An Assessment of the Benefits of Orbeseal® When Used in Combination with Dry Cow Antibiotic Therapy in Three Commercial Dairy Herds

NB Cook, *BSc, BVSc Cert CHP, DBR, MRCVS*;* **DA Pionek,** *DVM;* **P Sharp,** *BS*
School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706-1102
(*Corresponding author: University of Wisconsin-Madison, School of Veterinary Medicine, 2015 Linden Drive West, Madison, Wisconsin 53706-1102; Tel: 608 265 4981; Fax: 608 262 8595; Email: nbcook@wisc.edu

Abstract

An internal teat sealant containing bismuth subnitrate was used in combination with dry cow antibiotic therapy in a cow-level prospective case-control field trial in three herds with different dry cow management practices. Treated quarters (antibiotic + sealant) had a 59% lower odds of developing a new dry period infection at 1-3 days-in-milk (DIM) than control (antibiotic only) quarters (P<0.001). Herd-level variation in new intramammary infection (IMI) and mastitis treatment rates during the first 100 DIM were observed. Greatest benefits from internal teat sealant use were documented in herds with the highest rates of new IMI during the dry period, and for environmental streptococci. Economic benefit accrued from differences in the timing of treatment for mastitis was estimated to be $5.38 per cow in the herd.

Résumé

Une étude cas-témoin prospective au niveau de la vache a été menée sur le terrain dans trois troupeaux avec une régie différente du tarissement. Dans cette étude, on a combiné un traitement antibiotique au tarissement avec un scellant à trayons intramammaire contenant du sous nitrate de bismuth. Les chances de développer une nouvelle infection dans la période de tarissement durant les trois premiers jours en lait diminuaient de 59% dans les quartiers traités (antibiotique et scellant) par rapport aux quartiers non-traités (antibiotique seulement) (p < 0.001). On observait une certaine variation au niveau du troupeau dans le taux de nouvelle infection intramammaire et dans le taux de traitement contre la mammite pendant les 100 premiers jours en lait. Les plus grands bénéfices de l'utilisation du scellant à trayons intramammaire prenaient place dans les troupeaux avec le plus haut taux de nouvelle infection intramammaire durant le tarissement et pour les streptocoques environnemen-taux. Les bénéfices économiques qui découlent des différences dans le choix du bon moment pour traiter la mammite ont été évalués à $5.38 par vache du troupeau.

Introduction

Over the last few years there has been considerable interest in strategies to reduce the high rate of new intramammary infection (IMI) during the non-lactating period of the dairy cow. Susceptibility to infection with gram-negative bacteria,[3,15] streptococci and other gram-positive bacteria[4,14] has been repeatedly shown, and the cow-level factors contributing to this risk of infection have been thoroughly reviewed elsewhere.[5] Use of external and internal teat sealants to prevent new infections has been a subject of much interest, particularly in countries where widespread use of intramam-mary dry cow antibiotic therapy is becoming increasingly difficult to justify to concerned consumers.

A novel internal teat sealant, originally developed in Ireland in the mid-1970s,[11] has recently become commercially available. This teat sealant persists in the teat cistern throughout the entire dry period, and is now widely used on dairy farms across the world, including many in North America in the last year. The current product,[a] Orbeseal®, is marketed in a 4g intramammary syringe, and contains 2.6g (65% wt/wt) of bismuth subnitrate in an oily base.

In countries with a low legal limit for bulk-tank somatic cell count (BTSCC), such as New Zealand and the United Kingdom, the internal sealant has been marketed as a replacement for antibiotic use in the uninfected cow at dry-off. As such, several studies have shown that Orbeseal is more effective than no treatment, and is just

as effective, or more effective, than dry cow therapy alone for preventing non-lactating period new infections.[1,10,17]

In North America, which has a higher legal limit for BTSCC than many other areas of the world, and consequently a larger proportion of each herd potentially subclinically infected in late lactation, Orbeseal has been incorporated into dry cow management programs by administering the sealant after infusion of dry cow antibiotic. The strategy has been for the sealant to aid in preventing new IMI during the dry period, while the long-acting antibiotic remains to cure existing infections. Few studies have been done to show that the sealant is more effective than dry cow antibiotic therapy alone. In a New Zealand study using a split-udder design, no difference in new IMI rate was observed between quarters treated with a long-acting dry cow preparation (250 mg cephalonium) and quarters sealed after administration of 600 mg of cloxacillin.[17] In the only published US study using Orbeseal, which also used a split-udder design, quarters treated with 500 mg of cloxacillin alone[b] were compared with those which received both sealant and cloxacillin. Odds of developing a new IMI in the sealed quarters was reduced by 30%, suggesting benefits over and above that of dry cow therapy alone.[8] Preliminary data from a Canadian study also showed a very similar beneficial effect of a combination of sealant and antibiotic over antibiotic alone in infected quarters.[13] Economic benefit of using the sealant must come from reducing treatments for subclinical and clinical mastitis, and from improvements in the BTSCC. This is particularly challenging when the sealant is used concurrently with traditional dry cow antibiotic therapy.

One UK study[10] demonstrated that coliform infections responsible for mastitis up to 109 DIM were the result of infections which originally occurred in the dry period, and Green et al[9] recently showed that mastitic quarters infected with an udder pathogen during the dry period succumbed to clinical mastitis earlier in lactation, compared to quarters not infected in the dry period. These infections and mastitis cases may be influenced by the use of Orbeseal. Berry and Hillerton[1] found that clinical mastitis during the first 100 DIM was lower in sealant-treated cows than untreated control cows, and Godden et al[8] demonstrated a small, but significant reduction in clinical mastitis up to 60 DIM in quarters treated with sealant in combination with antibiotic, compared with antibiotic alone. However, trials using split-udder designs may not show the full benefit of treatment. Interdependence among quarters has been demonstrated[2] which would lead to a decrease in the number of cows with only one quarter infected, and an increase in the number of cows with either uninfected quarters, or two or more infected quarters. Thus, trials with split-udder designs may underestimate the true impact of a prevention strategy implemented at the cow-level. These differences may be due to an individual cow susceptibility effect, or due to a pathogen type and exposure effect. Awareness of these factors is necessary to predict the overall effect of a herd-level teat sealant strategy.

This study was conducted to examine the use of Orbeseal in commercial dairy herds at the cow level in combination with dry cow antibiotic therapy, and compared with antibiotic use alone. Herds with variation in dry cow management and housing were deliberately chosen, and application of the product was performed by trained herdsmen rather than the researcher in order to more accurately reflect field experiences with the product. To evaluate the economic return from using Orbeseal, differences in the rate of mastitis treatments to 100 DIM between treated (antibiotic + sealant) and control (antibiotic alone) groups were investigated.

Materials and Methods

Herd Selection and Management

Three herds in Wisconsin were selected on the basis of location, presence of monthly DHIA recording, adequacy of records and a willingness to follow the study protocol. Herd sizes were 309, 1337 and 1081 lactating cows at the start of the trial. Weighted mean annual somatic cell counts from monthly cow testing were 309,000/ml, 307,000/ml and 211,000/ml for herds A, B and C, respectively. Mean rolling herd average milk production was 25,032 lb (11,378 kg) with a range of 22,528-26,303 lb (10,240-11,956 kg). All lactating cows in each of the herds were housed year round in free stalls. All cows were dried off abruptly based on predicted calving date and milk yield, and blanket dry cow therapy was practiced using 1,000,000 units procaine penicillin G and one gram dihydrostreptomycin sulfate.[c] A J5 E.coli vaccination program was used on all farms, using three doses: at dry-off, at two weeks before calving, and at one-to-two weeks after calving.

All herds maintained two groups of dry cows. Following dry-off in the parlor, cows were transported to a separate dry cow facility away from the main dairy. In Herd A, far-dry cows were able to lie on unbedded free stalls fitted with rubber mats, but were predominantly exposed to a small dirt-lot area. The close-up group of cows (approximately 1-20 days prepartum) was able to lie on a loose housed bedded pack using straw or corn stalks. In Herd B, sand free stalls in a converted stanchion barn was the predominant form of housing for the far-off dry cows, while close-up cows were moved to a three-row pen with sand bedded free stalls at the main lactating cow facility. In Herd C, both far-off and close-up cows were managed on straw bedded packs, with the far-dry cows having occasional access to pasture.

In all herds, periparturient cows calved on a straw bedded pack maternity area and were transferred to free

stalls with either deep sand (Herd B) or mattresses bedded with sawdust (Herds A and C) at one to three days after calving.

Cow Selection and Sampling

Cows were eligible for inclusion in the trial provided they exhibited no clinical signs of disease, had four functional quarters free of teat abnormality (other than hyperkeratosis) or trauma, had not received antibiotic or anti-inflammatory treatment within the previous 30 days and were expected to calve within 100 days. Treatment allocation used a randomly generated assignment scheme established in advance, and cows were enrolled sequentially, starting with the first cow in the parlor, to either treatment (antibiotic + sealant) or control (antibiotic only) groups, according to the enrollment sheet.

Treatment administration was supervised by the researcher to ensure correct product allocation, but all sampling and product administration was performed by farm personnel following pre-arranged standard operating procedures. Disposable nitrile gloves were worn by all personnel for milk sampling and product administration. Single-quarter microbiological milk samples were collected from all cows fulfilling the enrollment criteria. Prior to sampling, teats were disinfected with an approved iodine pre-dip product and wiped dry with an individual cloth towel after a contact time of approximately 30 seconds. A 4x4 inch 8-ply gauze sponge, soaked in 70% alcohol, was used to scrub the teat ends. The alcohol was allowed to dry for at least one minute.

After milking, teat ends were again scrubbed with 70% alcohol prior to insertion of dry cow therapy. Partial insertion technique was used, and each teat and gland was massaged after infusion. Following dry cow antibiotic treatment, teat ends of cows allocated to the treatment group were scrubbed with 70% alcohol once more prior to insertion of an internal teat sealant containing 65% (wt/wt) bismuth subnitrate in an oily base (Orbeseal®).[a] The product was inserted into the teat cistern using a mid-length nozzle plastic tube, and care was taken not to massage the teat or gland after infusion. Cows in the control group received dry cow antibiotic alone. All teats received an approved non-barrier post-dip product prior to exiting the parlor.

Within three days of calving, single-quarter milk samples were taken from each cow by the herdsman on each farm, following similar sampling procedures as described above. Quarter milk samples were also requested from all cows identified with clinical mastitis during the first 100 days-in-milk, prior to the administration of treatment.

Cows were recruited into the trial between June 2002 and December 2002, and monitored for mastitis through June 2003.

Sample Handling and Bacteriology

The bacteriologist was blinded to the treatment group of each sample through coding of the sample vials prior to submission. Quarter-milk samples collected at dry-off were kept in a cool box in ice during transportation to the laboratory, where they were held frozen (-4°F; -20°C) until they were thawed and plated. Quarter-milk samples collected at calving time and from clinical mastitis cases were stored frozen (-4°F; -20°C) on farm, transported in a cool box in ice to the laboratory, where they were again held frozen (-4°F; -20°C) until they were thawed and plated. After thawing at room temperature, milk samples were vortexed until homogenous immediately prior to culturing. A 0.1 ml volume of each sample was inoculated onto the surface of a blood agar plate (BAP) using a calibrated pipette, and a sterile disposable inoculating loop was used to spread the sample evenly over the entire surface of each agar plate. All plates were inverted and incubated for 18-24 hours at 98.6°F (37°C). Plates that had no growth or no significant growth after 18-24 hours of incubation were incubated as above for an additional 24 hours and re-examined. Udder pathogen recognition was performed using the guidelines of the National Mastitis Council.[12]

Staphylococci (catalase-positive, gram-positive cocci) were tested for coagulase activity using rabbit coagulase plasma EDTA.[d] Coagulase-positive staphylococci were tested for acetoin production by inoculation of MRVP broth.[d] Those that were positive were classified as *Staphylococcus aureus*. Staphylococci that were coagulase-negative (CNS), and those that were coagulase-positive but VP-negative were classified as non-aureus staphylococci.

When significant growth of streptococci/enterococci (catalase-negative, gram-positive cocci) was present, the species was determined using the API 20 Strep kit.[e] Results were reported as *Streptococcus agalactiae* (none found), *Streptococcus uberis, Streptococcus dysgalactiae, Enterococcus* species, or *Streptococcus* non-*agalactiae* (SNAG).

An oxidase test was performed on gram-negative rods, which were identified using the API 20E kit.[f] Results were reported as *Escherichia coli* or by genus for other types. Oxidase-positive organisms often cannot be identified using this system; in that case, these bacteria were simply reported as "other gram-negative rods".

Determination of significance of growth was based on the number of colony forming units of each type of bacteria present, and the number of different types of bacteria present on a single plate. Predominant growth was generally considered significant. Any β-hemolytic gram-positive organism was viewed as potentially significant, with the exception of *Bacillus* species. The following were considered significant growth:

- ≥500 cfu/ml *Bacillus, Corynebacterium,* or *Nocardia* species, if present in pure culture.
- ≥500 cfu/ml CNS, coagulase-positive VP-negative *Staphylococcus* species, non-*agalactiae Streptococcus* species, or *Arcanobacterium pyogenes* if ≤50 cfu/ml other bacteria were present.
- Any amount of a coagulase-positive, VP-positive *Staphylococcus* or *Streptococcus agalactiae.*
- Any amount of a gram-negative rod (with the exception of *Proteus* species), or yeast if present in pure culture.

Additional Data Collection

Lactation number at dry-off, last DHIA (Dairy Herd Improvement Association) recorded actual milk yield and ICSCC (individual cow somatic cell count) prior to dry-off, drying-off date, calving date and first DHIA recorded ICSCC were recorded for each cow in DairyComp305 and transferred into an Excel spreadsheet. Data related to the removal of any cow from the study, including animals which were sold or died, were also recorded. In all herds, quarters were treated when clinically infected – with the observation of abnormal fore-milk at milking time. Treatment was also administered if quarters were found to be strongly positive to a California Mastitis Test (CMT) at 1-3 DIM, usually at the time of sampling for culture. All cows in the study were subjected to CMT at this time.

Definitions

The following definitions were used to define intramammary infection and mastitis:

New Dry Period IMI

A significant isolate of an organism found in a quarter at the 1-3 DIM sample that was not present in the quarter sample at dry-off.

Dry Period 'Cure'

Absence of an organism in a quarter at the 1-3 DIM sample that was present at dry-off.

Mastitis Treatment

A quarter treated with antibiotic because of detection of a positive reaction with the CMT at 1-3 DIM, or presence of abnormal milk up to 100 DIM.

Quarter Case of Mastitis

A mastitis event in a single quarter. Repeat cases occurring in the same quarter were considered a new clinical quarter case if the interval between events exceeded seven days.

Cow Case of Mastitis

A cow suffering mastitis in one or more quarters at the same time. Repeat cow cases were considered a new cow case if the interval between events exceeded seven days.

Economic Analysis

Frequency and distribution of first-cow cases of mastitis were used to model the cost benefit of using Orbeseal in each herd and for all herds. Cost of treatment and labor costs were not included in the assessment. The daily milk yield reduction model developed by Wilson *et al*[16] for lactation ≥2 cows was used to predict different lactation milk yield losses for mastitis cases occurring in weeks one to 15 after calving. Yield loss was corrected for residue withdrawal from dry cow antibiotic use for the first four days of lactation and for the use of intramammary lactational treatment (four days withdrawal). For example, lactational milk yield loss for a mastitis treatment in week one was calculated to be 1573 lb (715 kg), compared to a loss of 884 lb (402 kg) for a treatment occurring in week 14. Weekly distribution of cow cases per 100 cows was calculated for each herd, and cumulative lactational loss in milk potentially available for sale was calculated for treatment and control cows. A cost-benefit calculation for Orbeseal use was performed, off-setting the cost of the sealant at $1.80 per tube against the potential change in income from the sale of milk at a milk price adjusted for increased feed costs of $0.135 per lb ($0.297 per kg).

Statistical Analysis

Random allocation of cows by parity, proportion of cows with the last ICSCC greater than 200,000/ml and distribution of pathogens in quarters at dry-off between treatment groups was tested using Cochran-Mantel-Haenszel statistics based on table scores produced using the FREQ procedure in SAS version 8.0 (SAS, 1999). The Mixed Procedure in SAS (PROC MIXED, SAS, 1999) was used to compare dry period length, last DHIA recorded milk yield at dry-off and last DHIA ICSCC linear score between treatment groups. Effect of treatment on first ICSCC linear score was also examined in PROC MIXED, with calving month, dry period length, last recorded milk yield at dry-off and last recorded ICSCC linear score included as covariates and farm as a random effect.

Multivariate logistic binomial regression (PROC GENMOD in SAS, version 8.0) was used to investigate the relationship between treatment groups for new dry period IMI determined at 1-3 DIM. Farm was included as a fixed effect in all models, and a random term for cow was included in the repeated statement to account for clustering of infections in quarters within cow using an unstructured correlation format. Additional covariates used in the models included parity, calving month, last milk yield before dry-off, last ICSCC and dry period length. A backwards step-wise procedure was used to determine the final model. Fixed effects of farm, parity and a farm by treatment interaction were forced into all models. Statistical significance was declared at P<0.05.

Interactions between significant covariates were examined where appropriate. The analysis was repeated for new dry period infections caused by different pathogen groups, namely *Staphylococcus aureus*, gram-negatives, environmental streptococci, non-aureus staphylococci and other pathogens. A similar multivariate logistic binomial regression model was also developed to examine differences in dry period cure between treatment groups.

A χ^2 test was used to compare the rate of quarter cases of mastitis between treatment groups. The effect of treatment on the first cow case of mastitis was investigated using logistic binomial regression models in PROC GENMOD (SAS, 1999), controlling for the covariates previously stated in the manner described above.

Differences in the timing of the first clinical mastitis event between treatment groups were examined using a Cox proportional hazards regression model in the PHREG procedure of SAS (SAS, 1999). This described the survival distribution for control compared with treatment cows, using the date of the first clinical cow case of mastitis relative to calving. Cows were considered at risk of clinical mastitis from the date of calving (or the date of the first case of mastitis pre-calving in one cow) to 100 DIM. Cows and cow cases were classified as censored at the reported herd removal date or 30, 60 and 100 DIM in three separate models. Covariates included in the models were farm, parity, last ICSCC, dry period length, last milk yield before dry-off and calving month.

Results

Allocation of Cows to Treatment Groups

From an initial 608 cows enrolled from all three herds, data were available from a total of 528 cows (2112 quarters) calving between August 8, 2002 and February 22, 2003, after removal of animals with dry periods less than 42 and greater than 100 days (n=23), cows with missing data at dry-off (n=10) and cows that were missed for sampling at calving (n=47). There was no significant difference in the distribution of animals removed from the study between treatment and control groups, avoiding bias from omission of potential treatment failures.

The random allocation procedure at dry-off was successful in distributing cows evenly between treatment groups, with no significant differences (P>0.05) observed relative to parity, last milk yield, dry period length, last ICSCC and proportion of cows >200,000 at last ICSCC. Across all three herds, control cows averaged 59.6 lb (27.1 kg) milk at the last DHIA test before dry-off, and a mean dry period length of 60.8 days. Treatment cows averaged 55.9 lb (25.4 kg) milk and a dry period length of 60.2 days. A total of 258 control cows and 270 treatment cows were available for analysis. The data are summarized in Table 1.

Infection status at dry-off for 1032 control quarters and 1080 treatment quarters are summarized in Table 2. There were no overall significant differences (P=0.10) in pathogen profile between treatment groups, with 12.3% of quarters with an IMI overall. The treatment group had a numerically higher prevalence of quarter infection with *Enterococcus* spp than the control quarters, and conversely, the rate of infection with *Staphylococcus aureus* was higher in the control group.

Effect of Treatment on New Dry Period IMI

Significant herd (P=0.001) and treatment (P<0.001) effects on the proportion of quarters developing a new IMI between dry-off and 1-3 DIM were identified. Mean rates of new infection and odds ratios are presented in Table 3. For all herds, the mean rate of new IMI in control quarters was 16.5%, with a wide range between the three herds from 9.1 to 29.6%. Rate of new IMI in treat-

Table 1. Least squares mean (SE) parity, last DHIA recorded milk yield (lb), dry period length, last DHIA recorded ICSCC linear score and the proportion of cows with a last ICSCC greater than 200,000/ml by treatment group, by herd and for all herds combined.

	Herd A		Herd B		Herd C		All Herds	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
No. cows	39	37	123	135	96	98	258	270
Parity (SE)	2.2 (0.2)	2.3 (0.2)	1.7 (0.1)	1.8 (0.1)	2.0 (0.1)	1.7 (0.1)	1.9 (0.1)	1.9 (0.1)
Last DHIA milk yield (SE, lb)	64.5 (3.5)	60.3 (3.5)	66.2 (6.6)	60.1 (1.8)	48.4 (1.8)	56.1 (1.5)	59.6 (5.3)	55.9 (5.3)
Dry period length (SE, days)	61.1 (1.3)	60.5 (1.2)	64.9 (0.8)	63.9 (0.9)	56.4 (0.9)	56.1 (0.9)	60.8 (2.4)	60.2 (2.4)
Last DHIA linear score (SE)	3.88 (0.30)	3.93 (0.31)	3.63 (0.17)	3.79 (0.16)	2.78 (0.17)	2.79 (0.17)	3.42 (0.36)	3.50 (0.35)
No. (%) >200,000/ml last ICSCC	17 (43.6)	15 (40.5)	53 (41.3)	44 (32.8)	23 (24.0)	26 (26.5)	90 (35.2)	85 (31.6)

Table 2. Prevalence of IMI and profile of bacterial isolates present in control (n=1032) and treatment (n=1080) quarters at dry-off.[a]

	Control (n=1032)	Treatment (n=1080)	All (n=2112)
Quarters with no growth	882	943	1825
Total quarters with IMI	128	127	255
Quarters with mixed IMI	3	4	7
Quarters with contaminated sample	22	10	32
Percent of all quarters with IMI	12.7	11.9	12.3
Bacterial Isolates			
Staphylococcus aureus	15	9	24
Streptococcus dysgalactiae	1	1	2
Streptococcus uberis	0	1	1
Enterococcus spp	2	8	10
Other environmental streptococci	7	6	13
Total environmental streptococci	10	16	26
Escherichia coli	5	2	7
Klebsiella spp	2	5	7
Enterobacter spp	0	1	1
Pseudomonas spp	1	0	1
Other gram-negative rods	1	0	1
Total gram-negative pathogens	9	8	17
Non-aureus staphylococci	87	91	178
Corynebacterium spp	9	6	15
Nocardia sp	0	1	1
Pasteurella spp	1	0	1
Total Other	10	7	17

[a] Table reports all species cultured from single and mixed infections.

ment quarters was significantly lower (P<0.001), with a mean of 8.0% and a range from 6.9 to 11.9%. The odds of a treated quarter developing a new IMI compared with a control quarter were reduced by 59%, with a range from 28 to 72% between herds. These reductions were significant overall, and for Herds A and C, but not in Herd B, where the control rate of new IMI was the lowest. Parity was significant in the final model (P=0.026), with the rate of new IMI being lowest in cows beginning their second lactation and highest in cows beginning their fourth or greater lactation. Presence of an IMI at dry-off also significantly influenced the risk for new IMI independent of treatment (P=0.006). Specifically, the presence of *Staphylococcus aureus* or a non-aureus *Staphylococcus* spp reduced the rate of new IMI, and presence of an environmental streptococcus organism increased the risk relative to no infection or infection with a gram-negative organism. No other covariates were significant.

The unstructured model produced a correlation matrix to examine the interaction of new IMI between quarters within cow. The correlation coefficients suggested that both front quarters and both rear quarters were more closely correlated than interactions between front and rear quarters on either side of the udder.

Within new IMI pathogen groups, treatment effects were significant for the environmental streptococci (P<0.001) and for non-aureus staphylococci (P=0.029), but not for *Staphylococcus aureus* (P=0.102), gram-negative infections (P=0.102) and other pathogens (P=0.712). Table 4 summarizes the new IMI by bacterial isolate for all cows, and Table 5 documents the results of the multivariate regression analyses with odds ratios.

Effect of Treatment on First Linear Score

There was no significant difference in first ICSCC linear score between treatment groups (treatment=2.28, control=2.66, P=0.17), but last DHIA recorded ICSCC linear score before dry-off was significant in the final model (P<0.001).

Effect of Treatment on Risk of Dry Period Cure

The proportion of treated quarters experiencing a cure between dry-off and 1-3 DIM was not significantly different from control quarters (P=0.865), though numerically higher (90.1% treated v. 80.6% control). There were significant farm effects (P=0.03), with cure rates in Herd C being higher than Herd B, which was in turn higher than Herd A. Predictably, last DHIA recorded ICSCC was

Table 3. Results of multivariate regression analysis of odds of acquiring a new IMI between dry-off and 1-3 DIM for control and treatment quarters, for all quarters and by herd.

Herd	Control No. affected (%)	Treatment No. affected (%)	Estimate (SE)	Odds ratio$_{treatment}$ (95% confidence limits)	P value
All (control n=971, treatment n=1009)	160 (16.5%)	81(8.0%)	-0.88(0.17)	0.41 (0.30, 0.58)	<0.001
Herd A (control n=152, treatment n=143)	45 (29.6%)	17 (11.9%)	-1.26 (0.36)	0.28 (0.14, 0.57)	0.001
Herd B (control n=453, treatment n=489)	41 (9.1%)	34(6.9%)	-0.33 (0.26)	0.72 (0.43, 1.20)	0.211
Herd C (control n=366, treatment n=377)	74 (20.2%)	30(8.0%)	-1.05(0.26)	0.35 (0.21, 0.57)	<0.001

Models controlled for farm, parity, calving month, last DHIA ICSCC, dry period length and milk yield at the last DHIA test before dry-off.

significant in the final model (P<0.001), with cows with higher ICSCC at dry-off having lower cure rates.

Effect of Treatment on Risk of Mastitis to 30, 60 and 100 DIM

Quarter case rate for mastitis was 53.9 quarter cases per 100 cows in the control group and 42.6 quarter cases per 100 cows in the treatment group (χ^2 = 2.91, P=0.088). Unfortunately, culture samples were missed from 54.7% of first mastitis treatments, which hampered statistical analysis of mastitis data by pathogen type. Across all herds, 23.7% of cows in the treatment group were treated for mastitis within 100 DIM, compared with 29.1% of cows in the control group (P=0.140). No other covariates were significant in the logistic model, although there was a strong trend for a herd effect (P=0.077).

Timing of first treatment was examined more closely using a Cox proportional hazards model, the survival plot of which is shown in Figure 1 for all herds. Overall, for all cows treated within 100 DIM, the hazard ratio of 0.76 for treatment effect was not significant (P=0.106). However, there were differences by herd and by time after calving. The results of three models for all herds and for each herd censored by three different DIM – namely 30, 60 and 100 days after calving – are shown in Table 6. The 60-day model proved to be significant for all herds (P=0.03) with a hazard ratio of 0.65. Significance was also observed for Herd C at both 30 DIM and 60 DIM, with hazard ratios of 0.38 and 0.48, respectively. All hazard ratios favored the treatment group, except in Herd B up to 30 DIM.

Economic Analysis

Differences in the timing and frequency of the first cow case of mastitis led to an improvement in the milk available for sale prediction in the treatment group over the control group ranging from 3,120 to 15,585 lb (1,418 to 7,084 kg) milk per 100 cows, with an average for all herds of 9,321 lb (4,237 kg; Table 7). The cost benefit calculation for all three herds gave a mean benefit per cow of $ 5.38 for Orbeseal use. For Herds A and C, the benefit was $10.77 and $13.84 per cow, but in Herd B the improvement in milk available for sale was not sufficient to off-set the increased cost of using Orbeseal, resulting in a small net loss of $2.99 per cow.

Discussion

Quarters treated with Orbeseal combined with dry cow antibiotic therapy were at 59% lower odds of developing a new IMI at 1-3 DIM than quarters treated with antibiotic alone. This finding supports those of previous authors who similarly found that the internal sealant can help control new IMI during the non-lactating period. Two studies, the current one and one performed on two other US herds,[8] have shown there is a significant effect over and above that of antibiotic use alone. The latter study used a split-udder design and found a 30% lower odds of new IMI in treated quarters – this is approximately half the effect observed in the work described here using a whole-cow comparison. Both studies used a single sample at dry-off to classify quarters as infected and non-infected. The disadvantages of using this tech-

Table 4. Bacterial isolates from new IMI identified at 1-3 DIM from control and treated quarters and for all cows.[a]

	New IMI acquired between dry-off and 1 to 3 DIM		
	Control (n=971)	Treatment (n=1009)	All (n=1980)
Total quarters with new IMI	160	81	241
Quarters with mixed new IMI	9	2	11
Percent of all quarters with IMI	16.5	8.0	12.2
Bacterial Profile			
Staphylococcus aureus	22	11	33
Streptococcus dysgalactiae	10	1	11
Streptococcus uberis	12	3	15
Enterococcus spp	9	2	11
Other environmental streptococci	14	4	18
Total environmental streptococci	45	10	55
Escherichia coli	7	4	11
Klebsiella spp	1	0	1
Enterobacter spp	5	1	5
Proteus spp	1	0	1
Other gram-negative rods	5	4	9
Total gram-negative pathogens	19	9	27
Non-aureus staphylococci	78	47	124
Yeast	1	1	2
Bacillus spp	1	1	2
Corynebacterium spp	0	1	1
A.pyogenes	2	2	4
Blind quarter	0	1	1
Total other	4	6	10

[a] Table includes all species cultured from quarters with single and mixed new infections.

Table 5. Results of multivariate regression analysis of odds of acquiring a new IMI between dry-off and 1-3 DIM by pathogen group for control quarters and treated quarters.

Pathogen group	Control No. affected (%)	Treatment No. affected (%)	Estimate (SE)	Odds ratio$_{treatment}$ (95% confidence limits)	P value
Staphylococcus aureus	18 (1.8)	10 (0.99)	-0.84 (0.51)	0.43 (0.16, 1.18)	0.102
Environmental streptococci	41 (4.2)	10 (0.99)	-1.59 (0.38)	0.20 (0.10, 0.43)	<0.001
Gram-negative pathogens	15 (1.5)	7 (0.69)	-0.89 (0.54)	0.41 (0.14, 1.19)	0.102
Non-aureus staphylococci	73 (7.5)	46 (4.6)	-0.51 (0.23)	0.60 (0.38, 0.95)	0.029
Other pathogens	4 (0.41)	6 (0.59)	0.27 (0.72)	0.30 (0.32, 5.36)	0.712

Models controlled for farm, parity, calving month, last DHIA ICSCC, dry period length and milk yield at the last DHIA test before dry-off.

nique compared to using the culture information from multiple sample points prior to dry-off have been discussed elsewhere.[9] Using a single sample to diagnose IMI reduces the sensitivity of diagnosis. False negative samples are most likely to occur with coliforms and *Staphylococcus aureus*,[12] however, in both studies, large inoculum volumes were used (0.1ml) in order to reduce false negative results, and handling of samples was constant

between treatment groups so that any bias was equal between groups.

The proportion of quarters infected with a major or minor pathogen at dry-off was different between the two US studies. Godden *et al*[8] reported 33.3% of quarters in the control group were infected, compared with 12.7% in the current study. This may be explained by the difference in overall udder health between herds. Herd BTSCC

Table 6. Results of a Cox Proportional Hazards Regression Model for the occurrence of the first cow case of mastitis in a lactation. The estimates, standard error, hazard ratio and P value are presented for all herds and for each herd for three models run to 30 DIM, 60 DIM and 100 DIM.

	30 DIM				60 DIM				100 DIM			
	b	SE	HR	P	b	SE	HR	P	b	SE	HR	P
All herds	-0.45	0.25	0.64	0.07	-0.43	0.20	0.65	0.03	-0.28	0.17	0.76	0.11
Herd A	-0.66	0.71	0.52	0.35	-0.32	0.59	0.73	0.59	-0.50	0.52	0.61	0.33
Herd B	0.11	0.37	1.12	0.10	-0.18	0.30	0.83	0.53	-0.14	0.26	0.87	0.59
Herd C	-0.97	0.40	0.38	0.01	-0.73	0.32	0.48	0.02	-0.35	0.26	0.70	0.17

All models controlled for farm, parity, last DHIA recorded ICSCC, dry period length, last DHIA recorded milk yield and calving month.
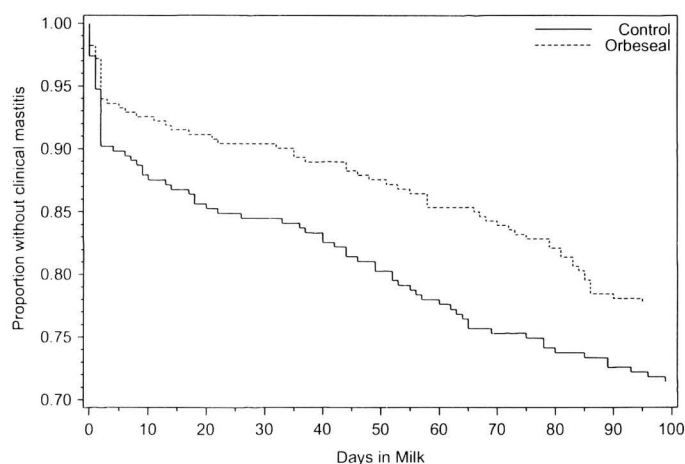
Figure 1. Survival distribution function for days to the first cow case of mastitis treatment through 100 days in milk (censor time) for all herds comparing treatment (dotted line = antibiotic + sealant) with control (continuous line = antibiotic only) groups.

values were higher in the herds used for the Minnesota trial, linear scores at dry-off were higher and *Streptococcus agalactiae* was also identified in several quarters, suggesting that the herds had less control of contagious pathogens than the herds used in the current study.

The average control rate of new IMI was lower in this study at 16.5%, compared with 25.4% in the other US study.[8] Control rates of new infection in quarters treated with dry cow antibiotic therapy have been reported infrequently. The new IMI rate in control quarters in a UK study was 39.3%,[10] compared to 2.7% in a New Zealand study,[17] a wide variation. In five herds in Canada and the US, the control rate of new IMI ranged from 8 to 18%,[7] with a mean of 11%, similar to the current study. Differences in culture technique may have contributed somewhat to differences in the reported rate of new IMI. However, herd level differences are likely the major contributing factor to the variation seen.

Cook *et al*[6] reported a wide range in the infection rate at first ICSCC test after calving in 145 Wisconsin dairy herds, and suggested that this might be related to differences in dry period management between farms. The variation observed in new IMI in this study appears to support this suggestion. Herd was included as a fixed effect in the new IMI model and was found to be significant. The herd with the lowest rate of new IMI in control quarters (9.1%) managed cows on sand bedded free stalls throughout the dry period. In contrast, the herd which managed cows intensively in a dirt lot area (Herd A), and the herd with a large straw bedded pack with pasture access (Herd C), had higher rates of new IMI at 29.6 and 20.2%, respectively. These latter two herds also showed the greatest benefit from Orbeseal use, with 72 and 65% lower odds of new infection in treated quarters, respectively. The herd with the lower control rate for new IMI received some benefit of Orbeseal use, but the difference was not statistically significant. Information regarding the degree of risk for new IMI during the dry period, such as ICSCC data and data from CMT use at dry-off and at calving, would therefore be of great value to a herd when deciding whether or not to use Orbeseal.

The benefits of Orbeseal in the current study were achieved with herdsmen administering the treatment, suggesting that when a reasonable standard of hygiene is practiced there does not appear to be any serious risk of infection associated with concurrent Orbeseal administration and dry cow antibiotic therapy.

The magnitude of reduction in new IMI risk may also be related to the type of trial used. Because there is interdependence between quarters within a cow, the actual reduction in new IMI could be reduced in a split-udder design study compared with a cow-level comparison. Correlations of new IMI between both rear and both front quarters were shown in the current study, and have been reported elsewhere. Thus, a cow may develop a new IMI in up to four quarters compared with no new IMI in any quarter in a cow-level comparison, but is only able to become infected in a maximum of two quar-

Table 7. Lactational milk yield loss assessment by the days-in-milk distribution of the first cow cases of mastitis in treatment (antibiotic + sealant) and control (antibiotic only) cows in all three herds combined.

Week of lactation	Lactational milk loss per mastitis event by week (lb)*	All herds			
		Treatment		Control	
		Mastitis cases per 100 cows	Lactational milk loss in mastitis cows (lb)*	Mastitis cases per 100 cows	Lactational milk loss in mastitis cows (lb)*
1	1573	8.1	12,738	11.2	17,611
2	1663	1.5	2497	2.3	3828
3	1639	0.7	1148	1.6	2625
4	1593	0.4	638	0.8	1274
5	1531	1.1	1685	0.4	614
6	1472	0.4	587	1.9	2794
7	1395	1.5	2094	1.9	2653
8	1320	1.1	1452	1.9	2510
9	1245	1.1	1371	1.6	1993
10	1175	1.5	1762	1.6	1881
11	1100	1.1	1210	0.4	440
12	1030	2.6	2675	1.2	1234
13	955	2.2	2099	1.2	1144
14	884	0.4	354	0.8	706
15	807	0	0	0.4	323
Total loss of milk for sale per 100 cows (lb)		32,309		41,631	
Increase in milk for sale per 100 cows for treatment cows (lb)		9321			
Benefit of Orbeseal use per cow in herd ($)#		5.38			

* Lactational milk loss was calculated using the daily milk yield reduction model of Wilson et al[16] corrected for antibiotic treatment withdrawal.

Benefit of Orbeseal use was calculated using a cost of $1.80 per tube and a milk price of $0.135 per lb ($0.297/kg).

ters in a spit-udder design study. The greater treatment effect in this study compared with that reported by Godden et al[8] may be explained, at least in part, by this clustering of new IMI within cow. Cow was included as a random term in all models to account for this correlation in the statistical analysis.

The overall effect of Orbeseal may also be related to the pathogen profile of new IMI in a herd, and different pathogen effects have been identified. A significant reduction in new environmental streptococcal infections was identified in the current study, consistent across all identified species. This effect was also found in other studies,[8,17] but Huxley et al[10] failed to show a significant effect on streptococci in a UK study. Orbeseal use tended to reduce CNS infections in the Minnesota study,[8] and there was a significant reduction in our study, but this effect was not seen in Orbeseal treated quarters alone in the UK study when compared with dry cow antibiotic treated quarters.[10] Orbeseal use has reportedly reduced new E. coli infections in herds not using a J5 vaccination program,[10] but no effect on gram-negative infections was

observed in the current study. No studies using Orbeseal have shown a significant reduction in infections caused by contagious pathogens. The reasons for the disparity in treatment effects between pathogen types and studies have yet to be determined, however the majority of studies agree that the sealant appears to significantly reduce IMI with the environmental streptococci.

Important covariates which were significant in the new IMI model were parity and infection status at dry-off. Parity effects on new IMI have been identified in some,[7] but not all studies,[8] but quarter infection status at dry-off has uniformly been identified as a significant factor, with several interesting interactions between pathogen types.[8,10] Last DHIA recorded ICSCC and milk yield effects were not significant. Significant effects of milk yield at dry-off on teat canal closure and new IMI rate have been documented,[7] but yield was measured on the day of dry-off rather than at the DHIA test conducted up to one month prior to dry-off, as was done in the current study. Dry period length averaged 60 days in our study, and was limited by the protocol to a range between

42 and 100 days. With trends toward shorter dry periods, the effect of Orbeseal will need to be further investigated in cows with dry periods of 30 days or less.

Although documentation of a reduction in new IMI at 1-3 DIM provides us evidence that the product functionally protects the udder from new infection during the dry period, the true impact of Orbeseal will be realized for the farmer due to a lower somatic cell count and a reduction in quarter treatments for clinical and subclinical mastitis.

Overall, first linear score was not significantly different in Orbeseal treated cows (2.3), compared to control cows (2.7). However, all herds screened quarters at calving with the CMT and treated positive quarters before the first DHIA test. Thus, treatment of infected control quarters may have masked any differences that might have been attributed to the use of Orbeseal. Significant differences in quarter level linear score have been shown in a split-udder design study, which perhaps more accurately represents the impact of the sealant.[8]

Quarters infected during the dry period have been shown to succumb to clinical mastitis earlier in lactation than culture-negative quarters.[9] Godden *et al*[8] also showed a benefit in the timing of treatment for quarters treated with Orbeseal and dry cow antibiotic, compared to dry cow antibiotic treated quarters alone. However, because the latter study was a split-udder design, the economics of this difference could not be calculated.

In the current study, there was a trend for a reduction in the proportion of cows treated for mastitis during the first 100 days of lactation, with 29.1% of control cows and 23.7% of treatment cows receiving treatment (P=0.14). There was also a trend for a reduction in total quarter cases treated per 100 cows, with 53.9 vs 42.6 for control and treatment cows, respectively. The focus of the analysis was on the first cow case treated in lactation. Results from the Cox proportional hazards modeling showed interesting differences in the timing of treatment between farms. Overall, there was a significant difference in the timing of the first cow case of mastitis between treatment groups for all herds to 60 DIM (P=0.03), and a trend for a difference to 100 DIM (P=0.11), with hazard ratios suggesting that the first mastitis treatment is delayed in treated cows. The effect was greatest in Herd C and least in Herd B. The difference in timing of the first cow case of mastitis, shown graphically in Figure 1, may be the most dramatic economic difference justifying Orbeseal use in some herds.

Using a yield reduction model for mature cows,[16] there was an improvement in predicted milk available for sale in all three herds in the treatment groups. There was a mean economic benefit from Orbeseal use of $5.38 per cow, with two herds receiving benefits over $10.00 per cow. However, in the herd with the lowest rate of IMI in control cows, and therefore the least potential for ben-

efit from the sealant program, the improvement in sale of milk in the treated group was not sufficient to offset the increased cost of Orbeseal use. If sealants are to be used in such a herd, other economic benefits (such as a lower BTSCC) must be realized in order to justify their use in combination with dry cow antibiotic treatment. Alternatively, the option to use the sealant alone, without dry cow antibiotic, in uninfected cows should be considered.

Conclusion

Orbeseal, when used in combination with dry cow antibiotic therapy, reduced the rate of new IMI during the dry period. The effect was greatest in herds with a high rate of new infection during the dry period, and in herds with a large amount of environmental streptococci infections. Economic justification for use of Orbeseal comes predominantly from a shift in the timing of mastitis treatment, particularly during the first 60 DIM. This difference in timing of first treatment resulted in a mean net benefit of $5.38 per cow. In herds with low rates of new IMI during the dry period, use of Orbeseal in combination with dry cow antibiotic may not be economically justifiable, and other strategies may need to be explored.

Acknowledgements

Footnotes

a Orbeseal®, Pfizer Animal Health, New York, NY
b Orbenin DC®, Schering-Plough Animal Health, Kenilworth, NJ
c Quartermaster®, Pfizer Animal Health, New York, NY
d Remel, Lenexa, KS
e bioMérieux, Inc., Hazelwood, MO
f bioMérieux, Inc., Hazelwood, MO

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Abstracts

Controlled Clinical Trial of the Effect of a Homoeopathic Nosode on the Somatic Cell Counts in the Milk of Clinically Normal Dairy Cows

Holmes M.A., Cockcroft P.D., Booth C.E., Heath M.F.
Veterinary Record 156:565-567, 2005

Cows in a 250-cow Holstein-Friesian herd were allocated at random to be treated with either a homoeopathic nosode or a negative control, both treatments being applied by means of an aerosol spray to the vulval mucous membranes. A total of six treatments were given over a period of three days and milk samples were taken for the determination of somatic cell counts (scc) on days -3, 3, 7, 9, 14, 21 and 28. Individuals applying the treatments or carrying out the scc determination were unaware of which animals were receiving which treatment. Owing to the wide natural variations in scc, the trial had only a 71 per cent possibility of detecting a 30 per cent difference in scc between the two groups. There were no significant differences between the scc of the two groups on any sample day, but there were significant variations between the scc on different days (P=0.003) in both groups.

Factors Associated with Lameness in Dairy Cattle

Blowey R.
In Practice 27:154-162, 2005

Many studies have shown that, despite years of research, the incidence of lameness in dairy herds in the UK remains unacceptably high. This is partly due to the impact of digital dermatitis, which was first reported in the UK in 1986 and now accounts for some 15 to 20 per cent of lameness cases in cows. More generally, increases in herd size and yield - factors which are themselves associated with an increased prevalence of lameness - have outstripped efforts in control. Lameness is most likely to result from pain in the limb, usually within the foot, and hindfeet are more commonly affected than front feet. It is not the purpose of this article to describe the range of lesions involved and their pathogenesis. Rather, the focus is on management factors involved in the aetiology of lameness, particularly as they relate to cow comfort, nutrition and routine husbandry.