J. Dairy Sci. 100:1–10
https://doi.org/10.3168/jds.2016-11193

Efficacy of a high free iodine barrier teat disinfectant for the prevention of naturally occurring new intramammary infections and clinical mastitis in dairy cows

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ABSTRACT

Using a natural exposure trial design, the goal of our study was to evaluate the clinical efficacy of an iodine teat disinfectant with barrier properties and a high level of free iodine relative to a conventional iodine teat disinfectant with no barrier properties and low levels of free iodine. During the 18 wk of the trial, quarter milk samples were collected every 2 wk from 385 dairy cows from 2 herds. Cows on both farms were assigned in a balanced way according to milk yield, number of lactation, days in milk, somatic cell count (SCC) and microbiology culture pretrial into one of following groups: nonbarrier post milking teat disinfectant (NBAR; n = 195 cows; 747 quarters) or barrier postmilking teat disinfectant (BAR; n = 190 cows; 728 quarters). Afterward, at each scoring date every 2 wk, milk SCC was quantified in samples from all mammary quarters and microbiologic culture was only performed on milk samples with SCC >200,000 cells/mL for multiparous cows and SCC >100,000 cells/mL for primiparous cows. A new intramammary infection (NIMI) was defined when a quarter had milk SCC <200,000 cells/mL for multiparous cows and <100,000 cells/mL for primiparous without microorganism isolation; NIMI was also defined as a subsequent sampling visit with milk SCC >200,000 cells/mL for multiparous cows and >100,000 cells/mL for primiparous cows along with positive microorganism isolation. A quarter could have several NIMI, but only 1 case per specific pathogen was considered. The most frequently isolated microorganism group on both farms was *Streptococcus* spp. (6.25% of total mammary quarters), followed by coagulase-negative staphylococci (3.6%) and *Corynebacterium* spp. (1.5%). In the present study, an interaction occurred between treatment and week of trial on the incidence risk of NIMI. Quarters disinfected with BAR had 54 and 37% lower odds of NIMI than quarters disinfected with NBAR at 8 and 16 wk of the trial, respectively; whereas at other weeks of the study both products had similar incidence risks of NIMI. Overall, teats disinfected with BAR had 46% lower odds of acquiring a clinical mastitis than those disinfected with NBAR. We concluded that the postmilking teat disinfectant with barrier properties and higher free iodine content reduced the risk of clinical mastitis, although differences in new infections were detected at only weekly time points.
Key words: clinical mastitis, free iodine, teat disinfection, efficacy, barrier

INTRODUCTION

Bovine mastitis remains a disease that negatively affects the dairy industry. Despite major advances for its control, it is a major cause of reduced milk yield, lower milk quality, cow culling, and, consequently, reduced profit for the dairy producer. Teat disinfection, both pre- and postmilking has been successfully used for many years to prevent new IMI (**NIMI**; Pankey et al., 1984). Iodine-based teat products are most commonly used to disinfect teats before and after milking. These are used by 66 (premilking) and 84% (postmilking) of large herd operations in the United States (Lopez-Benavides et al., 2016). The belief by some that the efficacy of a commercial iodine teat dip is determined by the amount of available iodine, that the higher the iodine content, the better, is erroneous (Gottardi, 2001); studies have shown otherwise, attributing little importance to crude iodine concentration, but more on the concentration of free iodine in the product (Gottardi, 2001; Murdough and Pankey, 1993). Free iodine is a measure of the concentration of uncomplexed or molecular iodine (Foret et al., 2005), which is highly reactive and therefore germicidal. In fact, free iodine is the only form of iodine with a proven correlation between equilibrium concentration and bactericidal

Received March 19, 2016.
Accepted December 15, 2016.
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activity that can be measured by 3 different methods: extraction with a nonpolar solvent, by dialysis, or by a potentiometric method (Gottardi, 2001). This concept was demonstrated in a natural exposure trial, in which a product with high (12–16 mg/kg) free iodine concentration reduced NIMI by 55% when compared with a lower (5–8 mg/kg) free iodine product (Foret et al., 2005).

Protection of the mammary gland against NIMI during intermilking periods can be extended by the use of barrier films. Teat disinfectants that have barrier properties contain film-forming ingredients that remain on the teat skin surface when applied postmilking. The use of barrier-type products was initially explored to protect teats during the dry period (Timms et al., 1987). When used at dry off and 10 d before calving, success in prevention of NIMI was observed when compared with nondipped teats (Timms, 1997). Poutrel et al. (1990) studied the inclusion of the barrier property in a product during lactation in a field study in 5 commercial herds and showed that an experimental chlorine dioxide barrier product reduced clinical mastitis (**CM**) by 33% compared with a 0.5% iodine teat disinfectant. Another study compared a barrier 0.3% chlorhexidine gluconate product versus a nonbarrier 1% iodine product and showed no beneficial effect of the barrier property (Nickerson and Boddie, 1995). Similarly, Hogan et al. (1995) showed no benefit of using a 0.55% chlorhexidine gluconate barrier product versus a nonbarrier 1% iodine product; however, when 2 products with equal levels of free iodine (12–20 mg/kg) were tested, a 24% reduction in CM and subclinical mastitis was observed in the group of cows dipped with a barrier product (Foret et al., 2006). Therefore, the presence of a film-forming agent that is able to stay on the teat surface combined with an effective, persistent, and active germicide is important for a true barrier product.

Formulation of a barrier-type product requires not only that the physical film adheres to and protects the gland from infection postmilking, but that the germicide remain active and kill microorganisms that come into contact with the film (Foret et al., 2006). This property could be advantageous in challenging weather conditions where high humidity, temperature, or mud put the mammary gland at high risk of infection due to the increased growth of environmental mastitis pathogens. Growth rates of coliforms and environmental streptococci are greatest during warm, wet weather (Hogan and Smith, 2012). It is desirable that the barrier product creates a persistent and active germicide film when applied to teat skin. The difference in persistence of iodine in the film was measured using an in vitro-excised teat model for 2 barrier products with differing iodine persistence (Buchalova and Rauer, 2013). When the same products were tested under a natural exposure trial, the barrier product with longer persistence resulted in a 30% lower infection rate (Lago et al., 2014).

Studies over the last 2 decades show that a higher free iodine content product is better than a lower content one, and that barrier products have merit in protecting teats from infection during the intermilking period (Foret et al., 2005; Lago et al., 2014). We hypothesized that the combination of barrier properties and high free iodine in a teat disinfectant product would show greater efficacy in preventing NIMI and CM as a result of higher and extended germicidal power during the intermilking periods, especially when weather conditions are challenging. The goal of the present study was to evaluate the clinical efficacy of an iodine product with barrier properties and a high level of free iodine relative to a conventional iodine product with no barrier properties.

MATERIALS AND METHODS

Study Design and Farm Information

An 18-wk split-herd efficacy trial was conducted on 2 commercial dairy farms in São Paulo State, Brazil, from August 2014 to January 2015 (Table 1). Within each farm, cows were assigned to 1 of 2 groups so that they were equally represented. Blocked randomization was based on milk yield (**MY**), number of lactations (**NL**), DIM, SCC, and microbiological status by aerobic culture before the beginning of the study (Table 2). Cows were assigned randomly to a group dipped with Della Soft (DeLaval, Tumba, Sweden; 0.5% iodine, 1–2 mg/kg free iodine, 2% emollients) postmilking (**NBAR**; n = 195 cows; 747 quarters) or the group dipped with Della Barrier (DeLaval; 1% iodine, 5–8 mg/kg free iodine, 10% emollients) postmilking (**BAR**; n = 190; 728 quarters).

Milking Routine

On both farms, the premilking routine included forestripping into a stripping cup, dipping with Della Soft (DeLaval; 0.5% iodine, 1–2 mg/kg free iodine, 2% emollients), wiping off teats with a single-use disposable paper towel, and finally attaching the milking machine. All milkers wore latex gloves during milking. At the end of milking, the assigned postmilking disinfectant (NBAR or BAR) was applied using a nonreturn dip cup to cover the whole barrel length of the teat. Dip cups were identified with color-coded (blue or red) ac-

Table 1. Description of farm characteristics and milking procedures during the pretrial period

Information	Farm A	Farm B
Production system	Freestall	Pasture-based
Bedding material	Sand	Pasture
Breed	Holstein	Holstein
Milk production (L/d)	30	23.5
Milking parlor type	Herringbone, 4 × 2	Herringbone, 6 × 1
Number of milking units	8	6
Number of lactating cows	176	159
Milking frequency (per day)	3	3
Number of milkers	2	2
Average bulk tank SCC (×1,000 cells/mL)	500	745
Average bulk tank ln SCC	2.7	2.9
Premilking teat disinfectant	Iodine (0.25%)	Iodine (0.25%)
Postmilking teat disinfectant	Iodine (0.5%)	Iodine (0.5%)

cording to each iodine teat disinfectants, cows were also identified using colored leg bands (blue or red) according to the study group assigned. Milking personnel and researchers were blinded to the origin of the iodine teat disinfectants.

A CM case was treated with intramammary antibiotics, according to farm-specific protocols, and both farms used blanket dry cow therapy, which consisted infusion of intramammary antibiotics and an internal teat sealant at dry off. A milking systems analysis was conducted at the beginning (wk 0) and during (wk 6) the trial, following Reinemann and Book (1994). At each time point, deviations from expected standards were corrected. Daily data on rainfall and temperature during the study period were collected from an official weather station located in Casa Branca (36.9 and 22 km away from farm A and B, respectively), Brazil (http://www.inmet.gov.br/portal/).

Table 2. Descriptive results (mean ± SD) of blocking data before the beginning of a randomized controlled trial to compare a high free iodine barrier postmilking teat disinfectant (BAR) to a lower free iodine, nonbarrier teat dip (NBAR)

Item	BAR	NBAR
Milk yield (L/d)	25.5 ± 10.58	26.5 ± 11.28
Parity[1]	2.1 ± 1.11	2.0 ± 1.09
DIM	205.8 ± 165.21	224.7 ± 191.17
SCC (×1,000 cells/mL)	621.4 ± 1,527.6	644.8 ± 1,640.82
ln SCC	2.8 ± 2.13	2.8 ± 2.17
Microbiology culture [no. (%)]		
Samples cultured	612 (100)	608 (100)
Culture negative	422 (68.95)	417 (68.59)
Culture positive	189 (30.88)	187 (30.76)
Contaminated	1 (0.16)	4 (0.66)
Streptococcus uberis	59 (9.64)	45 (7.40)
Streptococcus dysgalactiae	5 (0.82)	3 (0.49)
Streptococcus spp.	3 (0.49)	6 (0.99)
Staphylococcus aureus	1 (0.16)	2 (0.33)
Streptococcus agalactiae	1 (0.16)	1 (0.16)
Corynebacterium spp.	23 (3.76)	31 (5.10)
CNS	60 (9.80)	68 (11.18)
Prototheca spp.	3 (0.49)	3 (0.49)
Yeast	3 (0.49)	5 (0.82)
Enterococcus	7 (1.14)	3 (0.49)
Citrobacter	1 (0.16)	1 (0.16)
Gram-positive bacillus	23 (3.76)	19 (3.13)

[1]Number of lactation: 1 or ≥2.

Milk Sampling and Laboratory Analysis

We calculated the sample size using a one-sided significance level of 5%, an estimated power of 80, and Δ of 30% as a difference in proportion of NIMI (Ceballos-Marquez et al., 2013). Approximately 150 animals/600 quarters were estimated per group. Thus, quarter-level data were collected from a total of 385 cows (195 NBAR and 190 BAR) during the trial. Mammary quarters from all lactating cows were sampled every 2 wk during a period of 18 wk, providing up 10 periods of sample collection (wk 0–18). Cows that calved or were dried off during the experimental period were also included in the study, and their information was included in the analyses. However, cows that were present for only 1 of 10 scheduled sampling visits were not included in final data set used for statistical analysis.

To determine IMI status, milk samples were collected from all mammary quarters for SCC and aerobic microbiological culture. Before sampling, teat ends were disinfected with the premilking product and cleaned with a disposable paper towel. Next, each teat end was scrubbed vigorously with cotton soaked in an ethanol solution (70%) and air-dried. The first streams of foremilk were discarded, and 15 mL of milk were collected aseptically from each quarter into sterile vials. Samples were immediately stored in insulated boxes containing ice and stored at −20°C for 1 wk until microbiology culture was performed. For SCC analysis, 50 mL of milk were collected from individual quarters and preserved with 2-bromo-2-nitropropane-1,3-diol (0.05% wt/vol).

Milk SCC was determined using the flow cytometry method (Somacount 300, Bentley, Chasca, MN). All

milk samples collected every 2 wk were first submitted for determination of SCC, and only samples from quarters with SCC >200,000 cells/mL for multiparous cows and SCC >100,000 cells/mL for primiparous cows were selected and submitted for bacteriologic culture, as suggested by Ceballos-Marquez et al. (2013). These thresholds are useful for reducing costs associated with culturing, because low SCC quarters are most likely to not have an IMI. Aerobic culture was performed according to NMC standards (National Mastitis Council, 2004). Briefly, from each sample, 0.01 mL of milk was plated on blood agar and incubated aerobically for 24 and 48 h at 37°C. Samples yielding more than 2 different bacterial species were considered to be contaminated and removed from the statistical analysis. Bacteria were identified based on colony morphology and Gram staining. After Gram staining, the following biochemical tests for the identification of bacteria were performed: (a) positive catalase, coagulase, and acetoin for *Staphylococcus aureus* identification; (b) positive catalase and coagulase but negative acetoin for coagulase-positive staphylococci not *Staph. aureus*; (c) positive catalase and negative coagulase for CNS; (d) positive Christie, Atkins, Munch-Petersen (**CAMP**) test and negative esculin for *Streptococcus agalactiae*; (e) negative CAMP test and esculin for *Streptococcus dysgalactiae*; (f) positive esculin test and positive or negative CAMP, but negative bile esculin hydroxide for *Streptococcus uberis*; (g) positive esculin test and positive or negative CAMP, positive bile esculin hydroxide and negative pyrrolidonyl arylamidase (**pyr**) test (Probac do Brasil, São Paulo, Brazil) for *Streptococcus bovis*; and (h) positive esculin test and positive or negative CAMP, positive bile esculin hydroxide and pyr test for *Enterococcus* spp. Gram-negative bacteria were identified using MacConkey agar (Probac do Brasil, São Paulo, Brazil), and the following biochemical characteristics were evaluated using ENTEREX (Cefar Diagnóstica Ltda, São Paulo, Brazil): motility, enzymatic reactions, glucose and sucrose fermentation, sulfide acid production, gas formation, AA utilization, urea formation, and indole production. Yeast and *Prototheca* spp. were identified by morphological analyses using an optical microscope.

Determination of IMI

An IMI was detected for a quarter when the SCC milk sample had a value above its threshold and the culture result was positive. Interpretation of bacteriological results to determine an IMI followed recommendations by NMC (2012). Thus, an IMI existed when >1 colony (from a 0.01-mL milk sample) was isolated for any bacterial species, with the exception of CNS and *Corynebacterium* spp. For these, an IMI existed if ≥10 colonies from a 0.01-mL milk sample were observed.

A NIMI was identified when the microorganism causing the infection had not been isolated from the same quarter in the previous milk sampling at any visit through the study. Once a quarter was identified as being infected with a particular microorganism, any repeated infection of the same quarter with the initially identified pathogen was not considered a NIMI. A CM case could also be considered NIMI, as long as it was isolated a different microorganism from the previous bacteriologic culture result. The incidence risk per week of trial was calculated as the total number of NIMI divided by the total number of quarters at risk. Once a NIMI was identified, the quarter became eligible for infection (at risk) again for all organisms except the ones that the quarter had been infected with before (Ceballos-Marquez et al., 2013). When milk sample had 2 microorganisms isolated, it was considered as follows. (1) If 1 of 2 microorganism had been isolated in the same quarter, we designated it as an IMI caused by microorganism isolated before, but not NIMI. (2) If the 2 microorganisms were not isolated before in the same quarter, we considered it eligible for a NIMI. Samples with isolation of 1 major and 1 minor pathogen (Reyher et al., 2012) were designated as IMI caused by the major pathogen; samples with isolation of *Staph. aureus* and *Streptococci* were designated as IMI caused by *Staph. aureus*; samples with isolation of *Corynebacterium* spp. and CNS were designated as IMI caused by CNS.

Clinical Mastitis Identification

Milkers in both farms were trained to identify any abnormal milk or clinical symptoms associated with a CM case. To confirm CM, milkers discarded the first streams of milk in a strip cup before applying the premilking teat disinfectant. If a CM quarter was identified, milkers collected milk aseptically before mastitis treatment, and all CM milk samples were frozen for microbiology culture. Pertinent information from each CM episode was recorded. The CM treatment was based on specific protocols used by each farm.

Statistical Analysis

Analysis of NIMI. The treatment effect on the incidence risk of NIMI during the 2-wk study period was evaluated at the quarter level as binomial response variables, using logistic multivariable regression. We used PROC GLIMMIX of SAS version 9.3 (SAS Institute Inc., Cary, NC), according to following model:

$$\text{logit(pi)} = \beta_0 + \beta_1 \times \text{Treat} + \beta_2 \times \text{Visit} + \beta_3 \times (\text{Treat} \times \text{Visit}) + \beta_4 \times \text{DIM(covariate)} + \beta_5 \times \text{NL} + \beta_6 \times \text{QP} + \beta_7 \times \text{Herd} + \beta_8 \times (\text{Herd} \times \text{Treat}) + \text{Cow(random)} + \text{Re},$$

where logit(pi) is a link function of the probability to having a NIMI (1 or 0 outcome); β_0 is the intercept; β_1 refers to the regression coefficient for treatment (Treat); β_2 refers to the regression coefficient for week of trial (Visit); β_3 refers to the regression coefficient for the interaction between treatment and week of trial; β_4 refers to the regression coefficient for DIM; β_5 refers to the regression coefficient for parity (NL; primiparous or multiparous cow); β_6 refers to the regression coefficient for quarter position (QP; front vs. rear); β_7 refers to the regression coefficient for herd (CA or RD); β_8 refers to the regression coefficient for the interaction between herd and treatment. Cow was included as a random effect to account for the clustering of quarters within cows. The first-order autoregressive correlation structure was used. Re refers to the residual term in the model. A negative binomial distributed error term was used adopting a logit function, and the goodness-of-fit of models were evaluated by Hosmer and Lemeshow test.

Analysis of CM. The treatment effect on the incidence risk of CM **(IRCM**) during the 2-wk study period was evaluated at the quarter level as binomial response variables using logistic multivariable regression. Once a CM episode was identified, only cases that occurred after 14 d from a previous case in the same quarter were considered new for all organisms except the ones that the quarter had been infected with before. The treatment effect on the IRCM was analyzed using the PROC GLIMMIX of SAS version 9.3 (SAS Institute Inc.). The generalized linear mixed model was

$$\text{logit(pi)} = \beta_0 + \beta_1 \times \text{Treat} + \beta_2 \times \text{Visit} + \beta_3 \times \text{DIM(covariate)} + \beta_4 \times \text{NL} + \beta_5 \times \text{QP} + + \beta_6 \times \text{Herd} + \beta_7 \times (\text{Herd} \times \text{Treat}) + \text{Cow(random)} + \text{Re},$$

where logit(pi) is link function of the probability to having a CM case (1 or 0 outcome); β_0 is the intercept; β_1 refers to the regression coefficient for treatment (Treat); β_2 refers to the regression coefficient for week of trial (Visit); β_3 refers to the regression coefficient for DIM as covariate; β_4 refers to the regression coefficient for number of lactation (NL; primiparous or multiparous cow); β_5 refers to the regression coefficient for quarter position (QP; front vs. rear) effect; β_6 refers to the regression coefficient for herd (CA or RD); β_7 refers to the regression coefficient for the interaction between herd and week of trial. Cow were included as random effects to account for the clustering of quarters within cows. The first-order autoregressive correlation structure was used. Re refers to the residual term in the model. A negative binomial distributed error term was used adopting a logit function, and the goodness-of-fit of models were evaluated by Hosmer and Lemeshow test.

RESULTS

Bacteriological Culture

During the trial, a total of 12,408 milk samples were collected from individual mammary quarters for analysis of milk SCC and for microbiological culture (6,020 NBAR and 6,388 BAR). Around 67% of milk samples did not meet the SCC threshold criteria and were therefore not cultured. The most frequently isolated microorganism group on both farms from samples based on SCC threshold was *Streptococcus* spp. (6.25% of total mammary quarters), followed by CNS (3.6% of total mammary quarters) and *Corynebacterium* spp. (1.5% of total mammary quarters). Gram-negative bacteria were isolated in only 0.45% of total mammary quarters. Similarly, *Staph. aureus* was isolated with low frequency (0.3% of mammary quarters) in milk samples, whereas *Strep. agalactiae* was not isolated at all (Table 3).

NIMI

The overall NIMI incidence risk was 6.9 per 100 quarters at risk during the study period (Table 4). The majority (close to 90%) of NIMI in both treatment groups were caused by gram-positive microorganisms, namely environmental *Streptococcus* spp., CNS, and *Corynebacterium* spp. in 2.5, 1.8, and 1 per 100 quarters at risk, respectively. We found a higher proportion of NIMI caused by environmental streptococci in the NBAR group (42.6%) compared with the BAR group (36.3%). An interaction between treatment and week of trial on the incidence risk for NIMI was also observed ($P < 0.01$; Table 5). In wk 2, 4, 6, 10, 12, 14, and 18, the NIMI incidence risk was similar between treatments, whereas at wk 8 and 16 teats disinfected with BAR were less likely to have a NIMI compared with quarters disinfected with NBAR. Quarters disinfected with BAR had 54 and 37% lower odds of NIMI than quarters disinfected with NBAR product on wk 8 (OR = 0.46, 95% CI = 0.25–0.85) and 16 (OR = 0.63,

Table 3. Frequency of mastitis pathogens isolated from milk samples with SCC >200,000 cells/mL for multiparous and >100,000 cells/mL for primiparous cows, taken during 18 wk of a randomized controlled trial to compare a high free iodine barrier postmilking teat disinfectant (BAR) to a lower free iodine, nonbarrier teat dip (NBAR)

	BAR		NBAR		Total	
Samples status	No.	%	No.	%	No.	%
Total, all samples	6,388	100	6,020	100	12,408	100
Samples that did not meet SCC criteria	4,276	66.94	4,018	66.74	8,294	66.84
Samples cultured	2,112	33.06	2,002	33.26	4,114	33.16
Contaminated	27	0.42	15	0.25	42	0.34
Culture negative	1,193	18.68	1,100	18.27	2,293	18.48
Culture positive	892	13.96	887	14.73	1,779	14.34
Streptococcus uberis	239	3.74	207	3.44	446	3.59
Streptococcus dysgalactiae	40	0.63	45	0.75	85	0.69
Streptococcus spp.	144	2.25	101	1.68	245	1.97
Staphylococcus aureus	17	0.27	24	0.40	41	0.33
Escherichia coli	6	0.09	1	0.02	7	0.06
Klebsiella spp.	2	0.03	5	0.08	7	0.06
Corynebacterium spp.	79	1.24	104	1.73	183	1.47
CNS	213	3.33	235	3.90	448	3.61
Prototheca spp.	15	0.23	12	0.20	27	0.22
Yeast	7	0.11	17	0.28	24	0.19
Pseudomonas	7	0.11	6	0.10	13	0.10
Proteus	4	0.06	1	0.02	5	0.04
Nocardia	1	0.02	0	0.00	1	0.01
Enterococcus	6	0.09	4	0.07	10	0.08
Citrobacter	1	0.02	1	0.02	2	0.02
Enterobacter	4	0.06	8	0.13	12	0.10
Others enterobacteria	13	0.20	11	0.18	24	0.19
Gram-positive *Bacillus*	94	1.47	105	1.74	199	1.60

95% CI = 0.41–0.98), respectively. Days in milk had a positive association ($P < 0.0001$) with incidence risk of NIMI, and primiparous cows had 49% lower odds of NIMI than multiparous cows. Front quarters were 1.35 times more likely to have a NIMI than rear quarters. We noted an interaction between herd and treatment ($P = 0.036$) on NIMI. On farm CA (freestall system), we found no effect of postdipping on NIMI at 18 wk of trial on incidence risk of NIMI. Whereas, on farm RD (pasture-based) teats disinfected with BAR product

Table 4. Crude quarter level information of new intramammary infection (NIMI) and quarters at risk according to week of a randomized controlled trial to compare a high free iodine barrier postmilking teat disinfectant (BAR) to a lower free iodine, nonbarrier teat dip (NBAR)

	Week of trial									
Mastitis pathogen	2	4	6	8	10	12	14	16	18	Total
BAR										
Gram-positive	32	32	25	20	30	36	34	39	60	308 (88.8%)
Environmental *Streptococcus*	16	12	10	12	7	15	12	18	24	126 (36.3%)
Minor pathogens[1]	9	16	13	8	21	21	22	20	33	163 (50.0%)
Staphylococcus aureus	4	1	0	0	0	0	0	1	2	8 (2.3%)
Others[2]	3	3	2	0	2	0	0	0	1	11 (3.2%)
Gram-negative	0	3	4	3	4	9	6	6	4	39 (11.2%)
Total	32	35	29	23	34	45	40	45	64	347 (100.0%)
Quarters at risk (no.)	594	606	596	598	604	603	584	562	533	5,280
NBAR										
Gram-positive	47	21	37	26	39	35	28	55	39	327 (89.3%)
Environmental *Streptococcus*	25	13	15	12	16	17	9	29	20	156 (42.6%)
Minor pathogens[1]	18	7	19	14	20	16	19	24	19	156 (42.6%)
Staphylococcus aureus	2	1	2	0	0	1	0	1	0	7 (1.9%)
Others[2]	2	0	1	0	3	1	0	1	0	8 (2.2%)
Gram-negative	0	3	2	7	2	7	0	6	12	39 (10.7%)
Total	47	24	39	33	41	42	28	61	51	366 (100.0%)
Quarters at risk (no.)	588	547	558	545	576	537	533	548	534	4,966

[1]CNS and *Corynebacterium* spp.

[2]Others: *Nocardia* spp., includes *Prototheca* spp., and yeast.

had 31% lower odds of NIMI than those disinfected with NBAR during entire period. Overall, mammary quarters from cows on a freestall system had 26% lower odds of acquiring a NIMI than a pasture-based system (OR = 0.739, 95% CI = 0.604–0.90).

IRCM

In our study, the overall IRCM during entire study period was 0.8 per 100 quarters per 2 wk at risk (Table 6). Around 55% CM cases had culture-negative results, and the main pathogen group causing CM was environmental *Streptococcus* spp. (23.6% of total CM cases). The postmilking teat disinfectant used affected the IRCM. Overall, teats disinfected with BAR (0.33 cases CM/100 quarters at risk) had 46% lower odds of acquiring a CM (OR = 0.54; 95% CL = 0.35–0.85) than those disinfected with NBAR (0.61 cases CM/100 quarters at risk; $P < 0.01$; Table 7). Days in milk and quarter position did not affect the IRCM ($P = 0.348$ and $P = 0.185$, respectively), whereas primiparous cows had 54% lower odds of CM than multiparous cows. Week of trial had a significant effect on the overall IRCM ($P = 0.006$), with an increase of CM cases as the trial progressed. The average CM risk per 100 quarters at risk was 0.64 in the first 10 wk, and 1.07 in the last 8 wk. The IRCM did not differ between farms ($P = 0.499$) and we found no interaction between herd and postdip disinfectant ($P = 0.913$).

DISCUSSION

Results of present study showed that in wk 8 and 16, BAR teat disinfectant had a higher efficacy than NBAR for the prevention of NIMI, whereas no effect of teat disinfectant was observed during the others weeks of the trial. Additionally, IRCM during 18 wk of evaluation was lower for teats disinfected with BAR than NBAR product. The most commonly isolated mastitis pathogens in both dairy farms were environmental *Streptococcus* spp.

The higher efficacy of BAR compared with NBAR on preventing NIMI on wk 8 and 16 could possibly be associated with the increase in rainfall after wk 6 and a rise in ambient temperature after wk 14 (mean of 25.9°C) compared with previous weeks of trial (mean of 23.2°C). Average daily temperatures during the study period (August 2014–January 2015) ranged from 15 to 28°C and cumulative rainfall was 31.2, 27.8, 12.5, 47, 19,6 112, 68.8, 48, and 6.4 mm on wk 2, 4, 6, 8, 10, 12, 14, 16, and 18, respectively. Previous studies described higher growth rates of mastitis pathogens, especially coliforms and environmental streptococci, during rainy and hot weather (Hogan and Smith, 2012); increased risk of clinical mastitis was also reported by Oliveira et al. (2015) in Brazilian dairy cows during the rainy season. It is expected that when ambient humidity and temperature are high, the mammary gland is at a higher risk of infection due to the environmental challenge. A

Table 5. Multivariable logistic regression model[1] for treatment effects on odds of acquiring a new IMI during 18 wk of a randomized controlled trial to compare a high free iodine barrier postmilking teat disinfectant (BAR) to a lower free iodine, nonbarrier teat dip (NBAR)

	Model-based LSM of the incidence risk of new IMI						95% Confidence limit	
Item[2]	BAR	SEM	NBAR	SEM	β[3]	Odds ratio	Lower	Upper
Week at risk								
2	0.047[a]	0.009	0.070[a]	0.011	−0.411	0.663	0.403	1.090
4	0.054[a]	0.009	0.038[a]	0.008	0.351	1.420	0.815	2.476
6	0.044[a]	0.008	0.060[a]	0.010	−0.321	0.725	0.428	1.228
8	0.030[b]	0.006	0.056[a]	0.010	−0.767	0.464	0.255	0.847
10	0.046[a]	0.008	0.060[a]	0.010	−0.280	0.756	0.457	1.249
12	0.065[a]	0.010	0.067[a]	0.011	−0.038	0.962	0.601	1.541
14	0.060[a]	0.010	0.044[a]	0.009	0.331	1.393	0.827	2.345
16	0.066[b]	0.011	0.101[a]	0.014	−0.459	0.632	0.406	0.983
18	0.107[a]	0.014	0.091[a]	0.013	0.182	1.200	0.781	1.842
DIM	—	—	—	—	0.0024	1.002	1.002	1.003
Parity	—	—	—	—	−0.664	0.515	0.413	0.641
QP	—	—	—	—	0.303	1.353	1.106	1.656
Herd					−0.504	0.739	0.604	0.905

[a,b]Least squares means with different superscript letters within a row differ ($P < 0.05$).

[1]*P*-values of the multivariable logistic regression model: Treat = 0.109; Time <0.0001; Treat × Time 0.028; DIM <0.0001; NL <0.0001; QP = 0.003; Herd = 0.003; Herd × Treat = 0.036.

[2]Parity = primiparous or multiparous (referent); QP = quarter position (front or rear - referent); Herd = farm A (freestall) and farm B (pasture-based).

[3]β = regression coefficient.

Table 6. Numbers of clinical mastitis cases and quarters at risk of a randomized controlled trial to compare a high free iodine barrier postmilking teat disinfectant (BAR) to a lower free iodine, nonbarrier teat dip (NBAR)

	Week of trial (no.)									
Mastitis pathogen	2	4	6	8	10	12	14	16	18	Total
BAR										
Gram-positive	0	0	1	0	3	2	2	0	2	10 (30.3%)
Environmental *Streptococcus*	0	0	1	0	2	2	2	0	2	9 (27.3%)
Minor pathogens[1]	0	0	0	0	1	0	0	0	0	1 (3.0%)
Others[2]	0	0	0	0	0	0	0	0	0	0 (0.0%)
Gram-negative	0	0	0	0	0	0	1	0	0	1 (3.0%)
Negative growth	0	1	2	0	3	7	4	3	1	21 (63.6%)
Contaminated	0	0	1	0	0	0	0	0	0	1 (3.0%)
Total	0	1	4	0	6	9	7	3	3	33 (100.0%)
Quarters at risk (n)	636	654	644	644	632	640	621	606	579	5,656
NBAR										
Gram-positive	2	0	7	3	4	2	1	1	1	21 (37.5%)
Environmental *Streptococcus*	1	0	5	1	2	2	0	0	1	12 (27.4%)
Minor pathogens[1]	0	0	0	1	0	0	1	1	0	3 (5.4%)
Others[2]	1	0	2	1	2	0	0	0	0	6 (10.7%)
Gram-negative	0	0	1	0	1	2	0	0	1	5 (8.9%)
Negative growth	1	1	5	2	2	3	7	3	4	28 (50.0%)
Contaminated	0	1	0	0	1	0	0	0	0	2 (3.6%)
Total	3	2	13	5	8	7	8	4	6	56 (100.0%)
Quarters at risk	619	586	598	583	601	580	579	585	575	5,306

[1]CNS and *Corynebacterium* spp.

[2]Others: *Nocardia* spp., includes *Prototheca* spp., and yeast environmental *Streptococcus*.

Table 7. Multivariable logistic regression model[1] for treatment effects on odds of acquiring a clinical mastitis (CM) during 18 wk of a randomized controlled trial to compare a high free iodine barrier postmilking teat disinfectant (BAR) to a lower free iodine, nonbarrier teat dip (NBAR)

					95% CI	
Effect	Model-based LSM of the incidence risk of CM	SEM	β[2]	Odds ratio	Lower	Upper
Intercept	—	—	−6.491	—	−8.220	−4.762
Postdipping						
NBAR	0.006	0.001		Referent		
BAR	0.003	0.001	−0.634	0.541	0.346	0.844
Week						
2	0.001	0.001		Referent		
4	0.009	0.002	−0.380	0.684	0.114	4.097
6	0.003	0.001	1.732	5.654	1.643	19.459
8	0.001	0.001	0.574	1.775	0.424	7.429
10	0.007	0.002	1.503	4.495	1.277	15.826
12	0.009	0.002	1.732	5.655	1.642	19.478
14	0.008	0.002	1.608	4.991	1.429	17.441
16	0.004	0.002	0.916	2.499	0.644	9.705
18	0.006	0.002	1.322	3.752	1.029	13.676
DIM	—	—	0.001	1.001	0.999	1.002
Parity						
Multiparous	0.007	0.001		Referent		
Primiparous	0.003	0.001	−0.783	0.457	0.27	0.774
QP[3]						
Rear	0.002	0.001		Referent		
Front	0.008	0.001	1.154	3.171	0.576	17.464
Herd						
RD farm (pasture-based)	0.04			Referent		
CV farm (freestall)	0.05	−0.781	0.129	1.166	0.746	1.822

[1]*P*-values of the multivariable logistic regression model: Treat = 0.007; Time = 0.006; DIM = 0.348; NL = 0.004; QP = 0.184; Herd = 0.499; Treat × Herd = 0.913.

[2]β = regression coefficient.

[3]QP = quarter position (front or rear).

limitation of our study was that teat end cleanliness score was not evaluated, which could provide further information about the environmental infection pressure.

In the present study, we observed an interaction between herd and treatment on the incidence risk of NIMI, where BAR teat disinfectant was more effective than NBAR on the prevention of NIMI only on farm RD (pasture-based) at 18 wk of trial. These results may suggest that BAR teat disinfectant would offer an extra protection against new infections under a higher environmental challenge. Additionally, we observed that teats disinfected with BAR postdip had IRCM caused by environmental pathogens during 18 wk of study of 0.2%, whereas teats disinfected NBAR had IRCM of 0.5%, but no statistical analysis was done by group of pathogens.

Others studies found effects of high free iodine and barrier postdip on the prevention of NIMI (Foret et al., 2005, 2006). Foret et al. (2006) reported that NIMI was reduced by 21% when barrier disinfectants were used when compared with the nonbarrier postmilking teat disinfectant. Additionally, Foret et al. (2005) reported that teats disinfected with the higher free iodine concentration product had 55% less overall IMI and 58% less IMI caused by major pathogens compared with quarters disinfected with a lower free iodine concentration product.

The second most common microorganism isolated in milk samples was CNS. During the 18 wk of evaluation, teats disinfected with BAR and NBAR had similar incidence risk of NIMI caused by minor pathogens (3.08 and 3.14%, respectively). Quirk et al. (2012) reported that *Staphylococcus xylosus*, *Staphylococcus haemolyticus*, and *Staphylococcus hyicus* were sensitive to 1% iodine postdipping commercial product in comparison with undipped teats, whereas postdipping did not affect *Staphylococcus chromogenes* teat canal colonization and IMI. A study conducted in Brazil reported that *Staphylococcus chromogenes* was the most prevalent CNS species, and it was found that it increased SCC but had no effect on milk yield and composition (Tomazi et al., 2015).

As the treatment effect on the incidence risk of NIMI depended on the week of trial, and it was only significant at 2 out of 9 wk (wk 8 and 16), other factors not controlled in our study could have influenced the results. Several factors may contribute to the increase the risk of NIMI, such as the predominant mastitis pathogen group in farms studied, milking procedures, environmental cleanliness, and teat condition scores of the cows. In our study, 60% of teats evaluated had teat end orifice score $\geq$3 (data not presented here), which may increase the risk of IMI (Gentilini et al., 2016). Thus, with high environmental challenge, barrier postmilking teat disinfectant alone would be not sufficient to avoid IMI, especially in mammary quarters with hyperkeratosis.

It should be noted that both herds had high average bulk tank SCC (550 and 745 $\times$ 1,000 cells/mL) before the onset of the study, which could be a result of the high prevalence of IMI caused by gram-positive environmental pathogens, especially environmental streptococci. Therefore, results of our study should be interpreted with caution for herds with different etiological profile and with lower SCC.

CONCLUSIONS

We concluded that the studied postmilking teat disinfectant with barrier properties and higher free iodine content contributed to an overall lower incidence of CM during the study, although differences in NIMI were found at only 2 time points. Teat dip efficacy may depend not only on the formulation of products, but also on farm-specific variables, such as environmental conditions and the predominant mastitis pathogen group.

ACKNOWLEDGMENTS

We acknowledge the 2 dairy farms in São Paulo State that participated in this study. We also thank the Quality Milk Research Laboratory (Qualileite) team, at University of São Paulo, for helping data and sample collection. The authors acknowledge DeLaval Manufacturing (Kansas City, MO) for providing funding for the study.

REFERENCES

Buchalova, M., and E. S. Rauer. 2013. Improved persistence of iodine-based teat disinfectant: In vitro study. Pages 195–196 in NMC 52th Annual Meeting Proceedings, Verona, WI. Natl. Mastitis Counc. Inc., Madison, WI.

Ceballos-Marquez, A., T. Hemling, B. J. Rauch, M. Lopez-Benavides, and Y. H. Schukken. 2013. Noninferiority trial on the efficacy of premilking teat disinfectant against naturally occurring new intramammary infections using a novel 2-step diagnostic process. J. Dairy Sci. 96:8081–8092.

Foret, C., H. Aguero, and P. Janowicz. 2006. Efficacy of two barrier iodine teat dips under natural exposure conditions. J. Dairy Sci. 89:2279–2285.

Foret, C. J., C. Corbellini, S. Young, and P. Janowicz. 2005. Efficacy of two iodine teat dips based on reduction of naturally occurring new intramammary infections. J. Dairy Sci. 88:426–432.

Gentilini, M., M. L. Benavides, E. S. C. Pinheiro, C. M. M. R. Martins, and M. V. Santos. 2016. Quarters with high teat end condition scores are more likely to have new intramammary infections. Pages 124–125 in NMC 55th Annual Meeting Proceedings, Glendale, AZ. Natl. Mastitis Counc., Inc., Madison, WI.

Gottardi, W. 2001. Iodine and iodine compounds. Pages 159–183 in Disinfection, Sterilization, and Preservation. 5th ed. S. S. Block, ed. Lippincott Williams & Wilkins, Philadelphia, PA.

Hogan, J., and K. L. Smith. 2012. Managing environmental mastitis. Vet. Clin. North Am. Food Anim. Pract. 28:217–224.

Hogan, J. S., K. L. Smith, D. A. Todhunter, and P. S. Schoenberger. 1995. Efficacy of a barrier teat dip containing. 55% chlorhexidine for prevention of bovine mastitis. J. Dairy Sci. 78:2502–2506.

Lago, A., M. Lopez Benavides, M. Buchalova, and E. S. Rauer. 2014. Longer iodine persistence on teats improves prevention of new intramammary infections under natural exposure conditions. Pages 255–256 in NMC 53rd Annual Meeting Proceedings, Fort Worth, TX. Natl. Mastitis Counc. Inc., Madison, WI.

Lopez Benavides, M., J. Lombard, J. Raymond, T. C. Hemling, and L. Timms. 2016. Trends in the USA that are challenging the *status quo* in milking routines. Pages 29–37 in New Zealand Milk Quality Conference. New Zealand Veterinary Association, Hamilton, New Zealand.

Murdough, P. A., and J. W. Pankey. 1993. Evaluation of 57 teat sanitizers using excised cow teats. J. Dairy Sci. 76:2033–2038.

National Mastitis Council (NMC). 2004. Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determination of Milk Quality. 4th ed. NMC Inc., Madison, WI.

National Mastitis Council (NMC). 2012. Research committee report: Interpreting bacteriological culture results to diagnose bovine intramammary infections. Accessed Dec. 8, 2015. https://www.nmconline.org/wp-content/uploads/2016/08/Interpreting-Bacteriological-Culture-Results.pdf.

Nickerson, S. C., and R. L. Boddie. 1995. Efficacy of barrier-type postmilking teat germicides against intramammary infection. J. Dairy Sci. 78:2496–2501.

Oliveira, C. S. F., H. Hogeveen, A. M. Botelho, P. V. Maia, S. G. Coelho, and J. P. A. Haddad. 2015. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. Prev. Vet. Med. 121:297–305.

Pankey, J. W., R. J. Eberhart, A. L. Cuming, R. D. Daggett, R. J. Farnsworth, and C. K. McDuff. 1984. Uptake on postmilking teat antisepsis. J. Dairy Sci. 67:1336–1353.

Poutrel, B., F. Serieys, and M. Ducelliez. 1990. Efficacy of a germicidal post milking barrier-type teat dip in preventing intramammary infections. Vet. Rec. 126:638–640.

Quirk, T., F. K. Fox, D. D. Hancock, J. Capper, J. Wenz, and J. Park. 2012. Intramammary infections and teat canal colonization with coagulase-negative staphylococci after postmilking teat disinfection: species-specific responses. J. Dairy Sci. 95:1906–1912.

Reinemann, D. J., and J. M. Book. 1994. Airflow requirements, design parameters and troubleshooting for cleaning milking systems. Pages 26–35 in Proc. ASAE/NMC Dairy Housing Conference, January 31–February 4, 1994, Orlando, FL. American Society of Agricultural Engineers Inc., Madison, WI.

Reyher, K. K., D. Haine, I. R. Dohoo, and C. W. Revie. 2012. Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new intramammary infections with major mastitis pathogens-A systematic review and meta-analysis. J. Dairy Sci. 95:6483–6502.

Timms, L. L. 1997. Field Trial evaluation of a persistent barrier teat dip for preventing mastitis during the dry period. J. Dairy Sci. 80(Suppl. 1):225.

Timms, L. L., Z. Muyengwa, and G. Olaf. 1987. Persistency of barrier teat dips and other products on dry cows. J. Dairy Sci. 70(Suppl. 1):131.

Tomazi, T., J. L. Gonçalves, J. R. Barreiro, M. A. Arcari, and M. V. dos Santos. 2015. Bovine subclinical intramammary infection caused by coagulase-negative staphylococci increases somatic cell count but has no effect on milk yield or composition. J. Dairy Sci. 98:3071–3078.