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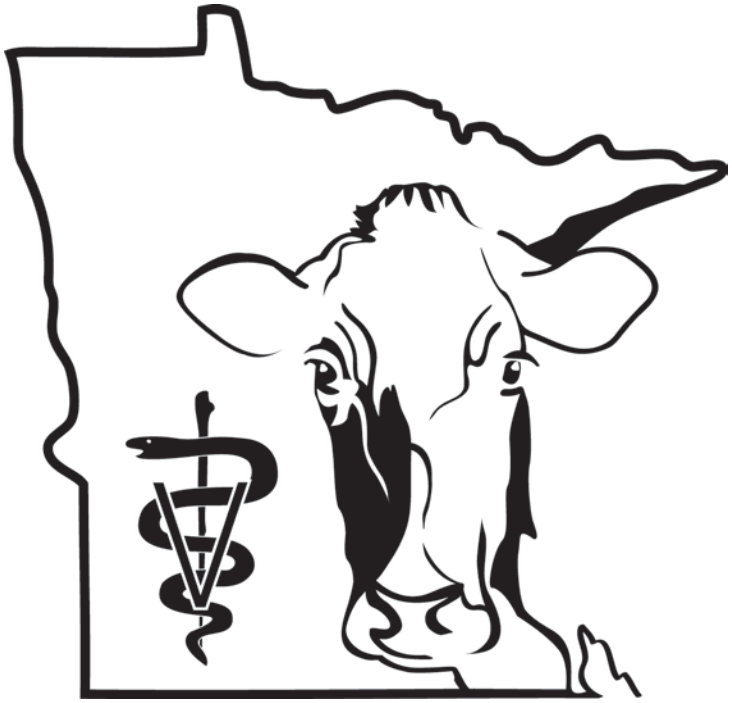

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Effectiveness of an Internal Teat Seal in the Prevention of New Intramammary Infections During the Dry and Early Lactation Periods in Dairy Cows When Used With a Dry Cow Intramammary Antibiotic

S. Godden,* P. Rapnicki,* S. Stewart,* J. Fetrow,* A. Johnson,‡ R. Bey,† and R. Farnsworth*

*Department of Clinical and Population Sciences, and
†Department of Veterinary Diagnostic Medicine,
Veterinary Teaching Hospital, University of Minnesota, St. Paul, MN 55108
‡Total Herd Management Services, Clintonville, WI 54929

ABSTRACT

The objectives of this study were to determine the effect of infusion with an internal teat seal at dry off, when used as an adjunct to long-acting antibiotic infusion at dry off, on the incidence of new intramammary infections (IMI) during the dry period, prevalence of IMI and LS after calving, and incidence of clinical mastitis between dry off and 60 DIM. A total of 437 cows from two dairy herds, with no clinical mastitis and four functional quarters, were enrolled at dry off. Prior to the final milking all quarters were sampled for bacteriological culture and SCC analysis. After milking all four quarters were infused with a commercially available long-acting dry cow antibiotic. Two contra-lateral quarters were then infused with an internal teat seal (Orbeseal®, Pfizer Animal Health, New York, NY). Following calving the teat seal was stripped out at first milking. Duplicate milk samples were collected between 1- 3 DIM and again between 6-8 DIM for culture and SCC analysis. Quarters treated with Orbeseal® had significantly lower prevalence of IMI at 1-3 DIM (tx = 22.8%, control = 29.1%), had significantly fewer quarters that acquired a new IMI between dry off and 1-3 DIM (tx = 20.2%, control = 25.4%), and had significantly fewer quarters affected by a clinical mastitis event between dry off and 60 DIM (tx = 5.9%, control = 8.0%). Multivariable analysis showed a significant effect of treatment, with treated quarters being 30 % less likely to develop a new IMI between dry off and 1-3 DIM, 31% less likely to have an IMI present at 1-3 DIM, 33% less likely to experience clinical mastitis between dry off and 60 DIM, and having significantly lower linear score measures at 1-3 and 6-8 DIM, as compared to control quarters.

INTRODUCTION

The mammary gland is particularly susceptible to new intramammary infections (**IMI**) during the early dry and late dry periods, correlating with involution and colostrogenesis, respectively (Smith et al., 1985; Eberhart, 1986; Todhunter et al., 1991; Hogan and Smith, 1998; Bradley and Green, 2000; Green et al., 2002). Some factors affecting this risk may include level of milk production at dry off, rapidity of udder involution, teat end condition, and level of contamination of teat ends (Oliver and Sordillo, 1989; Williamson et al., 1995; Dingwell et al., 2003). A major factor allowing the invasion of pathogens into the gland during the dry period may be that there is often a significant delay in the formation of a complete keratin plug in the streak canal

(Comalli et al., 1984; Williamson et al., 1995; Dingwell et al., 2003). In one study, Williamson et al. (1995) reported that 50 and 5% of teats had an incomplete keratin plug present after 7 and 50 days of the dry period, respectively. Dingwell et al. (2003) reported that 50% and 23% of teat ends were still open after one week and six weeks of the dry period, respectively. The latter study reported that quarters that remained open and quarters that had cracked teat-ends were both 1.7 times more likely to develop new IMI during the dry period compared to quarters that closed and that were not cracked (Dingwell et al., 2003). The practice of blanket dry cow antibiotic therapy (**DCT**), treatment of all quarters with a long-acting antibiotic at dry off, has been successful in curing many existing subclinical infections as well and offering short-term protection against new IMI when susceptible pathogens invade the gland during the early dry period (Natzke, 1981; Browning et al., 1990; Bradley and Green, 2001). However, new IMI may still occur if invading pathogens are not sensitive to the active ingredients in the antibiotic preparations being used and/or the antibiotic does not persist at therapeutic levels throughout the entire dry period (Smith et al., 1985; Bradley and Green, 2001).

One management tool that may be used to prevent new IMI during the dry period is an external teat sealant (Timms, 1997; Leslie et al., 1999; Lim et al., 2000). Once applied, these products dry to generate a latex, acrylic or other polymer-based film over the teat that prevents entry of pathogenic bacteria into the teat canal. Timms et al., (1997) reported that one product persisted for more than 3 d on 98% of teats and, with periodic reapplication, showed a reduction of up to 68% in new IMIs at calving. However, the extra labor and facilities required to frequently reapply these products may limit their potential for routine use on many dairies. An alternate management tool may be the use of an internal teat seal. Orbeseal® is an internal teat seal consisting of bismuth subnitrate in a paraffin base (65% w/w, 2.6 g in 4 g) (Pfizer Animal Health, New York, NY, 10017). This inert viscous paste is infused into the quarter at time of dry off forming an immediate physical barrier in the base of the teat cistern to prevent bacteria from ascending through the teat canal. Insoluble in milk, it has no antimicrobial properties and no residue or food safety risks (C.V.M.P., 1999). Woolford et al. (1998) reported that, of 19 treated quarters that were x-rayed, all had the internal teat seal present in the base of the teat sinus at 100 days dry. The majority of the Orbeseal® product is stripped out at first milking after calving, with some residual product removed in the subsequent several milkings after calving. This teat seal is currently approved and in use in the United Kingdom and New Zealand under the trade name TeatSeal® (Cross Vetpharm Group Ltd., Ireland; Bimeda Ltd., Auckland, NZ).

Studies in New Zealand and the U.K. have demonstrated that, when infused as the sole treatment in uninfected quarters at dry off, this internal teat seal has equal, if not better, efficacy in preventing new IMI during the dry period, as compared with DCT (Woolford et al., 1998; Berry and Hillerton, 2002; Huxley et al., 2002). Thus, an internal teat seal may be useful as an alternative to DCT for the prevention of new IMI during the dry period, when infused into uninfected quarters at dry off. Successful implementation of this strategic treatment approach may be a challenge, however, in many North American dairy herds. Strategic infusion of quarters with an internal teat seal, alone, will require careful attention to infusion techniques to avoid introducing pathogens into an unmedicated quarter. Quarters that are infected at dry off would still require DCT to achieve elimination of these existing infections. Furthermore, because there is still no quick, simple, inexpensive, and accurate on-farm method to differentiate uninfected from infected quarters at time of dry off, the standard industry recommendation of

blanket DCT is likely to continue. If this assumption is correct, then the next obvious question is whether there would be a benefit to using an internal teat seal as an adjunct to DCT at dry off. Ultimately this benefit should be measured from both an udder health and an economic standpoint.

The objective of this study was to describe the effectiveness of Orbeseal® in the prevention of new IMI during the dry and early lactation period when used as an adjunct to DCT at dry off. The primary hypothesis was that quarters treated with DCT and then Orbeseal® at dry off would experience a lower incidence of new IMI occurring between dry off and calving, as compared to quarters treated with DCT alone. Secondary hypotheses were that treatment with Orbeseal® and DCT at dry off would result in a lower prevalence of IMI after calving, a lower incidence of clinical mastitis between dry off and 60 DIM, and lower linear score (**LS**) after calving, as compared to quarters treated with DCT alone.

MATERIALS AND METHODS

Dry Off Enrollment, Treatment Assignment, and Sampling Strategy

Study participants included two commercial freestall Holstein dairy herds in Western Wisconsin, milking a total of approximately 2500 cows; 1000 from herd A and 1500 from herd B. To be eligible for enrollment cows had to be located at the home farm for at least three months prior to dry off, have received no parenteral or intramammary antibiotic or anti-inflammatory treatments within 30-days prior to dry off, have a projected dry period between 28 and 100 days, and have four functional quarters with no grossly abnormal milk or clinical mastitis present on the day of dry off. Teat end condition was not evaluated or considered among the inclusion/exclusion criteria.

Cows due to be dried off were brought into the parlor as a group, once per week on each dairy, for their last milking and routine DCT. Cows were visually identified using duplicate ear tags. Following teat preparation using the farm's usual premilking routine, which included pre-dipping with a 0.5% iodine-based teat dip, a California Mastitis Test (**CMT**) was performed on all four quarters (CMT results not reported in this paper). CMT results were not used to exclude cows from enrollment. Teat ends were then scrubbed with a 70% alcohol-soaked gauze and three foremilk samples aseptically collected from each quarter and placed directly into a chilled cooler (on ice). Immediately following the final milking out, all four quarters were again scrubbed with an alcohol-soaked gauze and then routinely dry treated by infusion of DCT (Orbenin-DC® (Cloxacillin (benzathine), 500 mg), Pfizer Animal Health, Groton, CT, 06340). All four quarters were massaged after DCT infusion. The treatment of infusion with Orbeseal® was then randomly assigned to two contralateral quarters (LH/RF or LF/RH) based on a randomly generated treatment assignment scheme that was established in advance and which began with the first cow entering the parlor. The alternate two contralateral quarters remained as control quarters. Orbeseal® treatment involved rescrubbing the teat end with an alcohol-soaked gauze and then infusing the Orbeseal® tube. The teats and quarters were not massaged after infusing the Orbeseal®. Following treatment all teats were post-dipped with a 1% iodine-based teat dip

and the cows then moved to a freestall pen where they were observed between 1 and 4 hours post-treatment for signs of adverse reactions.

Management of Dry and Fresh Cows

Dry cows were housed in either a pasture-based management system or freestall facility until approximately four weeks prior to the anticipated calving date when moved to a transition management facility (**TMF**). The TMF was a 400-stall sand freestall facility operated by the same owner of the participating milking dairies and managed in partnership with the College of Veterinary Medicine, University of Minnesota. Cows calved in individual maternity pens. Post-calving processing included attaching a collar with unique ID transponder, 16 ounces Propylene Glycol P.O., 500 ml 23% Calcium Borogluconate solution I.V., manually fore-stripping all four quarters followed by colostrum collection, and then relocation into a lactating cow pen. Cows remained at the TMF until between 10-14 DIM, at which time healthy cows were transported back to their dairy of origin.

Post-Treatment Sampling and Records

Post-calving duplicate foremilk samples were collected from all quarters in the parlor, once between 1-3 DIM, and once again between 6-8 DIM. Quarters were routinely prepped for milking, a CMT test performed, and, following cleaning of teat ends with a 70% alcohol-soaked gauze, three separate foremilk samples collected and then immediately chilled on ice. All clinical mastitis events and all culling, death, other disease, and treatment events occurring during the dry period and up to 60 DIM were recorded into on an-farm record keeping system used on all three sites (DairyComp305. Valley Agricultural Software, Tulare, CA, 93274). Milk samples were collected from all clinically affected quarters by farm staff at the time of detection.

Milk Sample Analysis

Somatic cell counts (SCC). One of the three quarter milk samples collected at the dry off, 1-3 DIM, and 6-8 DIM sampling points was preserved with a bronopol tablet (2-Bromo-2-Nitro-Propane-1,3 Diol: 6 mg/tablet), refrigerated, and then submitted unfrozen twice per week for SCC testing (MN DHIA Laboratory, Zumbrota, MN, 55992). Testing was performed using the Fossomatic 5000 Somatic Cell Count Instrument, using Opto Electronic Fluoro Flow Cytometry, with Ethidium Bromide and Buffer solution in staining of the white cells (Foss North America, Inc. Eden Prairie, MN, 55344).

Laboratory bacteriological culture. The two other duplicate milk samples collected at each sampling point were frozen and then submitted, once per week, for bacteriological culture (Laboratory for Udder Health, University of Minnesota, St. Paul, MN, 55108). One sample from each set of duplicate milk samples from individual quarters was thawed and, while still cold, 0.1 ml was plated onto MacConkey agar and Factor agar using sterile cotton tipped swabs. Factor Agar, similar to K.L.M.B. agar (Beatty et al., 1985), selects for gram-positive organisms while inhibiting the growth of gram-negative bacteria with antibiotics (Factor agar patent in process, University of Minnesota). A 0.1 ml inoculum volume was used to improve sensitivity (Buelow et al., 1996; Lam et al., 1996). Inoculated plates were incubated at 37°C. After incubation 18-24

hours all plates were observed for microbial growth. Those plates having growth were recorded and identification started. All plates were placed in the incubator for an additional 36-48 hours and the reevaluated for microbial growth. Colonies on MacConkey agar plates were presumptively identified based on colony morphology and colony color was used as a means of determining if the organism on the plate was a lactose-fermenting organism. Isolates were also gram stained to assist in organism identity. Organism identity was confirmed using the API 20E test (bioMerieux-Vitek, Inc. Hazelwood, MO). Colonies suspected as being staphylococci based on morphology were confirmed as staphylococci based on catalase reaction and microscopic morphology. Organisms suspected as being *Staphylococcus aureus* were confirmed using the tube coagulase reaction. Those organisms that were catalase positive and coagulase negative were classified as *Staphylococcus species*. Catalase negative streptococci were streaked onto TKT medium, which is selective for *Streptococcus* spp. only, to determine the esculin reaction and presumptive identification prior to organism confirmation using the API Streptococcus identification system (bioMerieux-Vitek, Inc. Hazelwood, MO, 63042).

On-farm culture of clinical cases. Milk samples collected from clinically affected quarters were cultured using an on-farm triplate culture system (Minnesota Easy Culture System II, 2000) and the results entered into the on-farm DairyCOMP 305 record-keeping system (Valley Agricultural Software, Tulare, CA, 93274). The three medias used in the Tri-plate system include Factor, TKT, and MacConkey agars, and so allow the differentiation of gram-positive from gram-negative pathogens, and then allows the differentiation between *Staph. aureus* and *Streptococcus* spp. Further speciation is facilitated by comparing colony morphology to color photographs provided in a laboratory manual. This on-farm system was already in routine use on study sites prior to initiating the study. In an effort to improve the quality of results from these on-farm cultures, one person on each of the three sites was made responsible for plating and then interpreting the culture results for all clinical mastitis events. These individuals were trained in culture and interpretation techniques by one of the study's principle investigators (a veterinarian) and all were provided with a manual, including color photos, which described culture methods and interpretation guidelines.

Definition of Terms used for Data Analysis

Intramammary Infection (IMI). The isolation of one or two bacterial species from a quarter milk sample. According to National Mastitis Council guidelines (N.M.C., 1999), a sample was considered "contaminated" if three or more bacterial species were isolated from the same sample, in which case the second (duplicate) sample was cultured. If the second sample was also contaminated, the results for that quarter were omitted from the final analysis. In cases of mixed infections a quarter was only counted once as being infected. However all bacterial species isolated were reported in the results.

New Intramammary Infection. A new IMI was defined as the appearance of bacterial growth in a quarter at either 1-3 DIM or at 6-8 DIM (evaluated independently) that was not previously infected at dry off, or the presence of a different bacterial species in a quarter after calving as compared to those species present at dry off. In cases of mixed infections, a quarter was counted only once as having a new IMI, even if two new bacterial species were cultured. However all new bacterial species isolated were reported in the results.

Cure. A cure was defined as the disappearance of growth of one or more bacterial species after calving that had been previously present in the quarter at dry off. By definition, it was possible for a cure (for one bacterial species) and a new IMI (for a different bacterial species) to occur in the same quarter.

Causes of Mastitis. Major pathogens included *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus bovis*, *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Bacillus* spp., *Pseudomonas* spp., and Yeast. Minor pathogens included *Corynebacterium bovis* and coagulase-negative staphylococci. Environmental pathogens were defined as all bacterial species with the exception of *Staphylococcus aureus* and *Streptococcus agalactiae*, which were defined as contagious pathogens. Two more general groups of mastitis pathogens used in the analysis included environmental gram- negative species (*Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Pseudomonas* spp.) and environmental streptococci species (*Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus bovis*, *Enterococcus* spp.).

Clinical Mastitis. A clinical mastitis event was defined as the presence of visibly abnormal milk (plus/minus abnormal quarter and/or signs of systemic illness). Analysis was performed at the quarter level, with a quarter experiencing one or more cases of clinical mastitis during the period between dry off and 60 DIM being classified as a positive mastitic quarter.

Data Management and Analysis

Data assembled into a database file (Microsoft Access, Microsoft Corp., Redmond, WA, US) included the following information for each quarter: treatment group assigned (control = DCT, treatment = DCT + Orbeseal®), herd, cow, parity, milk yield at dry off (mean kg/day for the seven days preceding dry off), dates for dry off, calving, 1-3 DIM sampling, and 6-8 DIM sampling events, days dry, and LS and bacteriological culture results from the dry off, 1-3 DIM, and 6-8 DIM sampling events. Additional data for quarters experiencing a clinical mastitis event between dry off and 60 DIM included the event date, bacteriological culture results, and treatment administered.

Chi-square analysis (Fisher's Exact Test; Proc FREQ in SAS, version 8.0) and then multivariate logistic binomial regression (Proc GENMOD in SAS, version 8.0) were used to investigate the relationship between treatment group (explanatory variable) and each of the following dependent variables of interest:

i. Prevalence of IMI: the presence of an IMI at dry off, 1-3 DIM, and 6-8 DIM (three time points an analyzed separately).
ii. Incidence of new IMI: the proportion of quarters developing a new IMI between dry off and 1-3 DIM or between dry off and 6-8 DIM (two time intervals analyzed separately).
iii. Incidence of clinical mastitis: the proportion of quarters experiencing a clinical mastitis event between dry off and 60 DIM.

iv. Cure rate: The proportion of quarters experiencing a cure between dry off and 1-3 DIM.

Analysis of bacteriological culture and clinical mastitis even data was first performed for all pathogens (overall), and then repeated for six general pathogen sub-groups: environmental, contagious, major, minor, environmental streptococci, and gram-negative spp.. Multivariate linear regression (Proc MIXED in SAS, version 8.0) was used to examine the relationship between treatment group (forced explanatory variable) and LS at dry off, 1-3 DIM, and 6-8 DIM (dependent variables). Least Squares Mean estimates for LS were reported in the results. A random term for cow was included in all logistic and linear regression models to account for the effect of clustering of quarters within cow. Farm level variation was accounted for by including 'herd' as a fixed effect in all models. Additional covariates were included in multivariate models if univariate analysis of the relationship between these covariates and the dependent variable of interest resulted in a P-value of < 0.25. Interaction terms were investigated, in all models, between treatment group and all other significant covariates. Final significance for testing the effect of treatment group was established at $P < 0.05$.

Clinical mastitis event data were analyzed using both the multivariate logistic regression approach (Proc GENMOD) described in the previous paragraph, as well as using a Cox Proportion Hazards Regression model (PHREG procedure in SAS, version 8.0), to describe the survival distribution function for treated vs control quarters for experiencing a case of clinical mastitis. For the latter analysis the date of origin was defined as the dry off date and the failure date was defined as the date when a quarter was first reported to be affected by a clinical mastitis event. Quarters were considered to be at risk of a clinical mastitis event only once during the period between dry off and 60 DIM. Quarters were classified as censored at either the cow's reported cull or died date, or 60 DIM, which ever came first. This regression model building approach (Proc PHREG) was the same as for the multivariate logistic regression models previously described (Proc GENMOD), in that it controlled for appropriate covariates. It also controlled for within-cow clustering of quarters by specifying the Covsandwich(aggregate) statement in the procedure statement and then specifying cow as the level of aggregate (id = cow). A plot of the estimated survival distribution function was created for days to a clinical mastitis event for control and treated quarters.

RESULTS

Of 437 cows (1748 quarters) initially enrolled at dry off between 3/27/2002 and 8/1/2002, 419 cows remained in the study, calving between 5/11/2002 and 10/5/2002. Three cows were omitted from final analysis because their dry period was fewer than 28 days in length. The remainder were removed from the study prior to calving primarily because of death or culling due to a variety of common disease ailments. Mastitis was not reported for any of the cows leaving the study prior to calving. Therefore, there should have been little opportunity for the introduction of bias due to inappropriate omission of treatment failures from the final analysis. Table 1 provides descriptive data comparing mean parity, milk yield at dry off (kg/d), LS at dry off, and days dry, for cows and quarters enrolled from both herds. Table 2 describes the bacterial species cultured from quarters at the dry off, 1-3 DIM and 6-8 DIM sampling points. Table 3

describes the bacterial species representing new IMI acquired between dry off and 1-3 DIM and between dry off and 6-8 DIM. Records were omitted from culture analyses when results were missing or culture results contaminated. Only 5% of quarters cultured contained mixed results (two or more pathogens) and only 0.5% of quarters cultured were excluded from analysis because of contaminated results in both duplicate samples collected (> 2 pathogen species cultured).

Linear Score and IMI Status at Dry Off

The prevalence of IMI (overall) in treated and control quarters at dry off was 31.1% and 33.3%, respectively, and the Least Squares Mean LS at dry off was 5.4 (s.e. 0.08) for both treated and control quarters. Neither of these parameter values were statistically different between treated and control quarters ($P > 0.05$), indicating that the randomization procedure used for treatment allocation was successful. Quarters had significantly higher LS measures at dry off if they were infected with either a major pathogen species (estimate (SE) = 0.76 (0.10)) or a minor pathogen species (estimate (SE) = 0.54 (0.11)), as compared to LS in uninfected quarters. There was a negative relationship between LS and milk yield (kg/d) at dry off (estimate (SE) = -0.13 (0.007), and a positive association between LS and parity (estimate (SE) = 0.28 (0.05)).

Prevalence of IMI at 1-3 and 6-8 DIM.

Chi-square and multivariate logistic regression analysis showed that the prevalence of IMI (overall) was significantly higher for control vs. treated quarters at 1-3 DIM (29.1% vs. 22.8%, $P < 0.05$), and at 6-8 DIM (25.9% vs. 20.6%, $P < 0.05$) (Table 2). The odds of having an IMI present at 1-3 DIM were 31% lower for treated vs. control quarters at 1-3 DIM (estimate (SE) = -0.37 (0.11), odds $\text{ratio}_{tx} = 0.69$, 95% CI = (0.56, 0.85)) ($P = 0.0008$) (Table 5), and 26% lower for treated vs. control quarters at 6-8 DIM (estimate (SE) = -0.30 (0.11), odds $\text{ratio}_{tx} = 0.74$, 95% CI = (0.60, 0.91))($P = 0.004$).

Quarters dried off during the months of May or June were at significantly higher risk for presence of an IMI (overall) after calving than the month of August (referent) ($P < 0.05$). In univariate analysis both the presence of an IMI at dry off and LS at dry off were each associated with an increased risk for an IMI present at 1-3 DIM ($P < 0.05$). However, when included together in the multivariate model, the presence of an IMI at dry off was no longer significant ($P = 0.16$). The latter model was selected because it yielded a better model fit according to the log-likelihood ratio. Covariates describing herd, parity, days dry and milk yield (kg/day) at dry off were not significant covariates in these models ($P > 0.05$). No interactions existed between Orbeseal® treatment and any other covariates.

Further regression analysis showed that treatment with Orbeseal® was associated with a significant reduction in prevalence of IMI at 1-3 DIM for the following general pathogen sub-groups: major pathogens, minor pathogens, environmental pathogens, and environmental streptococci spp.. There was no effect of treatment on prevalence of IMI at 1-3 DIM when considering IMI caused by either contagious pathogens or gram-negative spp. (Table 5).

Incidence of New IMI between Dry Off and 1-3 and between Dry Off and 6-8 DIM.

Chi-square and multivariate logistic regression analysis showed that the incidence of new IMI (overall) was significantly lower for treated vs. control quarters when assessed at 1-3 DIM (20.2% vs. 25.4%, $P < 0.05$), and also tended to be lower when assessed at 6-8 DIM (18.8% vs. 21.7%, $P < 0.10$) (Table 3). This second interval (dry off to 6-8 DIM) is a less accurate estimate of the apparent incidence of new IMI between dry off and calving because of new infections and cures occurring between 1-3 DIM and 6-8 DIM. The odds of developing a new IMI (overall) between dry off and 1-3 DIM were 30% lower for treated vs. control quarters (estimate (SE) = -0.36 (0.11), odds $ratio_{tx} = 0.70$, 95% CI = (0.56, 0.87)) ($P = 0.002$) (Table 5).

In the final multivariate model, the presence of an IMI at dry off was associated with an increase in risk for acquiring a new IMI (overall) by 1-3 DIM (estimate (SE) = 0.31 (0.16), $P < 0.05$). More specifically, this association was related to previous IMI with a major, but not a minor, pathogen species. Quarters dried off during the months of May or June were at significantly higher risk for developing a new IMI between dry off and 1-3 DIM, as compared to quarters dried off during the month of August (referent) ($P < 0.05$). Furthermore, there was a positive association between LS at dry off and risk for developing a new IMI by 1-3 DIM (estimate (SE) = 0.08 (0.04), $P = 0.05$), even when controlling for IMI status at dry off in the same model. This increased LS could have represented the presence of an IMI that went undetected with culture. Another possibility is that it was a residual elevation in LS in a quarter that had recently eliminated an IMI, and that the same unexplained variable that had predisposed the teat to the earlier IMI would also predispose it to an increased risk for a new IMI during the dry period (e.g. teat end condition). Other covariates describing herd, parity, days dry, and milk yield at dry off were not significant covariates in these models ($P > 0.05$). No interactions existed between Orbeseal® treatment and any other covariates.

Further regression analysis showed that treatment with Orbeseal® was associated with a significant reduction in the incidence of new IMI at 1-3 DIM for the following general pathogen sub-groups: major pathogens, environmental pathogens, and environmental streptococci spp.. The was also a strong tendency for a reduction in the incidence of new IMI caused by minor pathogens ($P = 0.08$). However there was no effect of treatment on incidence of new IMI caused by either contagious pathogens or gram-negative spp. (Table 5).

Incidence of Clinical Mastitis between Dry Off and 60 DIM.

The overall incidence of clinical mastitis between dry off and 60 DIM was significantly lower for treated (5.9%) vs. control quarters (8.0%) (Table 4). Of the 69 cases and 51 cases occurring in control and treated quarters, respectively, only two control quarters and one treated quarter experienced a case during the dry period, occurring at 4 days, 6 days, and 44 days precalving, respectively. All remaining cases occurred after calving. The odds of experiencing a case of clinical mastitis (overall) between dry off and 60 DIM were 33% lower for treated vs. control quarters (odds $ratio_{tx} = 0.67$, 95% CI = (0.48, 0.93)) ($P = 0.02$) (Table 6). Survival analysis using the Cox Proportional Hazards Regression model produced an identical estimate for odds of failure (clinical mastitis event) by 60 DIM (estimate (SE) = -0.40 (0.16), Hazard Ratio = 0.67, $P < 0.05$) (Figure 1).

In the final multivariate logistic regression model the presence of an IMI with a major, but not a minor, pathogen species at dry off was associated with an increase in risk for a clinical mastitis event by 60 DIM (estimate (SE) = 0.63 (0.22), $P < 0.05$). Also, LS at dry off had a positive association with risk for a clinical mastitis event (estimate (SE) = 0.20 (0.06), $P < 0.05$). Quarters dried off during the months of May, June, or July were at significantly higher risk for clinical mastitis than for the month of August (referent) ($P < 0.05$). Finally, there was a strong tendency for cows with longer days dry to be at increased risk for a clinical mastitis event (estimate (SE) = 0.04 (0.02), $P = 0.06$). Variables describing parity and milk yield at dry off were not significant covariates in these models ($P > 0.05$). No interactions existed between Orbeseal® treatment and any other covariates.

Further regression analysis showed that treatment with Orbeseal® was associated with a significant reduction in the risk for a clinical mastitis event between dry off and 60 DIM for clinical cases caused by the following general pathogen sub-groups: major pathogens, environmental pathogens, and environmental streptococci spp.. There was no effect of treatment on the incidence of clinical cases caused by minor pathogens, contagious pathogens, or gram-negative spp. (Table 5).

Linear Score at 1-3 and 6-8 DIM

Quarters treated with Orbeseal® had a significantly lower mean LS at both 1-3 DIM and 6-8 DIM (Least squares mean (SE) LS = 5.1 (0.17) and 2.8 (0.18)) than for control quarters (Least squares mean (SE) LS = 5.4 (0.17) and 3.1 (0.18)) ($P < 0.0001$). There was a positive association between LS at dry off and LS at both 1-3 DIM (estimate (SE) = 0.17 (0.03), $P < 0.05$) and 6-8 DIM (estimate (SE) = 0.19 (0.03), $P < 0.05$). Also, the presence of an IMI at dry off caused by a major, but not a minor, pathogen species was associated with an increase in LS at 1-3 DIM (estimate (SE) = 0.27 (0.10), ($P < 0.05$) and at 6-8 DIM (estimate (SE) = 0.19 (0.10), ($P < 0.05$). Quarters dried off during the months of April or May had a significantly lower LS than for August (referent) ($P < 0.05$). Parity had a positive effect on LS at 1-3 DIM (estimate (SE) = 0.20 (0.05), $P < 0.05$) and 6-8 DIM (estimate (SE) = 0.13 (0.05), $P < 0.05$). Days dry had a negative association with LS at both 1-3 DIM and 6-8 DIM (estimate (SE) = -0.03 (0.008), $P < 0.05$). Neither herd nor milk yield at dry off were significant covariates in these models. No interactions existed between Orbeseal® treatment and these other covariates.

Incidence of Cures between Dry Off and 1-3 DIM.

The apparent incidence of cures (overall) occurring between dry off and 1-3 DIM was not different between treated quarters (91.3%) and control quarters (88.2%) ($P > 0.05$). There was a significantly lower risk for a cure if the IMI infection at dry off was caused by a contagious pathogen (estimate (SE) = -1.28 (0.35), $P < 0.05$), as compared to IMI caused by other pathogen groups. There was a negative relationship between LS at dry off and risk for a cure by 1-3 DIM (estimate (SE) = -0.25 (0.08), $P < 0.05$). No other covariates tested were associated with risk for an apparent cure between dry off and 1-3 DIM.

DISCUSSION

This is the first study performed in North America to test the efficacy of an internal teat seal as an adjunct to DCT to prevent new IMI during the dry period. The results of this study demonstrate that the additional infusion with Orbeseal® had a significant effect on reducing the incidence of new IMI acquired between dry off and 1-3 DIM, reducing the prevalence of IMI at both 1-3 and 6-8 DIM, reducing the incidence of clinical mastitis events between dry off and 60 DIM, and reducing LS at 1-3 and 6-8 DIM. The odds of an Orbeseal®-treated quarter acquiring a new IMI between dry off and 1-3 DIM, having an IMI present at 1-3 DIM, or experiencing a clinical mastitis event between dry off and 60 DIM, were reduced by an estimated 30%, 31%, and 33%, respectively, as compared to control quarters. The protective effect of Orbeseal® treatment is presumed to be mediated by acting as a functional keratin plug and so reducing access to the gland by environmental pathogens.

There was no treatment effect when considering IMI caused by contagious mastitis pathogens (primarily *S. aureus* in this study). This may be explained by the fact that cows are at a reduced risk for transmission of contagious pathogens during the dry period. Rather, the protective effect of treatment was mediated primarily through a reduction in the rate of new IMI caused by environmental pathogens, both major and minor, and particularly by the some of the environmental streptococci species. While no analysis was performed for any one specific bacterial species, the latter relationship appeared to be driven by a numerical reduction in new IMI caused by *S. dysgalactia, S. bovis*, and *Enterococcus* spp., but not *S. uberis*. In fact, there was a numerical increase in treated quarters for new IMI caused by *S. uberis*. It is uncertain why there should be a difference between these species, since all are thought to be transmitted primarily from the environment (N.M.C., 1999).

Similarly, it is not clear why there was no treatment effect observed when considering the gram-negative pathogen group, particularly when Huxley et al. (2002) reported that treatment of uninfected quarters with teat seal resulted in significantly fewer new IMI caused by *E. coli* and all *Enterobacteriaciae*, as compared to uninfected quarters treated with antibiotic alone. Possible explanations for differences between this and the Huxley et al., (2002) study could include that the latter study was of a very different population of quarters and cows (targeted uninfected cows and quarters), used a different study design, and may have been in herds and/or climates with exposure to a different pathogen profile mix. The current study found no interaction between treatment effectiveness and covariates such as LS, IMI or milk yield at dry off. However, it is possible that, teat seal treatment aside, there may be differences between studies with regards to some of these other factors that could affect the risk for new IMI by a particular bacterial species or group during the dry period. This could include cow factors at dry off such as IMI status (overall), IMI status for specific pathogen species or groups (e.g. minor pathogen species), LS, immune system function, milk production, or teat end condition. This could also include differences in herd management factors such as dry cow housing environment, bedding type, season, ambient temperature, humidity, nutrition, vaccination programs, and possibly the duration of action and effectiveness of the dry cow antibiotic selected. These factors could create differences in teat-end exposure to different bacterial species or groups, as well as differences in the cow/quarter's ability to defend against invasion and infection by specific bacterial species or groups. Studies are lacking to address whether the effectiveness of

Orbeseal® varies if used with different commercially available preparations of long-acting antibiotics. Furthermore, while it was not recorded in this study, future studies should seek to describe if the teat seal is still present in the teat at the time of calving.

The ideal post-calving sampling point for culture would have occurred at the first milking when the Orbeseal was stripped out, as the estimate of new IMIs occurring between dry off and the 1-3 DIM sampling point could be influenced by some cures or new IMIs occurring after removal of the teat seal. However, there were concerns about sample quality, as a several different facility staff routinely processed fresh cows. Therefore it was decided that the same study technician would collect post-fresh samples at both the 1-3 DIM and 6-8 DIM sampling points. The estimate of incidence of new IMI calculated using the samples collected at 1-3 DIM would be more accurate than that produced using the samples collected at 6-8 DIM, as several new IMIs and cures could have occurred between the 1-3 DIM and 6-8 DIM sampling points.

Previous research on accuracy of the on-farm triplate culture system (Minnesota Easy Culture System II) has demonstrated that very good agreement occurred in differentiating between general pathogen categories when the technician is provided with training and specific protocols to guide interpretation (Bey and Farnsworth, 2000). Errors that did occur were often the result of attempting to "over-read" the identification system (e.g. trying to differentiate between a *S. uberis* vs. an *Enterococcus* spp.). With this in mind, the authors took a conservative approach to the analysis of clinical mastitis data by investigating the relationship between treatment and both the total number of cases (overall) and cases caused by broader pathogen sub-groups. As such, there is good confidence in the validity of the study results and inferences investigating the relationship between treatment and clinical mastitis events.

Dry period cure rates for this study were similar to the study by Huxley et al., (2002), which reported a cure rate of 93.6% in quarters receiving antibiotic alone. Similar to results reported by Huxley et al., (2002), the current study found no effect of Orbeseal® treatment on apparent cure rates. This is logical given that the product contains no antimicrobial properties, and given that all four quarters were treated with DCT.

It was not an objective of this study to describe associations between other covariates and the dependent variables of interest, but rather just to control for them in the analyses. However, there were some interesting associations identified, many of which have been reported in previous studies. For example, quarters dried off in the months of May or June were at higher risk, than quarters dried off in August (referent), for having an IMI present at 1-3 DIM, acquiring a new IMI between dry off and 1-3 DIM, and experiencing a clinical mastitis event between dry off and 60 DIM. An explanation for this could be that the heat and humidity of the summer months could have contributed to a greater pathogen load in the environment. Heat stress could also have compounded the degree of immunosupression that frequently occurs in periparturient cows, resulting in a reduced ability to eliminate infection if a pathogen did invade the gland and/or increased shedding from infected glands (Nardone et al., 1997; Mallard et al., 1998; Kimura et al., 1999). Furthermore, heat stress, changes in nutritional management, or other management changes occurring during this period could contribute to the development of other metabolic diseases in the periparturient period, which can then affect immune function (Kehrlie et al., 1990; Zerbe et al., 2000). A final contributing factor could be differences in the types of

facilities (freestall, pasture) where far-off dry cows were housed. Unfortunately this hypothesis could not be investigated because the study did not capture records describing each cow's far-off housing location nor how long she stayed at that location.

Readers should be cautious about extrapolating the results of this study, performed in two purposively selected herds, to other dairy herds. Ongoing teat seal studies in multiple herds in Canada and the U.S. will be necessary to determine if the levels of subclinical and clinical mastitis and the magnitude of treatment response observed in this study are representative of other commercial North American dairy herds. Readers should also be cautious about making direct comparisons between the results of this study and other recent teat seal studies performed in New Zealand (Woolford et al., 1998) and the U.K. (Berry and Hillerton, 2002; Huxley et al., 2002), given the very different nature of the study populations used, study designs, and objectives. In particular, these earlier studies targeted the use of this internal teat seal as the sole treatment in uninfected quarters, as compared to either DCT as sole treatment, or no treatment. It is reassuring that there is some consistency, in terms of IMI prevalence and rates, between this and at least one other study: The overall prevalence of IMI (all quarters) at dry off for quarters in the current study was 32.2%, as compared to an average of 37.6% for all quarters included in the Huxley et al. (2002) study. Similarly, the current study reported an incidence of new IMI in antibiotic-treated quarters of 25.4%, as compared to 39.3% in antibiotic-treated quarters in the Huxley et al. (2002) study. By contrast, however, Woolford et al., (1998) reported only a 2.3% new IMI rate in antibiotic-treated quarters in three New Zealand herds. It is clear that more studies are needed to describe if the results of the current study will be repeated in other commercial North American dairy herds. Similarly, further studies with treatment applied at the cow-level, and not the quarter-level, will be necessary to accurately describe whether the biological benefits associated with using an internal teat seal in addition to an antibiotic are cost effective. Economic benefits measured at the cow-level could include lowered SCC and associated milk quality premiums, a reduction in milk lost due to reduced rates of subclinical mastitis, and a reduction in costs associated with a lower clinical mastitis rates, including discarded milk, drug costs, labor, veterinary fees, culling, death, genetic loss, and antimicrobial residue risks (Fetrow et al., 2000).

CONCLUSIONS

The results of this study demonstrate that infusion with Orbeseal®, as an adjunct to DCT at dry off, had a significant effect on reducing the incidence of new IMI acquired between dry off and 1-3 DIM, reducing the prevalence of IMI at both 1-3 and 6-8 DIM, reducing the incidence of clinical mastitis events between dry off and 60 DIM, and reducing LS at 1-3 and 6-8 DIM. Quarters treated with Orbeseal® and DCT were 30% less likely to develop a new IMI between dry off and 1-3 DIM, 31% less likely to have an IMI present at 1-3 DIM, 33% less likely to experience a clinical mastitis event between dry off and 60 DIM, and had significantly lower LS measurements at both 1-3 and 6-8 DIM, as compared to quarters treated with DCT alone. While further studies are needed to describe if similar performance can be attained in other commercial North American dairy herds, and to accumulate data defining the cost-benefit of using the teat seal as an adjunct to DCT at dry off, the results of this study suggest that Orbeseal® is very

promising as an additional management tool to assist in the prevention of new intramammary infections during the dry period.

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Table 1. Description of Study Cows and Quarters that Calved Successfully, by Herd of Origin

	Herd A Mean (SE) (range)	Herd B Mean (SE) (range)
Number of cows enrolled	239	180
Parity at dry off	2.5 (0.09) (1 to 8)	2.4 (0.09) (1 to 8)
Milk yield at dry off (kg/day)	19.6 (0.6) (1.4 to 39.4)	14.7 (0.7) (0.8 to 36.9)
Dry period (days)	55.1 (0.5) (33 to 97)	56.0 (0.7) (33 to 91)
LS at dry off (mean (SE)(range))	5.2 (0.07) (0.1 to 10.9)	5.5 (0.08) (0.1 to 11.1)

Table 2. IMI Prevalence and Description of Bacterial Species Present in Control (DCT) and Treated (DCT + Orbeseal® Quarters at Dry off, 1-3 DIM, and 6-8 DIM

	IMI present at dry off		IMI present at 1-3 DIM		IMI present at 6-8 DIM	
	Control (n=828)	Treated (n=834)	Control (n=812)	Treated (n=821)	Control (n=811)	Treated (n=809)
Quarters with no growth	552	575	576	634	601	642
Total quarters with IMI	276	259	236	187	210	167
Quarters with mixed IMI	49	32	39	40	30	31
% of all quarters with IMI	33.3%[a]	31.1%[a]	29.1%[a]	22.8%[b]	25.9%[a]	20.6%[b]
Bacterial species cultured						
Staphylococcus aureus	32	27	21	15	16	11
Streptococcus agalactiae	0	0	1	0	0	0
Total contagious pathogens	32	27	22	15	16	11
Streptococcus dysgalactiae	3	1	8	2	7	1
Streptococcus uberis	42	38	19	36	44	35
Enterococcus spp.	27	31	47	22	27	27
Streptococcus bovis	0	0	9	2	10	5
Total environmental Streptococci	72	70	83	62	88	68
Escherichia coli	30	18	12	14	14	10
Klebsiella spp.	28	25	27	21	13	17
Enterobacter spp.	14	9	31	30	25	29
Proteus spp.	0	0	2	2	4	2
Citrobacter spp.	1	1	0	0	0	0
Pseudomonas spp.	0	0	4	2	1	1
Total gram-negative pathogens	73	53	76	69	57	59
Yeast	6	6	4	7	5	7
Bacillus spp.	0	1	3	3	2	2
Total major pathogens	183	157	188	156	168	147
Coagulase-negative staphylococci	133	129	85	66	72	49
Corynebacterium spp.	9	5	2	5	0	2
Total minor pathogens	142	134	87	71	72	51
Total pathogen count	325	291	275	227	240	198

Note: reports all species cultured from quarters with single and mixed infections.

a, b % of all quarters with IMI between columns are significantly different ($P < 0.05$)

Table 3. Description of Bacterial Species Isolated in New Intramammary Infections from Control (DCT) and Treated (DCT + Orbeseal®) Quarters at 1-3 DIM and 6-8 DIM

	New IMI acquired between dry off and 1-3 DIM		New IMI acquired between dry off and 6-8 DIM	
	Control (n = 812)	Treated (n = 821)	Control (n = 811)	Treated (n = 809)
Number of quarters with new IMI	206	166	176	152
Number of quarters with mixed new IMI	34	36	21	27
% of quarters with new IMI	25.4%[a]	20.2%[b]	21.7%[a]	18.8%[c]
Bacterial species cultured in new IMI				
Staphylococcus aureus	10	7	6	4
Streptococcus agalactiae	2	0	0	0
Total contagious pathogens	12	7	6	4
Streptococcus dysgalactiae	8	2	7	1
Streptococcus uberis	18	35	38	32
Enterococcus spp.	46	22	25	25
Streptococcus bovis	9	2	10	5
Total environmental *Streptococci* spp.	81	61	80	63
Escherichia coli	11	14	13	10
Klebsiella spp.	26	19	11	16
Enterobacter spp.	28	29	24	29
Proteus spp.	2	2	4	2
Citrobacter spp.	0	0	0	0
Pseudomonas spp.	4	2	1	1
Total gram negative pathogens	71	66	53	58
Yeast	4	7	5	7
Bacillus spp.	3	3	2	2
Total major pathogens	171	144	146	134
Coagulase-negative staphylococci	67	53	51	43
Corynebacterium spp.	2	5	0	2
Total minor pathogens	69	58	51	45
Total pathogen count	240	202	197	179

Note: reports all species cultured from quarters with single and mixed new infections.
a, b % of all quarters with new IMI between columns are significantly different at $P < 0.05$
a, c % of all quarters with new IMI between columns are different at $P < 0.10$

Table 4. Description of Pathogens Cultured from Control Quarters (DCT) and Treated Quarters (DCT plus Orbeseal®) for Clinical Mastitis Events Occurring Between Dry Off and 60 DIM

Organism cultured from clinical mastitis event	Control Quarters n (% of all affected)	Treatment Quarters n (% of all affected)
Staphylococcus aureus	3 (4.3)	3 (5.9)
Environmental *streptococci spp.*	17 (24.6)	3 (5.9)
Escherichia coli	11 (15.9)	8 (15.7)
Klebsiella spp.	1 (1.4)	4 (7.8)
Pseudomonas spp.	0 (0)	1 2.0)
Total gram-negative pathogens	12 (17.4)	13 (25.5)
Total major pathogens	32 (46.4)	19 (37.3)
Coagulase-negative *staphylococci*	7 (10.1)	9 (17.6)
Corynebacterium spp.	1 (1.4)	0 (0)
Total minor pathogens	8 (11.6)	9 (17.6)
Total pathogens identified	40 (58.0)	28 (54.9)
No growth	14 (20.3)	10 (19.6)
Contaminated sample	0 (0)	1 (2.0)
No results available	15 (21.7)	12 (23.5)
Total affected	69 (100)	51 (100)
Total at risk	862	862
% of total affected	8.0% [a]	5.9% [b]

Note: Only first clinical mastitis event is reported for any one quarter

a, b % of all quarters with a clinical mastitis event between columns significantly different ($P < 0.05$)

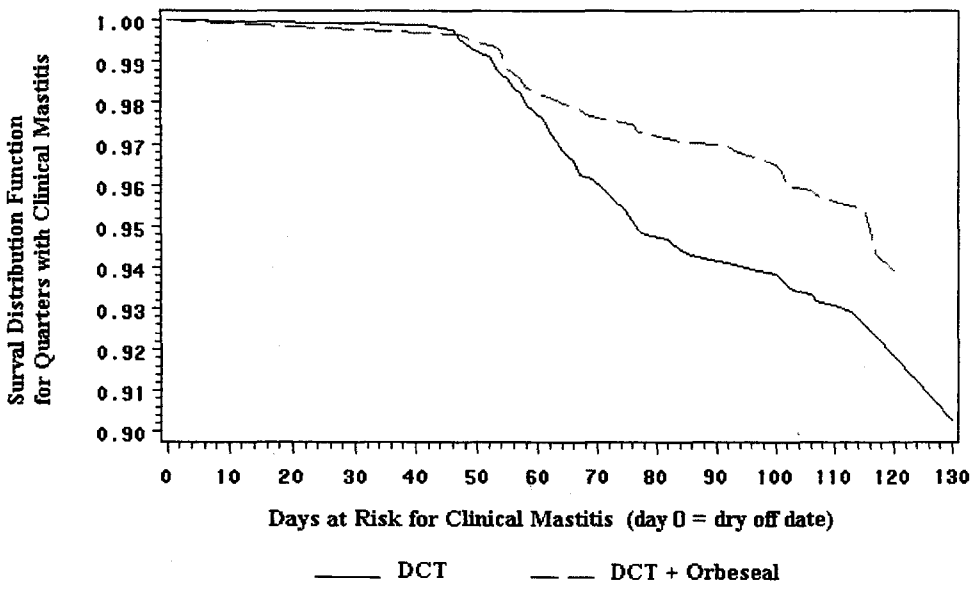

Figure 1. Survival distribution function for days to a clinical mastitis event between dry off (day 0) and 60 DIM (censor date), for quarters treated with DCT or DCT plus Orbeseal®. Cox Proportional Hazard regression model controls for herd, month of dry off, days dry, parity, infection status at dry off, and LS at dry off.

Table 5. Results of Multivariate Regression Analysis of Odds for Presence of an IMI at 1-3 DIM and Odds of Acquiring a New IMI between Dry off and 1-3 DIM for Control Quarters (DCT) and Treated Quarters (DCT plus Orbeseal®)

Parameter of Interest	Control (n = 812) n affected (%)	Treated (n = 821) n affected (%)	Estimate (SE)	Odds Ratio $_{treatment}$ (95% Conf. Limits)	P value
IMI present at 1-3 DIM					
All pathogens	236 (29.1%)	187 (22.8%)	-0.37 (0.11)	0.69 (0.56, 0.85)	0.0008
Major spp.	149 (18.3%)	116 (14.1%)	-0.36 (0.13)	0.70 (0.54, 0.91)	0.009
Minor spp.	73 (9.0%)	51 (6.2%)	-0.41 (0.19)	0.66 (0.46, 0.97)	0.03
Environmental spp.	214 (26.3%)	172 (20.9%)	-0.34 (0.11)	0.71 (0.57, 0.88)	0.002
Contagious spp.	19 (2.3%)	14 (1.7%)	-0.28 (0.34)	0.76 (0.39, 1.47)	0.42
Environmental streptococci spp.	83 (10.2%)	61 (7.4%)	-0.38 (0.17)	0.68 (0.49, 0.95)	0.02
Gram Negative spp.	76 (9.3%)	70 (8.5%)	-0.14 (0.16)	0.87 (0.63, 1.20)	0.40
New IMI acquired between dry off and 1-3 DIM					
All pathogens	206 (25.4%)	166 (20.2%)	-0.36 (0.11)	0.70 (0.56, 0.87)	0.002
Major spp.	135 (16.6%)	107 (13.0%)	-0.35 (0.14)	0.71 (0.54, 0.93)	0.01
Minor spp.	58 (7.1%)	42 (5.1%)	-0.37 (0.22)	0.69 (0.45, 1.06)	0.09
Environmental spp.	193 (23.4%)	159 (19.4%)	-0.32 (0.12)	0.73 (0.58, 0.92)	0.007
Contagious spp.	10 (1.2%)	6 (0.7%)	-0.53 (0.41)	0.59 (0.26, 1.33)	0.20
Environmental streptococci spp.	81 (10.0%)	60 (7.3%)	-0.39 (0.17)	0.68 (0.49, 0.95)	0.02
Gram Negative spp.	71 (8.7%)	66 (8.0%)	-0.14 (0.17)	0.87 (0.63, 1.21)	0.41

Models control for herd, parity, month, LS and infection status at dry off

Table 6. Results of Multivariate Regression Analysis of Odds for Experiencing a Clinical Mastitis Event Between Dry Off and 60 DIM for Control Quarters (DCT) and Treated Quarters (DCT plus Orbeseal®)

Pathogen Cultured	Control (n = 862) n affected (%)	Treatment (n = 862) n affected (%)	Estimate (SE)	Odds Ratio $_{treatment}$ (95% Conf. Limits)	P value
All pathogens	69 (8.0%)	51 (5.9%)	-0.40 (0.17)	0.67 (0.48, 0.93)	0.02
Major spp.	32 (3.7%)	19 (2.2%)	-0.73 (0.28)	0.48 (0.28, 0.83)	0.008
Minor spp.	8 (0.9%)	9 (1.0%)	-0.02 (0.44)	0.98 (0.42, 2.30)	0.96
Environmental spp.	37 (4.3%)	25 (2.9%)	-0.59 (0.25)	0.55 (0.34, 0.90)	0.02
Contagious spp.	3 (0.4%)	3 (0.4%)	0.006 (0.70)	1.01 (0.25, 4.0)	0.99
Environmental streptococci spp.	17 (2.0%)	3 (0.4%)	-1.79 (0.68)	0.17 (0.04, 0.63)	0.0009
Gram Negative spp.	12 (1.4%)	13 (1.5%)	-0.057 (0.41)	0.94 (0.43, 2.10)	0.89

Models control for herd, days dry, and parity, month, LS, and infection status at dry off