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Randomized noninferiority study evaluating the efficacy of a postmilking teat disinfectant for the prevention of naturally occurring intramammary infections

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ABSTRACT

The objective of this study was to complete a positive-control, natural exposure, noninferiority design field study to test the efficacy of a novel glycolic acid-based postmilking teat disinfectant as compared with a previously proven iodine-based postmilking teat disinfectant (positive control). The primary outcome of interest was the effect of treatment on incidence of new intramammary infections. Secondary outcomes included the effect of treatment on prevalence of infection, somatic cell count, and teat condition. After blocking by parity, approximately 300 early- to mid-lactation cows on a large Wisconsin dairy farm were randomly assigned to 1 of 2 groups. For a 12-wk period between May and August 2014, the 2 groups were dipped after each milking with either the experimental (EX) or positive control (PC) product. Individual quarters were sampled to establish bacteriological infection status at the beginning of the study, and every 2 wk thereafter, by use of a 2-stage process evaluating somatic cell count (SCC), and then culturing milk samples only when SCC exceeded a parity-specific threshold. Teat condition scoring was completed at the beginning of the study and on wk 4, 8, and 12. Mixed logistic regression was used to evaluate the effect of treatment on dichotomous outcome measures including the odds of acquiring a new infection during a given 2-wk sampling interval (incidence), the odds for presence of infection at sampling (prevalence), and odds for a normal teat skin condition score. Mixed linear regression was used to evaluate the effect of treatment on somatic cell count. For the noninferiority analysis, the upper bound of the 95% confidence interval for the difference in new infection rate between the 2 treatments (EX − PC), had to be to the left of the critical value d (0.035) to conclude that EX was noninferior relative to PC with respect to risk for new infections. Results showed that the incidence of new infections was not different for quarters dipped with EX (3.2%) as compared with PC (4.2%). Similarly, the prevalence of infection tended to be lower for quarters dipped with EX (3.92%) as compared with PC (5.03%). No overall difference was found between treatments when evaluating somatic cell count measures and teat condition scores. Because the upper bound of the 95% confidence interval of the new IMI rate difference was smaller than the predefined noninferiority limit, it was concluded that the experimental product was not inferior compared with the positive control. As such, the glycolic acid-based teat disinfectant evaluated in this study can be considered an effective postmilking teat disinfectant, as well as safe, in so far as the product was not irritating to teat skin and did not negatively affect skin condition measures, as compared with the positive control group.

Key words: teat disinfectant, glycolic acid, iodine, intramammary infection, noninferiority

INTRODUCTION

Despite continued advances in prevention and treatment during the past several decades, mastitis in dairy cows remains as one of the leading causes of decreased milk production, lower milk quality, animal loss and ultimately reduced profit for the dairy producer (Ruegg, 2012). One recommendation in the National Mastitis Council (**NMC**) Recommended Mastitis Control Program to prevent new intramammary infections (**NIMI**) is to routinely apply pre- and postmilking teat disinfectants after each milking (NMC, www.nmconline.org). It is in the best interest of udder health solution companies to develop teat disinfectants that meet producer needs, address societal demands, and fulfill regulatory requirements. A teat disinfectant must meet several

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requirements to serve its purpose in modern dairies: (1) have proven germicidal efficacy, (2) prevent NIMI, (3) maintain optimal teat condition and promote lesion healing, (4) not irritate the cow or the user, and (5) leave no residues in milk that could affect human health.

Germicidal efficacy can be confirmed using standard in vitro tests (e.g., EN1656; CEN, 2000), although it must be clarified that these results do not guarantee mastitis control. The true test of mastitis prevention is achieved when products are tested in the field against a product of proven efficacy. The NMC has made protocols available to achieve these comparisons (Nickerson et al., 2004). In the last few years, adjustments to existing protocols have been proposed to take into account more robust statistical approaches, appropriate logistics to run these types of trials, and economic considerations that are more realistic for the sponsor of the product evaluation (Ceballos-Marquez et al., 2013; Schukken et al., 2013). Although these protocol adjustments have been made by the NMC, they are not yet formally endorsed by the NMC. Maintaining healthy teat skin reduces bacterial colonization and possible IMI. Practical troubleshooting guidelines to score teat condition have been published by the NMC (Ohnstad et al., 2007). Irritation to the cow or the user should be addressed by the developer of the product during early stages of development. Finally, it is the responsibility of the manufacturer to guarantee that none of the components used in the formulation compromise human health.

Globally, producers and governing bodies are trending to more regulations for teat disinfectant raw materials. For example, the European Union has implemented rigorous controls for biocides that come into contact with food-producing animals. Under the biocidal products regulation, new products require review and authorization before they can be placed on the market, and the active substances contained in them must be previously approved. This strict regulation includes teat disinfectants used in the dairy industry.

Formulating teat disinfectants with chemicals that naturally occur in milk is an interesting opportunity for the udder health solutions industry, because concerns about residues in milk are minimized. Glycolic acid is naturally present in milk and has been widely used in cosmetic skin care products, in food processing as a flavoring agent, as a preservative, and as a kitchen cleaner. Moreover, glycolic acid was recently approved by biocidal products regulation for use as a germicide in teat dips. However, glycolic acid alone shows limited germicidal efficacy, hindering its chances to meet a teat disinfectant's minimal requirements for the product's intended purpose. GlyTec (DeLaval, Kansas City, MO), which includes 3% glycolic acid, is a proprietary blend of glycolic acid and other ingredients that overcomes the named limitations related to germicidal efficacy. Field-based studies are needed to measure the efficacy of postmilking teat disinfectants using germicides that naturally occur in milk, as well as to evaluate safety to ensure that the product is not irritating to teat skin.

The aim of this study was to measure the efficacy of a novel glycolic acid-based teat disinfectant, OceanBlu Pre Post, when applied postmilking. The primary objective of the study was to demonstrate noninferiority of this experimental (**EX**) test product when compared with a previously proven iodine-based positive control (**PC**) product with regard to the incidence of NIMI that occurred under natural challenge conditions on a commercial dairy farm. Secondary objectives included describing the effect of treatment on prevalence of IMI, SCC, and teat condition throughout the trial period. Our null hypothesis was that treatment EX was inferior to treatment with PC, or $P(NIMI)_{Test} - P(NIMI)_{Control} \geq \Delta$, where Δ = the prespecified margin of inferiority. Our alternative hypothesis, if we rejected the null, was that treatment with EX was not inferior compared with treatment with PC, or $P(NIMI)_{Test} - P(NIMI)_{Control} < \Delta$.

MATERIALS AND METHODS

Study Farm and Study Pens

This randomized, noninferiority, positive-control field study was conducted on a commercial Holstein dairy farm in western Wisconsin. The farm milks approximately 1,500 lactating cows 3 times daily in a double-20 parallel parlor. The herd was enrolled on a monthly DHIA testing program, with herd average milk production, SCC, butterfat percent, and protein percent being 41.3 kg/d, 229,000 cells/mL, 3.9%, and 3.2%, respectively. Lactating cows are housed in 1 of 8 freestall pens with reclaimed deep sand bedding. Alleyways are scraped between milkings. After calving, cows spend approximately 30 d in a fresh cow pen, after which they are moved into early lactation pens.

Cow Enrollment

Two weeks before study initiation, study cows were initially identified as all 297 early-to-mid-lactation animals already housed within 2 pens on the farm (pens 4 and 5), each with 150 freestalls. These pens included predominantly older cows, but also some first lactation animals. No inclusion or exclusion criteria were applied to be eligible for enrollment. After blocking by parity (lactation 1 vs. lactation $\geq$2), cows were assigned to