared with negative control quarters (Timms, 2000); however, long-term persistence of the sealant on the teat remains a problem (Hemling et al., 2000; Leslie et al., 1999). The efficacy of internal teat sealers containing between 25 and 37%, wt/wt, bismuth subnitrate in preventing the acquisition of new dry period IMI was demonstrated in the 1970s (Meaney, 1976, 1977, 1993). At that time, the prevalence of contagious mastitis pathogens and the rapid expansion in antibiotic dry cow therapy usage limited the interest in this approach (Meaney, 2000, personal communication). However, a combination product comprising a short-acting cloxacillin tube infused immediately before a bismuth subnitrate sealer has been available in Ireland since that time (Osmonds Teatseal, Cross Vetpharm Group Ltd., Ireland).

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Recently, the efficacy of a reformulation of the seal containing 65%, wt/wt, bismuth subnitrate in a paraffin base without antibiotic was studied in New Zealand. The seal was significantly better than a negative control (no therapy) and equivalent to a positive control (antibiotic dry cow therapy) in preventing new IMI during the dry period and clinical mastitis during the following lactation (Woolford et al., 1998).

The aim of the research outlined in this paper was to assess the ability of an internal teat sealer containing 65%, wt/wt, bismuth subnitrate at preventing new IMI during the dry period compared with an antibiotic tube containing a cephalosporin (Cepravlin Dry Cow, Schering-Plough Ltd, UK), under UK field conditions.

MATERIALS AND METHODS

Herd Selection

Sixteen herds were selected on the basis of location (South West England), 12-mo geometric mean BMSCC (<200,000 cells/ml), regular cow level SCC sampling, good farm records, and likelihood of compliance with the study protocol. Herds were not selected on the basis of previous mastitis incidence or etiology.

A calculation to determine the number of cows needed to provide adequate statistical power to demonstrate equivalence between the two treatment groups was based on regional data of dry period IMI rates and etiology (Bradley and Green, 2000).

Herd Management

The 16 herds were between 100 and 350 cows in size; 305-d adjusted milk yields ranged from 5000 to 8500 L, and 12-mo geometric mean BMSCC ranged between 54,000 and 163,000 cells/ml. In 15 herds, cows were managed at pasture during the summer months and in either covered stalls or straw yards during the winter. One herd (farm 7) practiced an extended grazing system cows were kept at pasture and only housed on covered straw yards during the wettest part of winter, typically 2 mo. Dry cows on all farms were kept at pasture during the summer months and in covered stalls or straw yards during the winter. Periparturient cows were kept at pasture or in loose boxes. All herds practiced postmilking teat disinfection (either dipping or spraying) and blanket antibiotic dry cow therapy. Mastitis vaccines were not used on any farm. Cow level SCC were recorded monthly on 15 farms and on alternate months on one farm (farm 8).

Cow Level Selection Criteria

Cows were deemed eligible for consideration if they had no signs of clinical disease, had four functional

quarters free of teat abnormalities, had not received antibiotic or anti-inflammatory treatment during the previous 30 d, and had a predicted dry period of greater than or equal to 51 d. A maximum predicted dry period length was not stipulated. Enrollment criteria specified were all routine (monthly/bimonthly) cow level SCC \leq 200,000 cells/ml and no cases of clinical mastitis during the preceding lactation.

The first cow enrolled on each farm was allocated to a treatment group by the toss of a coin; cows were then alternately allocated to either treatment group A or B as they entered the study. This method was employed to ensure that the two groups were temporally matched even when low numbers of cows were being enrolled, thus ensuring that the environmental exposure was as similar as possible between the two groups.

The herdspersons were blinded with respect to product administration. Animals not deemed eligible for inclusion in the study received conventional antibiotic dry cow therapy.

Dry Cow Products

Product A. An internal teat sealer with no antibacterial properties containing 65%, wt/wt, bismuth subnitrate in an oily base; 4-g product in a mid-length-nozzled plastic tube for aseptic infusion into the teat cistern (Teatseal, Cross Vetpharm Group Ltd., Ireland).

Product B. 250 mg of cephalonium in a mid-length-nozzled plastic tube (Cepravlin Dry Cow, Schering-Plough Ltd., UK).

Sampling Protocol and Product Administration

Duplicate microbiological and single SCC "screening" samples were collected aseptically from all quarters at two time points: 1) Drying off samples—after last milking, immediately before dry cow product infusion; 2) Calving samples—after calving and before the first machine milking.

At drying off, duplicate quarter microbiological milk samples were collected aseptically from all cows fulfilling the enrollment criteria, following a protocol previously described by Bradley and Green (2000). After microbiological sampling, quarter milk samples were drawn into pots containing bronopol and natamycin preservative tablets for SCC analysis.

Following sampling, before the teat was infused with either product A or B, the teat end was scrubbed with 70% ethanol and allowed to dry. Product A (teat sealer) was deposited into the teat cistern; product B (antibiotic) was infused into the teat cistern and massaged up into the quarter. The teats were then dipped in a solution containing 2800 mg/kg of available chlorine (Agri-

sept, Pharmacia Animal Health, UK); cows were confined to a loafing yard for at least half an hour after product administration. Disposable latex gloves were worn throughout the sampling and treatment procedure. The first author collected all drying off samples and administered dry cow product. Calving samples were collected following an identical sampling protocol by trained field technicians. All the samples were kept in a cool box during transportation to the laboratory where microbiological samples were frozen (-20°C) and SCC samples refrigerated ($+2$ to $+8^{\circ}\text{C}$) immediately upon arrival.

Clinical Mastitis Sampling Strategy

Clinical mastitis was monitored for the first 100 d of the next lactation. Herdpersons were trained in the identification, grading, and aseptic sampling of clinical mastitis. Clinical mastitis was identified in quarters on the basis of the presence of abnormal secretion and/or udder changes (e.g., pain, heat, swelling). Mastitis severity was graded according to the following scheme: Grade 1—milk changes only (e.g., clots), Grade 2—milk and udder changes (e.g., heat, swelling); and Grade 3—systemic signs (e.g., depression, pyrexia). Secretion samples were collected, before administration of treatment, using a protocol similar to that used for collection of screening samples (Bradley and Green, 2000). Samples were frozen on farm immediately after collection and returned to the laboratory on dry ice.

Bacteriology

Each week, frozen screening and clinical mastitis samples were submitted to the Langford Veterinary Laboratories Agency for bacteriological analysis. Ten microliters of secretion was inoculated onto sheep blood agar and Edward's agar, and 100 μl of secretion was inoculated onto MacConkey agar to enhance the detection of *Enterobacteriaceae*. Plates were incubated at 37°C and read at 24 and 48 h. Organisms were identified and quantified using standard laboratory techniques (Quinn et al., 1994).

SCC Analysis

Each week, SCC samples were submitted to ON MeRiT Laboratories, Newbury, UK for analysis by the Fossomatic method (Anon, 1981; Anadis SCC 300, France).

Additional Data Collection

Parity, last recorded yield, drying off date, calving date, parenteral antibiotic, and anti-inflammatory

treatments and withdrawal of any cow from the study as a result of death, sale, or culling throughout the dry period and first 100 d of lactation were recorded for all study animals.

Definition of Terms used for Analysis

IMI: Diagnosis. Isolation of an organism was considered an infection. A sample was considered "contaminated" if more than three organisms were isolated. In this case, the duplicate microbiological sample was cultured and the infection status was based on reculture of organisms.

IMI: New infection during the dry period. An organism not present at drying off but identified in a quarter at calving.

IMI: "Cure" during the dry period. An organism present at drying off and not identified in the same quarter at calving.

IMI: "No cure" during the dry period. An organism present at drying off and subsequently identified in the same quarter at calving.

IMI: Cause of clinical mastitis. When an organism was isolated in pure growth it was considered to be the cause of mastitis. A sample was a "mixed growth" if there was growth of two or three known mastitis pathogens. A sample was considered "contaminated" when more than three organisms were isolated.

Clinical mastitis—quarter case. A single recorded occurrence of clinical mastitis in a quarter, including all multiple quarter and recurrent cases. Recurrent cases were classified as cases recurring in the same quarter greater than 5 d after the end of the last clinical episode and caused by the same pathogen.

Clinical mastitis—cow case. A cow experiencing a mastitis event, regardless of the number of quarters affected or the number of times each quarter was affected (if caused by the same pathogen). Synonymous with the proportion of cows affected with each pathogen.

Major pathogen IMI. IMI caused by all microbiological isolates recognized as capable of inducing mastitis in cattle, excluding minor pathogens.

Minor pathogen IMI. IMI caused by coagulase-negative staphylococci, *Corynebacterium* spp., and *Micrococcus* spp.

Enterobacterial IMI. IMI caused by any member of the family *Enterobacteriaceae*, a taxonomic group of organisms similar to but not entirely synonymous with the collection of organisms often referred to as coliforms.

Parity. Parity number refers to the lactation the animal entered when calving occurred at the end of the dry period in the study (i.e., a first-lactation animal

dried off during the study was assigned parity number two). Parity groups two to four were assessed separately, parities five and greater were assessed as one group.

Data Collation and Statistical Analysis

Data were collated and analyzed using Excel and Access 2000 (Microsoft Corporation), Stata version 6 (Stata Corporation, Texas), Epi-Info version 6.04b (Centers for Disease Control and Prevention, Atlanta), and Egret version 2.0.3 (Cytel Software Corporation, Cambridge, MA). The χ^2 test was used to compare proportions; the Mann-Whitney test was used to compare non-parametric continuous data. The significance probability was set at $P \leq 0.05$ for a two-tailed test. All 16 farms were used in all analysis except that farm 11 was excluded from analysis involving last recorded yield, as this data was not recorded.

Unconditional logistic regression was used to model the occurrence of new IMI and the cure of existing IMI at the quarter level to control for potential confounding factors. A strategy was employed as described by Kleinbaum (1994). Outcome variables, which tended towards significance ($P < 0.25$) on univariate analysis, were considered for modeling (Hosmer and Lemeshow, 1989). Models were fitted with the following outcome variables i. New major pathogen IMI at calving; ii. New enterobacterial IMI at calving; iii. New *Escherichia coli* IMI at calving; iv. New minor pathogen IMI at calving; v. All major pathogen cure; vi. All minor pathogen cure; and vii. *Corynebacterium* spp. cure. Treatment group was used as an exposure variable and cow identity was fitted as a random effect to account for the clustering of quarters within cows. The confounding influence of herd, parity, last recorded yield, month of drying off, month of calving, quarter SCC at drying off, and dry period length were assessed. Confounders were left in the model when there was judged to be a biological improvement in the model as calculated by either the likelihood ratio statistic (LRS; significance probability set at 0.05 for a two-tailed test) or a narrowing of the confidence interval of product (Kleinbaum, 1994). Interactions between covariates were examined and left in the model when there was a significant improvement in the model according to the LRS (Kleinbaum, 1994). Similarly, the effect of treatment group was deemed significant when its inclusion significantly improved the model.

RESULTS

A total of 1056 cows was dried off between September 30, 1999 and April 7, 2000. Of these animals, 505

(47.8%) fulfilled the enrollment criteria; 252 received product A (teat sealer) and 253 received product B (antibiotic). The number and proportion of animals enrolled on each farm ranged from 10 to 62 and 23.8 to 71.3%. A further 22 animals (11 in each group) were enrolled between April 8 and June 2, 2000, on farm 1. Only data on clinical mastitis incidence in the first 100 d of lactation were collected from these animals.

There was no significant difference between the treatment groups in the last recorded yield before drying off, dry period length, parity number, and SCC at drying off measured using the Mann-Whitney test (Table 1), suggesting the randomization procedure was successful.

Data from 467 (232 product A and 235 product B) and 479 animals (237 product A and 242 product B) were available for dry period IMI and clinical mastitis analysis, respectively.

Univariate Analysis

New IMI acquired during the dry period. The number of new IMI acquired during the dry period by each treatment group is outlined in Table 2. Cows that received product A (teat sealer) acquired significantly fewer IMI caused by *E. coli* ($P < 0.001$), all *Enterobacteriaceae* ($P < 0.001$), and all major pathogens combined ($P < 0.01$) at the quarter level, and *E. coli* ($P < 0.001$) and all *Enterobacteriaceae* ($P < 0.001$) at the cow level. There were no significant differences between the groups in the number of new IMI caused by any other major or minor mastitis pathogen.

Clinical mastitis. Two cases of clinical mastitis occurred in cows that received product B (antibiotic) during the dry period, compared with no cases in product A (teat sealer) animals, the difference was not significant. The number of cases and causes of clinical mastitis during the first 100 d of lactation is outlined in Table 3. There was no significant difference between the groups in the number of cases by quarter (product A 30/948 cf. product B 35/968) or cow (product A 25/237 cf. product B 31/242). There was no difference between the groups in severity of mastitis (Mann-Whitney); product A (teat sealer): grade 1, 40.0%; grade 2, 50.0%; grade 3, 10.0%; product B (antibiotic): grade 1, 55.9%; grade 2, 23.5%; and grade 3, 20.6%.

IMI present at drying off. Sixty out of 1868 quarters (3.2%) were infected with one or more major pathogens at drying off, 27 (2.9%) in product A (teat sealer) and 33 (3.5%) in product B (antibiotic) animals (Table 4). Of these quarter infections, 10 were identified at calving in each group. The difference in cure rate was not significant. Of the 20 infected quarters that remained at calving, 15 occurred on one farm (farm 6).

Table 1. Median, mean, and SD of parity, last recorded yield, dry period length, and drying off SCC by treatment group.

	Group A (Teat sealer)				Group B (Antibiotic)			
	n	Median	Mean	SD	n	Median	Mean	SD
Parity	252	3	3.6	1.6	253	3	3.4	1.5
Last recorded yield (L)	238	14.4	14.4	5.02	241	14.0	14.3	4.55
Dry period length (d)	241	62	68.0	21.8	242	62	68.7	22.2
Drying off SCC (000/ml) ¹	1002	251	253.8	1250.5	992	242.5	225.3	850.4

¹Quarter geometric mean of samples taken at drying off (>200,000 cells/ml, likely result of concentration effect caused by low milk yield before drying off and effect of geometric mean of quarter data not a composite quarter sample).

Out of 1868 quarters examined, 645 (34.5%) were infected with one or more minor pathogens at drying off, 310 in product A (teat sealer) and 335 in product B (antibiotic) animals (Table 4). The quarter cure rate for *Corynebacterium* spp. was significantly greater in cows that received product B ($P < 0.001$).

Logistic Regression Analysis

New IMI acquired during the dry period. Cows that received product A (teat sealer) were significantly less likely to acquire new quarter infections during the dry period caused by all major pathogens combined

Table 2. Number of quarter and cow IMI acquired during the dry period by causative organism.

Diagnosis	Group A (Teat sealer)		Group B (Antibiotic)	
	New quarter IMI during dry period (n = 928)	New cow IMI during dry period (n = 232)	New quarter IMI during dry period (n = 940)	New cow IMI during dry period (n = 235)
Coagulase-positive staphylococci	10	9	7	6
<i>Streptococcus dysgalactiae</i>	2	2	0	0
<i>Streptococcus uberis</i>	11	11	12	11
<i>Streptococcus</i> spp. (Other)	11	11	14	13
<i>Enterococcus</i> spp.	20	18	35	31
<i>Escherichia coli</i>	13 ^a	11 ^a	42 ^b	35 ^b
<i>Klebsiella</i> spp.	0	0	1	1
<i>Serratia</i> spp.	0	0	2	2
<i>Pantoea</i> spp.	1	1	0	0
<i>Citrobacter</i> spp.	1	1	0	0
<i>Morganella</i> spp.	0	0	1	1
<i>Proteus</i> spp.	2	2	7	7
<i>Enterobacter</i> spp.	0	0	1	1
<i>Hafnia</i> spp.	0	0	1	1
All <i>Enterobacteriaceae</i>	17 ^a	15 ^a	55 ^b	48 ^b
<i>Ochrobacter</i> spp.	1	1	1	1
<i>Chryseomonas</i> spp.	1	1	0	0
<i>Aerococcus</i> spp.	0	0	2	2
<i>Acinetobacter</i> spp.	14	13	14	12
Nonfermenters	11	10	11	10
<i>Bacillus</i> spp.	8	7	5	4
Other nonspecified organisms	0	0	1	1
<i>Aspergillus</i> spp.	1	1	2	2
Yeast	0	0	1	1
<i>Mucor</i> spp.	0	0	4	4
All yeast and fungi	1	1	7	7
All major pathogens	103 ^a	81	145 ^b	100
<i>Micrococcus</i> spp.	19	14	13	12
Coagulase-negative staphylococci	162	108	184	126
<i>Corynebacterium</i> spp.	48	39	39	31
All minor pathogens	218	135	224	142

^{a,b}Numbers within rows and between treatment groups with different superscripts differ ($P < 0.01$).

Table 3. Number of clinical mastitis cases in the first 100 d of lactation, by causative organism.

Organism	Group A (Teat sealer)		Group B (Antibiotic)	
	Quarter cases (n = 948)	Cow cases (n = 237)	Quarter cases (n = 968)	Cow cases (n = 242)
<i>Escherichia coli</i>	7	6	7	7
<i>Streptococcus uberis</i>	8	5	3	3
Coagulase-positive staphylococci	0	0	1	1
<i>Streptococcus dysgalactiae</i>	1	1	2	2
<i>Enterococcus</i> spp.	1	1	1	1
<i>Acinetobacter</i> spp.	1	1	0	0
Coagulase-negative staphylococci	2	2	2	2
<i>Corynebacterium</i> spp.	1	1	0	0
Mixed growth	1	1	4	4
No growth	3	3	8	6
No sample	5	4	7	6
Total	30	25	35	31

(odds ratio = 0.66, 95% CI = 0.47 to 0.93, $P = 0.02$, Table 5), all *Enterobacteriaceae* (odds ratio = 0.30, 95% CI = 0.16 to 0.55, $P < 0.001$, Table 5) and *E. coli* (odds ratio = 0.29, 95% CI = 0.14 to 0.63, $P = 0.002$, Table 5). Model 2 (New IMI caused by all *Enterobacteriaceae* during the dry period) was confounded by last recorded yield. Cows were 1.06 times more likely to become infected with an enterobacterial IMI during the dry period for every 1-L increase in last recorded milk yield ($P = 0.036$). There was no significant difference between the groups in the number of new IMI caused by minor pathogens.

IMI cure during the dry period. There was no significant difference between the groups in the proportion of IMI caused by all major pathogens cured during the

dry period. Cows that received product A (teat sealer) were significantly less likely to cure IMI caused by *Corynebacterium* spp. (odds ratio = 0.06, 95% CI = 0.02 to 0.13, $P < 0.001$, Table 5) during the dry period.

DISCUSSION

This paper reports the first controlled study to demonstrate the efficacy of an internal bismuth subnitrate teat sealer in protecting quarters against new dry period IMI caused by major mastitis pathogens, particularly environmental organisms, under UK field conditions. Internal bismuth sealers were first developed by Meaney in Ireland, as an approach to decreasing dry

Table 4. Number of IMI present at drying off and number and percentage cured during the dry period.

Diagnosis	Group A (Teat sealer)		Group B (Antibiotic)	
	Quarter IMI present at drying off (n = 928)	Quarter IMI cured during dry period (%)	Quarter IMI present at drying off (n = 940)	Quarter IMI cured during dry period (%)
Coagulase-positive staphylococci	8	1 (12.5)	13	5 (38.5)
<i>Streptococcus uberis</i>	0	0 (0)	2	2 (100)
<i>Escherichia coli</i>	0	0 (0)	3	3 (100)
<i>Streptococcus</i> spp. (Other)	3	1 (33.3)	2	0 (0)
<i>Enterococcus</i> spp.	6	6 (100)	6	4 (66.7)
<i>Klebsiella</i> spp.	1	1 (100)	0	0 (0)
<i>Pantoea</i> spp.	0	0 (0)	1	1 (100)
<i>Pseudomonas</i> spp.	1	1 (100)	0	0 (0)
<i>Acinetobacter</i> spp.	2	2 (100)	1	1 (100)
Nonfermenters	2	1 (50.0)	0	0 (0)
<i>Aspergillus</i> spp.	1	1 (100)	1	1 (100)
Yeast	1	1 (100)	1	1 (100)
<i>Bacillus</i> spp.	5	5 (100)	3	3 (100)
All major pathogens	27	17 (63.0)	33	23 (69.7)
<i>Micrococcus</i> spp.	8	8 (100)	12	12 (100)
Coagulase-negative staphylococci	79	54 (68.4)	118	76 (64.4)
<i>Corynebacterium</i> spp.	237	135 ^a (57.0)	219	205 ^b (93.6)
All minor pathogens	310	183 ^a (59.0)	335	279 ^b (83.3)

^{a,b}Numbers within rows and between treatment groups with different superscripts differ ($P < 0.001$).

period IMI. Their efficacy was established using challenge dip models (Meaney, 1976, 1977, 1993). More recently, the efficacy of a reformulated product was demonstrated under New Zealand field conditions (Woolford et al., 1998). In the New Zealand study, quarters containing the seal acquired significantly fewer new dry period IMI compared with negative control quarters. The effect was particularly marked against streptococci, especially *S. uberis* the predominant mastitis pathogen under New Zealand field conditions. There was no significant difference between quarters containing the teat sealer and those containing a long-acting antibiotic tube.

In this UK study, the protective effect was particularly marked for environmental organisms. Environmental bacteria are opportunistic invaders of the mammary gland following migration into the teat cistern via the sphincter. The nonlactating gland is particularly prone to infection. In a recent UK study, 12.8% of quarters acquired new enterobacterial infections during the dry period (Bradley and Green, 2000). The early and late dry periods are the times of greatest risk (Smith et al., 1985). The mammary gland is separated from the external environment by the formation of a keratin "plug," which seals the teat sphincter; a functional plug was demonstrated to be present within 16 d of drying off in one study (Comalli et al., 1984). More recently, it has been reported that approximately 50 and 5% of teats were still 'open' (had an 'inadequate' keratin plug) after 7 and 50 d of the dry period, respectively. Furthermore, 97% of the clinical dry period IMI occurred in these 'open' quarters (Williamson et al., 1995). The internal teat sealer used in this study sinks to the base of the teat cistern and covers the teat sphincter. Radiographical examination has demonstrated the presence of the sealer at the base of the teat cistern 100 d after infusion (Woolford et al., 1998). In agreement with the previous work conducted in Ireland and New Zealand, the results of this UK study indicate that the sealer in its current formulation effectively prevents the entry of mastitis pathogens, particularly environmental organisms, during the dry period.

Last recorded milk yield prior to drying off was significantly related to the risk of acquiring a new enterobacterial infection during the dry period. For every 1-L increase in final yield, cows were 1.06 times more likely to acquire an enterobacterial IMI. Our data would suggest that management at the end of lactation should include strategies to minimize milk yield before drying off to reduce this risk. However the logistic regression model used here did not contain total lactation yield as a potential confounding factor. The possibility remains that the increased risk of acquiring a new enterobacterial IMI is a function of high yielding cattle rather than

of yield prior to drying off. Further work is needed to investigate this relationship.

The dry period cure rate of *Corynebacterium* spp. was significantly lower in quarters receiving the teat sealer (57.0%) compared with quarters receiving the antibiotic tube (93.6%). The cure rate in quarters receiving teat sealer is similar to that described previously for quarters receiving no dry cow therapy (Harmon et al., 1986). Antibiotic dry cow therapy is an efficient method of eliminating IMI caused by *Corynebacterium bovis* (Bramley et al., 1976; Honkanen-Buzalski et al., 1984), our results agree with these previous findings. It has been demonstrated by some authors that quarters infected with *C. bovis* are significantly less likely to become infected with major pathogens (Black et al., 1972; Lam et al., 1997; Rainard and Poutrel, 1988). Others, however, have demonstrated that *C. bovis*-positive quarters were significantly more likely to become infected with environmental streptococci (Hogan et al., 1988) and *Streptococcus agalactiae* (Pankey et al., 1985). The role of *C. bovis* in increasing or decreasing the susceptibility of quarters to subsequent infection with other mastitis pathogens undoubtedly remains a complex issue in need of further investigation. It remains a possible explanation for the lower proportion of major pathogen infections acquired by cows that received the teat sealer.

Infections acquired during the dry period can cause clinical mastitis during the following lactation (Bradley and Green, 2000; Smith et al., 1985). However, there was no significant difference between the groups in the number of cases of clinical mastitis during the first 100 d of lactation despite the significantly greater number of new infections acquired during the dry period by product B (antibiotic) cows. Woolford et al. (1998) also demonstrated a nonsignificant decrease in the number of cases of clinical mastitis between the teat sealer and antibiotic groups. The total number of cases of mastitis in our UK study was low; only 3.4% of quarters and 11.7% of cows were affected compared with 6.5% of quarters and 25.1% of cows suffering clinical mastitis over an identical time period in another recent UK study (Bradley and Green, 2001b). This may have been indicative of the "resistant" subset of animals studied (i.e., cows that did not suffer clinical mastitis during the previous lactation). Alternatively, it could have been a reflection of the clinical significance of the infections acquired. This study was designed to demonstrate equivalence between the groups in new dry period IMI rather than clinical mastitis; a significant difference may have been seen in larger group sizes. If the difference in quarter cases of clinical mastitis between the groups were a real effect, a quarter group size of approximately 12,000 (equivalent to 3000 cows in each group)

Table 5. Logistic regression models used to assess the efficacy of product A in reducing dry period IMI caused by major and minor pathogens.

Variable	Coefficient	SE	Odds Ratio	95% CI	P value
Model 1: Outcome variable—New IMI caused by all major pathogens during dry period					
Constant	-1.99	0.14			
Exposure variable					
DRY COW PRODUCT					
B (Antibiotic) (Reference category)	
A (Teat sealer)	-0.42	0.17	0.66	0.47–0.93	0.02
% SCL ¹	0.96	0.14			
Model 2: Outcome variable—New IMI caused by all <i>Enterobacteriaceae</i> during the dry period					
Constant	-4.10	0.53			
Confounders					
LAST RECORDED YIELD					
Every ↑ in 1L	0.06	0.03	1.06	1.00–1.12	0.036
Exposure variable					
DRY COW PRODUCT					
B (Antibiotic) (Reference category)	
A (Teat sealer)	-1.21	0.31	0.30	0.16–0.55	<0.001
% SCL	0.98	0.31			
Model 3: Outcome variable—New IMI caused by <i>Escherichia coli</i> during the dry period					
Constant	-4.05	0.42			
Exposure variable					
DRY COW PRODUCT					
B (Antibiotic) (Reference category)	
A (Teat sealer)	-1.22	0.38	0.29	0.14–0.63	0.002
% SCL	1.52	0.34			
Model 4: Outcome variable—All minor pathogen IMI cured during the dry period					
Constant	1.91	0.22			
Exposure variable					
DRY COW PRODUCT					
B (Antibiotic) (Reference category)	
A (Teat sealer)	-1.41	0.26	0.24	0.15–0.40	<0.001
% SCL	1.07	0.24			
Model 5: Outcome variable— <i>Corynebacterium</i> spp. IMI cured during the dry period					
Constant	4.07	0.63			
Confounders					
MONTH OF CALVING					
1	0.00	1.00	
2	-0.14	0.66	0.87	0.24–3.17	0.827
3	-1.61	0.61	0.20	0.06–0.66	0.008
4	-1.72	0.57	0.18	0.06–0.54	0.003
5–7	-1.57	0.69	0.21	0.05–0.80	0.022
11	0.19	0.90	1.20	0.21–7.07	0.837
12	-0.53	0.53	0.59	0.21–1.65	0.314
Exposure variable					
DRY COW PRODUCT					
B (Antibiotic) (Reference category)	
A (Teat sealer)	-2.89	0.42	0.06	0.02–0.13	<0.001
% SCL	0.90	0.28			

¹% SCL is a scalar term for the random effect of cow (Egret Manual Version 2.0.3, Cytel Statistical Software Corp., USA).

would have been necessary to detect a significant difference.

Selecting cows suitable for nonantibiotic dry cow therapy (such as teat sealers) is dependent on reliably identifying uninfected quarters at drying off. The cost and practicalities of quarter bacteriology make it an

unrealistic method on commercial UK dairy units. Historical mastitis and SCC data are available on many farms and may provide a practical alternative method to identify eligible cows. The use of these data will inevitably lead to the misclassification of both false-positive (not infected, but excluded by the enrollment

criteria) and false-negative cows (infected, but selected by the enrollment criteria). In this study, the use of a cow SCC threshold of 200,000 cells/ml led to 11.3% of cows (60 quarters in 53 cows) being incorrectly identified as uninfected with major pathogens. It is not possible, using the data collected, to calculate the number of false-positive cows that were excluded inappropriately. However, there was no significant difference in the apparent quarter cure rate between the two treatment groups (63% teat sealer vs. 70% antibiotic tube). If the difference in cure rate seen here is a real effect (as would seem likely because the teat sealer has no antibacterial properties) it would be necessary to recruit nearly 12,800 quarters (3200 cows) to each group before the difference could be shown to be significant.

Across the 16 herds, the proportion of cows eligible for recruitment to the study ranged from 24 to 71%, with a mean value of 47.8%. Herd BMSCC, mastitis incidence, and particularly the proportion of herd affected with mastitis had the most bearing on numbers enrolled. Dry period length (less than 51 d), treatment within the previous 30 d, and minor teat abnormalities did, however, exclude significant numbers of cows on some farms. Calving seasonality was an issue on farms 5, 12, and 14 because the recruitment period was late in the season for these herds, biasing the sample towards older (and therefore higher SCC) cows, resulting in a lower recruitment proportion in these herds.

It must be reiterated that nonantibiotic dry cow therapies (such as the teat sealer used in this study) are not designed to treat existing infections present at drying off. For animals not deemed eligible to receive nonantibiotic therapies, antibiotic dry cow treatment will remain vital. Failure to treat these animals effectively could lead to the build-up of a reservoir of subclinical infection within herds, which will increase BMSCC and the incidence of clinical mastitis.

The introduction of an internal teat sealer without antibacterial properties into the mammary gland represents a potential risk to the quarters treated. Pathogens present around the teat sphincter or in the environment could be inoculated into the quarter or the seal material may act as a nidus for infection if the product is contaminated during the infusion process. The teat disinfection process employed during this study before product infusion was fastidious. Users in the field need to be made aware of the potential dangers and adequately trained to maintain aseptic infusion techniques of the highest standards. These precautions will minimize the potential risks of inoculating pathogenic organisms into the quarter, at the time of product administration.

If historical mastitis and SCC data alone are used to select cows suitable for nonantibiotic dry cow therapy, lowering the SCC threshold to reduce the number of

false-negative cows included will increase the number of false-positive cows excluded to the point that the total number of animals eligible may be unacceptably low in commercial situations. Sealing a small number of infected quarters is acceptable providing the potential benefits (e.g., reduced numbers of new dry period infections) outweighs either the impact of missing real infections or the cost of identifying uninfected cows absolutely, e.g., quarter sample bacteriology. More research is needed to identify the most suitable regimes for using historical SCC and mastitis data for identifying uninfected cows at the end of lactation.

If the use of nonantibiotic dry cow therapies becomes widespread, the ability to diagnose quarter infection status by methods other than bacteriology (which remains expensive and time consuming) or analysis of historical data (as used in this study) will become increasingly important. California mastitis test results (Poutrel and Rainard, 1981; Rindsig et al., 1978) and N-acetyl-beta-D-glucosaminidase levels (Hassan et al., 1999) at drying off have previously been used to diagnose cows suitable for selective dry cow therapy regimes. Milk conductivity (Chamings et al., 1984; Hillerton and Walton, 1991) and, more recently, acute-phase proteins (Gronlund et al., 2001) have also been suggested as detection methods for subclinical mastitis. The relative merit of these methods is dependent on their abilities to detect subclinical major pathogen infections present at drying off with a high degree of sensitivity and specificity. This remains an area in need of further research.

New dry period IMI were diagnosed with a single bacteriological isolation of a pathogen at calving in quarters uninfected at drying off. The authors accept that this may lead to an increase in the proportion of both false-positive (transient infections) and false-negative quarters in both groups, compared with methods based on reisolation of causal pathogens in second or third samples. However, the increase in accuracy and repeatability between these methods can be as little as 5% (Griffin et al., 1987) providing precautions such as careful aseptic sampling technique, use of accredited laboratories and "blinding" of microbiological operators are taken to minimize risks, these were used in this study. Quarter SCC thresholds can be used in conjunction with single bacteriological isolation of a pathogen in an attempt to decrease the number of false-positive and -negative diagnoses; levels of 125, 250, 500, or 1000 $\times 10^3$ cells/ml have been suggested (Griffin et al., 1987). Adding any of these SCC thresholds using the quarter level SCC data from the drying off and calving samples to the data produced in this study does not alter either the outcome or significance of the results.

The authors accept that assessing bacteriological cure rate during the dry period on single isolation after the dry period, especially for organisms such as *S. aureus*, which are intermittently shed from infected glands, will lead to an overestimation of the efficacy of a product. This study was designed to investigate the new dry period infection rate, not dry period cure rate; cure rate results from this study should be interpreted with care.

Antibiotic dry cow therapies were originally designed to control contagious mastitis; most products have limited activity against gram-negative bacteria. Gram negative and environmental bacteria are becoming increasingly important causes of clinical mastitis in the UK as levels of contagious mastitis fall (Bradley and Green, 2001a). This combined with the drive to reduce the use of routine prophylactic antibiotic treatments in animals (in an attempt to prevent the build up of antimicrobial resistance in humans) will increase the pressure to prescribe dry cow therapy rationally, justifiably, and to the individual not the herd. It is likely that the rationale for the use of high doses of long-acting antibiotics in cows uninfected with major pathogens at drying off will become increasingly untenable if effective alternatives for the prevention of new infections during the dry period are available. The internal teat sealer used in this study could be one such viable alternative.

CONCLUSIONS

This study has demonstrated that an internal teat sealer dry cow therapy based on bismuth subnitrate can significantly reduce the number of new IMI acquired during the dry period compared with the UK market leading antibiotic dry cow therapy under UK field conditions. In the light of these findings, the continued blanket use of antibiotic dry cow therapy in low SCC cows needs to be questioned and reevaluated. Further work to support the usage of internal teat sealers is needed in this novel and evolving area of dry cow therapy.

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