

An assessment of the benefits of Orbeseal® when used in combination with dry cow antibiotic therapy in three commercial dairy herds

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ABSTRACT

An internal teat sealant (Orbeseal®, Pfizer Animal Health, New York, NY) 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 infection at 1-3DIM than control (antibiotic only) quarters ($P < 0.001$). Herd level variation in new IMI and mastitis treatment rates during the first 100DIM were observed. The greatest benefits from Orbeseal use were documented in herds with the highest rates of new IMI during the dry period, and for the 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 using a model for milk yield reduction.

(Key words: internal teat sealant, dry period, mastitis)

Key: IMI – intra-mammary infection, BTSCC – bulk tank somatic cell count, ICSCC – individual cow somatic cell count, DHIA – Dairy Herd Improvement Association

INTRODUCTION

Over the last few years there has been considerable interest in strategies to reduce the high rate of new intra-mammary 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^{14 4} has been repeatedly shown, and the cow level factors contributing to this risk of infection have been thoroughly reviewed elsewhere⁵. The use of external and internal teat sealants to prevent these new infections has been a subject of much interest, particularly in countries where the widespread use of intra-mammary dry cow antibiotic therapy is becoming increasingly difficult to justify to a concerned consumer.

The recent commercial availability of a novel internal teat sealant originally developed in Ireland in the mid-1970s¹¹, which is able to persist in the teat cistern throughout the entire dry period, has led to its widespread use in dairy farms across the world, including many of those in North America in the last year. The current product; Orbeseal® (Pfizer Animal Health, New York, NY), consists of a 4g intra-mammary syringe containing 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 at preventing non-lactating period new infections^{10 11 7}.

In North America, with a higher legal limit for BTSCC, and consequently a larger proportion of each herd potentially sub-clinically infected in late lactation, Orbeseal has been incorporated into dry cow management programs by administering the sealant after infusion of dry cow antibiotic. The aim has been for the sealant to aid in the prevention of new IMI during the dry period, while the long acting antibiotic remains to cure existing infections. Fewer studies have been carried out

to show that the sealant has effects over and above that of 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 (250mg Cephalonium) and quarters sealed after administration of 600mg Cloxacillin¹⁷. In the only published study using Orbeseal performed in the US to date, which also used a split udder design, quarters treated with 500mg Cloxacillin alone (Orbenin DC®, Shering-Plough Animal Health, Kenilworth, NJ) were compared with those which received both sealant and cloxacillin. A significant 30% lower odds of developing a new IMI was observed in the sealed quarters, suggesting benefits over and above that of dry cow therapy alone⁸. 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 quarters¹³.

The studies reported so far ably demonstrate the ability of the internal sealant to prevent new infection of the non-lactating mammary gland. However, the economic benefit of the sealant must come from reducing treatments for sub-clinical and clinical mastitis and from improvements in BTSCC. This is particularly challenging in a situation where the sealant is used in addition to traditional dry cow antibiotic therapy.

One UK study¹⁰ demonstrated that coliform infections responsible for mastitis up to 109 days in milk (DIM) were due to 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 that were not infected in the dry period. It is these infections and mastitis cases that may be influenced by the use of Orbeseal. Berry and Hillerton¹ found that clinical mastitis during the first 100DIM 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 60DIM 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 between quarters has been demonstrated² 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. An awareness of these factors is necessary if we are to predict the overall effect of a herd level teat sealant strategy.

Quarter infection status at dry off, milk yield at dry off, and teat end condition are all individual cow factors which have been shown to influence teat end openness during the dry period and susceptibility to infection⁷. The majority of the studies investigating the use of Orbeseal have used cows with 305 day lactation milk yields less than 8,500 kg, with low milk yields at dry off. The study by Godden et al.⁸, in two herds with BTSCC ranging from 275,000 to 450,000, is the only study reported thus far using Orbeseal in cows with high milk yields at dry off, in intensively managed free stall housed US dairy herds, rather than herds which graze at pasture during the spring and summer months.

Between herd variation in new IMI rate during the dry period, driven by factors such as differences in housing, vaccination and hygiene, has not been adequately described in a large

number of farms. Relatively few herds have been intensively monitored using bacteriology, and rates of new infection in control quarters receiving dry cow antibiotic therapy in studies across the world to date have varied widely from 2.7 to 39.3%. Cook et al. ⁶ described the use of individual cow somatic cell counts (ICSCC) at first Dairy Herd Improvement Association (DHIA) test as a crude monitor of udder infection dynamics at a herd level. In 145 Wisconsin dairy herds, infection rates (defined as an ICSCC > 200,000/ml) at first test in uninfected cows at dry off (defined at an ICSCC < 200,000/ml at the last test before dry off), varied from 0 to 71% - suggesting a wide variation in risk of new infection. Although new infections defined using ICSCC data do not accurately identify infections solely occurring during the non-lactating period, different management strategies and environments for dry cows may have contributed to the wide variation. These factors will certainly influence the impact of Orbeseal at a herd level.

Specific pathogen effects of Orbeseal have also varied in the studies reported thus far. In the New Zealand study ¹⁷ and in the US study reported by Godden et al. ⁸, the protective effect of Orbeseal was targeted predominantly at the environmental streptococci. In contrast, no significant effect on streptococci was demonstrated in a United Kingdom study ¹⁰, but this study did find a significant effect on coliform bacteria in herds which did not practice vaccination with a J5 bacterin, which has not been repeated elsewhere.

Finally, in many of the studies previously reported, it should be noted that a researcher with a vested interest in the performance of the product carried out application at dry off. The sealant does not contain an antibiotic and thus poor hygiene at the time of insertion may be a potential risk factor for new infection ⁵. This risk has yet to be quantified in commercial dairy herds.

The current study aimed to examine the performance of Orbeseal in commercial dairy herds when applied at the cow level in combination with dry cow antibiotic therapy and compared with antibiotic use alone. Herds with a 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. Differences in the rate of mastitis treatments to 100DIM between treated (antibiotic + sealant) and control (antibiotic alone) groups were investigated in order to evaluate the economic return from using the product.

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 and weighted mean annual somatic cell counts from monthly cow testing were 309,000/ml, 307,000/ml and 211,000/ml for herds B, L and N respectively. Mean (range) rolling herd average milk production was 11,378 kg (10,240 – 11,956 kg). All lactating cows on each of the herds were housed all year round in free stalls. All cows in all of the herds 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 1 gram Dihydrostreptomycin sulfate (Quartermaster®, Pfizer Animal Health, New York, NY). A

label dosing program of J5 *E.coli* vaccination was used on all farms, using three doses; at dry off, at 2 weeks before calving and at 1-2 weeks after calving.

All herds operated 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 B, far-dry cows were able to lie on un-bedded free stalls fitted with rubber mats, but were predominately exposed to a small dirt-lot area. The close-up group (approximately 20-1 days pre-partum) consisted of a loose housed bedded pack using straw or corn-stalks. In herd L, sand free stalls in a converted stanchion barn were 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 N 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, peri-parturient cows calved on a straw bedded pack maternity area and were transferred to free stalls with either deep sand (Herd L) or mattresses bedded with sawdust (Herds B and N) at 1-3 days after calving.

Cow Selection and Sampling

Cows were eligible for inclusion in the trial provided that 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 and the alcohol was allowed to dry for at least one minute.

After milking, the 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, the 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®; Pfizer Animal Health, New York, NY). 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 3 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 occurring 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 (-20°C) until they were thawed and plated. Quarter milk samples collected at calving time and from clinical mastitis cases were stored frozen (-20°C) on farm, transported in a cool box in ice to the laboratory, where they were again held frozen (-20°C) until they were thawed and plated. After thawing at room temperature, milk samples were vortexed until homogenous immediately prior to culturing. A 0.1ml 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 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 Council¹².

Staphylococci (catalase-positive, Gram-positive cocci) were tested for coagulase activity using rabbit coagulase plasma EDTA (Remel, Lenexa, KS, USA). Coagulase-positive *Staphylococci* were tested for acetoin production by inoculation of MRVP broth (Remel, Lenexa, KS, USA). 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 (bioMérieux, Inc., Hazelwood, MO, USA). The 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 (bioMérieux, Inc., Hazelwood, MO, USA). Results were reported as *E. 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. All of 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 recorded actual milk yield and ICSCC 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 Milk Test (CMT) at 1-3DIM, usually at the time of sampling for culture.

Definitions

The following definitions were used to define intra-mammary infection and mastitis:

New Dry Period Infection.

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

Dry Period ‘Cure’.

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

Mastitis Treatment

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

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 7 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 7 days.

Economic Analysis

The 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. The 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 1 to 15 after calving. Yield loss was corrected for residue withdrawal from dry cow antibiotic use for the first 4 days of lactation and for the use of intra-mammary lactational treatment (4 days withdrawal). For example, lactational milk yield loss for a mastitis treatment in week 1 was calculated to be 715Kg, compared to a loss of 402Kg for a treatment occurring in week 14. The weekly distribution of mastitis treatments per 100 cows was calculated for each herd and the 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.

Statistical Analysis

Random allocation of cows by parity, the proportion of cows with the last ICSCC greater than 200,000/ml, and the 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 recorded milk yield at dry off and last ICSCC linear score between treatment groups. The 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 infection 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 100DIM. Cows were classified as censored at the reported herd removal date or 30, 60 and 100DIM 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 ($n=47$), cows with missing data at dry off ($n=10$) and cows that were missed for sampling at calving ($n=23$). 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 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 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 infection

Significant herd ($p=0.0012$) and treatment ($P<0.0001$) effects on the proportion of quarters developing a new IMI between dry off and 1-3DIM 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 treatment quarters was significantly lower ($P<0.0001$) 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 B and N, but not in herd L, where the control rate of new IMI was the lowest. Parity was significant in the final model ($P=0.0263$), 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 ($P=0.0057$). Specifically, the presence of *Staphylococcus*

aureus or a non-aureus *staphylococcus* spp reduced the rate of new IMI and the 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 matrix is presented in Table 4. The correlation coefficients suggest that both front quarters and both rear quarters are 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 ($P < 0.0001$) and for non-aureus *staphylococci* ($P = 0.0292$), but not for *Staphylococcus aureus* ($P = 0.1015$), gram negative infections ($P = 0.1018$) and other pathogens ($P = 0.7123$). Table 5 summarizes the new IMI by bacterial isolate for all cows and Table 6 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, $P = 0.1698$), but last DHIA recorded ICSCC linear score before dry off was significant in the final model ($P < 0.0001$).

Effect of Treatment on Risk of Dr Period Cure

The proportion of treated quarters experiencing a cure between dry off and 1-3DIM was not significantly different from control quarters ($P = 0.8646$), though numerically higher (90.1% treated v. 80.6% control). There were significant farm effects ($P = 0.03$), with cure rates in herd N being higher than herd L, which was in turn higher than Herd B. Predictably, last DHIA recorded ICSCC was significant in the final model ($P < 0.0001$), with cows with higher ICSCC at dry off having lower cure rates.

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

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 ($\chi^2 = 2.91$, $P = 0.088$). 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 100DIM, compared with 29.1% of cows in the control group ($P = 0.1404$). No other covariates were significant in the logistic model, although there was a strong trend for a herd effect ($P = 0.0774$).

The 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 100DIM, 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 7. 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 N at both 30DIM and 60DIM, with hazard ratios of 0.38 and 0.48 respectively. All hazard ratios favored the treatment group, except in herd L up to 30DIM.

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 1,418 to 7,084 kg milk per 100 cows, with an average for all herds of 4,237 kg (Table 9). The cost benefit calculation for all three herds gave a mean benefit per cow of \$ 5.38 for Orbeseal use. For herds B and N, the benefit was \$10.77 and \$13.84 per cow, but in herd L, 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 1-3DIM than quarters treated with antibiotic alone. This finding supports those of previous authors who similarly found that the internal sealant has a role to play in the control of new IMI during the non-lactating period. Two studies; the current one and one performed on two other US herds⁸, have shown that 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 technique compared to using the culture information from multiple sample points prior to dry off have been discussed elsewhere⁹. 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, which would lead to an over diagnosis of new IMI post-partum. 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 infected, compared with 12.7% in the current study. This may be explained by the difference in overall udder health between the herds. Herd BTSCC 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 study⁸. 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 a rate of 2.7% in a New Zealand study¹⁷ – a wide variation. In 5 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 a little to differences in the reported rate of new IMI. However, herd level differences are likely the major contributing factor to the differences seen.

Cook et al. ⁶ reported a wide variation in infection rate at first ICSCC test after calving, and suggested that this was 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 on a dirt lot area (Herd B), and the herd with a large straw bedded pack with pasture access (Herd N), had higher rates of new IMI at 29.6% and 20.2% respectively. These latter two herds also received the greatest benefit of Orbeseal use, with a 72% and 65% lower odds of new infection in treated quarters respectively. The herd with a low control rate of 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 were achieved in the current study with herdsmen applying the treatment – suggesting that provided a reasonable standard of hygiene is practiced, there does not appear to be any serious risk of infection associated with Orbeseal administration in combination with dry cow antibiotic therapy.

The size of the 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 two or more 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 quarters in a spit udder design study. The greater treatment effect found in this study compared with that of 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 effect of Orbeseal may also be related to the pathogen profile of new IMI in a herd. A significant reduction in new environmental streptococcal infections was identified in the current study, consistent across all identified species. This was also found in New Zealand ¹⁷, and in the study by Godden et al. ⁸ However, Huxley et al. ¹⁰ failed to show any significant effect on streptococci other than a numerical reduction in *Enterococcus* spp. A significant effect on non-aureus staphylococci was also found in this study. A trend for reduction in coagulase negative staphylococcus infection was reported in the Minnesota study ⁸, but no such effect was identified in Orbeseal treated quarters alone, when compared with dry cow antibiotic treated quarters in the UK ¹⁰. A reduction in new infections with *E.coli* in herds which did not use a J5 vaccination program has been an infrequent finding and no effect on gram negative infections was observed in the current study. None of the studies using Orbeseal have demonstrated a significant

protective effect for contagious pathogens, although there was a numerical reduction in the number of infections in this study.

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 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 up to one month prior, as it was in the current study. Dry period length averaged 60 days in the current study and was limited to a range between 42 and 100 days by the protocol used. 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 1-3DIM 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 by a lower somatic cell count and a reduction in quarter treatments for clinical and clinical mastitis.

First linear score was not significantly different in Orbeseal treated cows (2.3) compared to control cows (2.7) overall. However, all herds screened quarters at calving with the CMT and treated positive quarters before first DHIA test. Thus, treatment of infected control quarters may have masked any differences that might have been apportioned 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.

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=0.1404). There was also a trend for a reduction in total quarter cases treated per 100 cows from 53.9 in controls to 42.6 in treatment cows. The focus of the analysis was on the first cow case treated in lactation. Results from the Cox proportional hazards modeling showed that there were interesting differences in the timing of treatment between farms. Overall, there was a significant difference in the timing of first cow cases of mastitis for all herds to 60DIM (P=0.03), and a trend for a difference to 100DIM (P=0.11), with hazard ratios suggesting that the first mastitis treatment is delayed in treated cows. The effect was greatest in herd N and the least in herd L. 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 benefit of Orbeseal use of \$5.38 per cow, with 2 herds receiving benefits of over \$10.00 per cow. However, in the herd with the lowest rate of IMI in control cows, and therefore the least potential for benefit from the sealant program, the improvement in sale of milk in the treated group was not sufficient to off-set the increased cost of Orbeseal use. Use of Orbeseal alongside dry cow antibiotic could therefore be justified in two herds, but not in the herd with a low control rate of IMI. In this herd, and other similar herds, economics dictate that if Orbeseal is to be used, administration instead of, rather than in addition to, dry cow antibiotic therapy in uninfected cows should be considered, rather than the blanket approach used in the current study.

Conclusion

Orbeseal, when used in combination with dry cow antibiotic therapy, reduced the rate of new IMI during the dry period. The effect appeared to be greatest in herds with a high rate of new dry period infection and in herds with a large amount of environmental streptococci infections. Economic justification for the use of Orbeseal comes from a reduction in the rate of treatment for clinical and sub-clinical mastitis and a shift in the timing of that treatment, particularly during the first 60DIM. These changes carry 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

This study was funded by Pfizer Animal Health (New York, NY). The authors wish to thank Ann Wilkinson and Kim Gajewski for assistance in data management and review of procedures, and acknowledge the help of Dan Weigel and Joe Boucher for assistance with statistical analyses. The authors are grateful to the owners and employees of the three Wisconsin dairy herds for their contribution to the project.

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Table 1. Least squares mean (SE) parity, last DHIA recorded milk yield (kg), 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 B		Herd L		Herd N		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, kg)	29.3 (1.6)	27.4 (1.6)	30.1 (3.0)	27.3 (0.8)	22.0 (0.8)	25.5 (0.7)	27.1 (2.4)	25.4 (2.4)
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.

	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
% of all quarters with IMI	12.7	11.9	12.3
Bacterial Isolates			
<i>Staphylococcus aureus</i>	15	9	24
<i>Streptococcus dysgalactiae</i>	1	1	2
<i>Streptococcus uberis</i>	0	1	1
<i>Enterococcus spp.</i>	2	8	10
Other Environmental streptococci	7	6	13
Total environmental streptococci	10	16	26
<i>Escherichia coli</i>	5	2	7
<i>Klebsiella spp.</i>	2	5	7
<i>Enterobacter spp.</i>	0	1	1
<i>Pseudomonas spp.</i>	1	0	1
Other gram negative rods	1	0	1
Total gram negative pathogens	9	8	17
Non-aureus staphylococci	87	91	178
<i>Corynebacterium spp.</i>	9	6	15
<i>Nocardia</i>	0	1	1
<i>Pasteurella spp</i>	1	0	1
Total Other	10	7	17

Note table reports all species cultured from single and mixed infections

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

Herd	Control n affected (%)	Treatment n 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 B (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 L (Control n=453, Treatment n=489)	41 (9.05%)	34 (6.9%)	-0.33 (0.26)	0.72 (0.43, 1.20)	0.211
Herd N (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

Table 4. Correlation coefficients between quarters (LF, LR, RF, RR) within cow for new IMI at 1-3DIM.

Quarter	LF	LR	RF	RR
LF	1.00	0.04	0.19	0.09
LR	0.04	1.00	0.09	0.21
RF	0.19	0.09	1.00	-0.02
RR	0.09	0.21	-0.02	1.00

Table 5. Bacterial isolates from new IMI identified at 1-3DIM from control and treated quarters and for all cows.

	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
% of all quarters with IMI	16.5	8.0	12.2
Bacterial Profile			
<i>Staphylococcus aureus</i>	22	11	33
<i>Streptococcus dysgalactiae</i>	10	1	11
<i>Streptococcus uberis</i>	12	3	15
<i>Enterococcus spp.</i>	9	2	11
Other Environmental streptococci	14	4	18
Total environmental streptococci	45	10	55
<i>Escherichia coli</i>	7	4	11
<i>Klebsiella spp.</i>	1	0	1
<i>Enterobacter spp.</i>	5	1	5
<i>Proteus spp.</i>	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
<i>Bacillus</i>	1	1	2
<i>Corynebacterium</i>	0	1	1
<i>A.pyogenes</i>	2	2	4
Blind Quarter	0	1	1
Total Other	4	6	10

Note table includes all species cultured from quarters with single and mixed new infections

Table 6. Results of multivariate regression analysis of odds of acquiring a new IMI between dry off and 1-3DIM by pathogen group for control quarters and treated quarters.

Pathogen Group	Control n affected (%)	Treatment n affected (%)	Estimate (SE)	Odds Ratio^{treatment} (95% Confidence Limits)	P value
<i>Staphylococcus aureus</i>	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

Table 7. 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 30DIM, 60DIM and 100 DIM.

	30DIM				60DIM				100DIM			
	<i>b</i>	SE	HR	P	<i>b</i>	SE	HR	P	<i>b</i>	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 B	-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 L	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 N	-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.

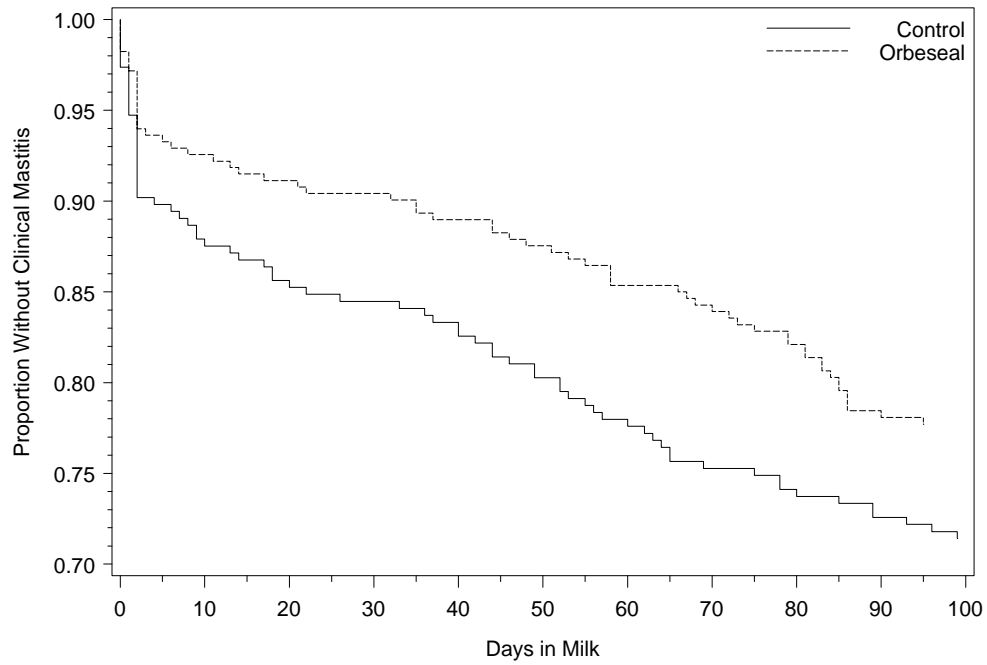


Table 9. Lactational milk yield loss assessment by the days in milk distribution of the first cow cases of mastitis in treatment (antibiotic + sealant) and control (antibiotic only) cows in all three herds.

Week of Lactation	Lactational milk loss per mastitis event by week (kg)*	All Herds			
		Treatment		Control	
		Mastitis Cases per 100 cows	Lactational Milk Loss in Mastitis Cows (kg)*	Mastitis Cases per 100 cows	Lactational Milk Loss in Mastitis Cows (kg)*
1	715	8.1	5,790	11.2	8,005
2	756	1.5	1,135	2.3	1,740
3	745	0.7	522	1.6	1,193
4	724	0.4	290	0.8	579
5	696	1.1	766	0.4	279
6	669	0.4	267	1.9	1,270
7	634	1.5	952	1.9	1,206
8	600	1.1	660	1.9	1,141
9	566	1.1	623	1.6	906
10	534	1.5	801	1.6	855
11	500	1.1	550	0.4	200
12	468	2.6	1,216	1.2	561
13	434	2.2	954	1.2	520
14	402	0.4	161	0.8	321
15	367	0	0	0.4	147
Total loss of milk for sale per 100 cows (kg)		14,686		18,923	
Increase in milk for sale per 100 cows for Treatment Cows (kg)		4,237			
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 \$0.297 per kg