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Effect of double-premilking teat disinfection protocols on bacterial counts on teat skin of cows and milker gloves in a free-stall-housed dairy herd

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Research Article

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Abstract

This research communication addresses the hypothesis that double premilking teat disinfection (DD) is more effective in reducing soiling and bacterial count on the cows' teat skin and milkers' gloves than conventional disinfection (CONV). The design was a 3 × 3 Latin square (three groups of cows and three treatments) with conventional teat disinfection (CONV, lactic acid application after forestripping), double teat disinfection using the same disinfectant (DD1D, lactic acid application before and after forestripping), and double teat disinfection using two different disinfectants (DD2D, application of lactic acid before and chlorine-based disinfectant after forestripping). All groups were assigned for six days for each treatment, and the evaluations and samples were collected on the last day, before and after treatment. We evaluated the teat cleanliness score (TCS), count of Gram-negative bacteria (coliforms and noncoliforms), *Staphylococcus* spp., *Streptococcus* spp., and total bacterial count (TBC) on the cows' teats and TBC on the milkers' gloves. TCS after premilking was lower in DD than CONV treatment. The reduction of *Staphylococcus* spp. count was greater in the DD treatment and tended to be higher in the DD2D. The TBC reduction on the cows' teats was greater in the DD treatments. The TBC on the milkers' gloves was lower for DD before and after premilking. In conclusion, DD can be an alternative for reducing some bacterial populations on cow teats and preventing the transmission of microorganisms between cows *via* the milkers' hands.

Greater soiling of teats is associated with a higher somatic cell count (SCC) in milk (Kappes *et al*., 2020), and cows with a dirty udder are at a higher risk of intramammary infection than those with a clean udder (Schreiner and Ruegg, 2003). Cross-transmission of microorganisms among cows by milkers' hands during premilking management can also increase milk SCC. Therefore, the disinfection of milker gloves during the premilking routine and teat disinfection can contribute to reducing the bacterial load and SCC in milk (Islam *et al*., 2009).

Double premilking disinfection is utilized on some farms and is recommended by technicians. However, no studies have evaluated the efficacy of double premilking disinfection compared to conventional disinfection (once). Our objective was to test the hypothesis that double premilking teat disinfection (DD) is more effective in reducing soiling and bacterial count on the cows' teat skin and milkers' gloves than conventional disinfection (CONV), and double premilking teat disinfection, using two different disinfectants (DD2D), is more effective in reducing the bacterial count on the cows' teats.

Materials and methods

Experiment design

The trial was conducted on a commercial dairy farm in southern Brazil (latitude 27°00′43.9″ ‘, longitude 51°23′18.4‴, and altitude 796 m above sea level). The Ethics Committee of the Universidade approved all animal procedures do Estado de Santa Catarina (protocol n. 398240423).

The herd comprised 115 Holstein cows housed in a free-stall with tunnel ventilation. The cows were milked thrice daily in 2 × 6 parallel milking parlours. The bedding consisted of mattresses with sawdust treated with 6–8% quicklime. Three groups of cows were formed based on milk yield and parity: group 1 comprised 49 high-yielding multiparous cows (mean of 43.7 kg

milk/cow/day), group 2 consisted of 34 low-yielding multiparous cows (mean of 30.5 kg milk/cow/day), and group 3 included 32 primiparous cows (mean of 38.7 kg milk/cow/day).

We used a 3 × 3 Latin square design (three groups of cows and three treatments), with groups corresponding to those previously established on the farm. The treatments were conventional disinfection (CONV) as a control, double-teat disinfection using the same disinfectant (DD1D), and double-teat disinfection using two disinfectants (DD2D). In the CONV treatment, after forestripping, we applied a 40% lactic acid disinfectant and dried the teats after 30 s with a paper towel. For DD1D, we applied a 40% lactic acid disinfectant, followed by forestripping, applying the same disinfectant, and drying it after 30 s with a paper towel. DD2D followed a similar protocol to DD1D, differing only in using a second disinfectant (1500 ppm chlorine-based). Post-milking disinfection was standardized across all treatments, entailing immersion of the teats in an iodine-based disinfectant. Initially, each group was randomly assigned to one treatment, with all cows receiving the same treatment before each milking for six days. On the seventh day, the group was assigned to another treatment for six more days. This rotation was repeated once more for an equal period, resulting in the three groups undergoing the three treatments for 18 d (three periods of six days each).

On the second milking of the final day of each period (the sixth, twelfth, and eighteenth day of the experiment), we assessed the teat cleanliness score (TCS) before (PRE) and after (POST) treatment. Additionally, samples were taken from the cows' teats and milkers' gloves using sterile swabs, both PRE and POST treatment, for microbiological counts.

Teat cleanliness score

The TCS was assessed using a damp towel marked with the cow's number identification. Before treatment, the moist towel was rubbed three times from the base to the apex of the left front teat. After treatment, the same process was repeated on the right rear teat. The towels were air-dried at room temperature and categorized into five scores, ranging from 0 (clean teats) to 4 (extremely dirty; Hovinen *et al.*, 2005).

Teat bacterial count

Microbiological samples were randomly collected from the right front and left rear teats of the last cow on each side of the milking parlour. Before treatment, a sterile swab moistened with buffered peptone water was rubbed on the cranial surfaces of both teats. After treatment, the same procedure was repeated on the external side of the same teats. After sample collection, the swabs were placed in 4 ml tubes with buffered peptone water and refrigerated for subsequent analysis on the same day. The samples were vigorously vortexed, and serial dilutions were performed to identify and enumerate the colonies. Serial dilutions of 1:10, 1:100, and 1:1000 were prepared for PRE samples, while POST samples underwent a dilution of 1:10.

Baird Parker agar enriched with Tellurite Egg Yolk was used to enumerate *Staphylococcus* spp. Modified Edwards agar supplemented with 5% sterile bovine blood was utilized to enumerate *Streptococcus* spp. MacConkey agar was utilized for enumerating coliform (lactose-fermenting in red or pink colour) and Gram-negative non-coliform bacteria (colourless colonies). Plate Count Agar (PCA) was used to determine the total bacterial count (TBC).

We used 1:100 and 1:10 dilutions for PRE and POST samples, respectively (previously tested in a pilot study). We plated one inoculum of 100 µl onto the Baird Parker and modified Edwards plates, one inoculum of 50 µl onto MacConkey, and one inoculum of 10 µl onto PCA plates. The plates were incubated at 37 ± 1 °C for 24 h to count the colonies. Colony-forming units (CFU) per ml were calculated by multiplying the number of colonies, the dilution factor, and the correction factor by ml. The correction factors for the Baird Parker, modified Edwards, MacConkey, and PCA were 10, 10, 20, and 100, respectively.

Gloves bacterial count

We collected samples from the milkers' gloves before and after the premilking management on the last cow on each side of the milking parlour. A sterile swab moistened with buffered peptone water was rubbed onto the milkers' gloves (palm and fingers). Swabs were placed in 4 mL tubes with buffered peptone water and refrigerated for subsequent analysis. The samples were vigorously shaken and serially diluted from 1:10 to 1:1000. We used dilutions of 1:100 for both the PRE and POST samples. A 10 µl inoculum was plated on a PCA plate and incubated at 37 ± 1 °C for 24 h to count the colonies.

Statistical analysis

Analysis of variance was performed using the GLM procedure of the SAS® statistical package after testing the data for normality of residuals with the UNIVARIATE procedure, with a significance level of $P < 0.05$, using the Shapiro–Wilk test. The effects of group and treatments were tested using orthogonal contrasts. We compared CONV vs. DD and DD1D vs. DD2D.

To obtain the normality of the residues, the bacterial count was transformed into a logarithm of base 10. The variables of teat bacterial count and TCS were analysed for PRE and POST treatment, as well as the reduction of these values in the logarithm of base 10. The variable milker glove bacterial count was evaluated using PRE and POST. Negative values indicated a reduction in the contamination of teats and TCS. Statistical differences were defined at the 5% level, and a tendency was determined at the 10% level.

Results and discussion

We did not observe any group effect. A total of 340 evaluations of TCS was performed, with 108 for CONV, 102 for DD1D, and 130 for DD2D. The mean TCS PRE was higher than three for all treatments (Table 1), indicating that the teats were dirty, which was very important for challenging the treatments. Sawdust on the surface of the free-stall bed is likely to have a large influence on the TCS (Schreiner and Ruegg, 2003). Cows from the DD treatment had cleaner teats POST ($P = 0.04$) and showed a tendency towards a greater reduction in TCS ($P = 0.09$) compared to those from the CONV treatment (Table 1), with no difference between DD1D and DD2D. This is likely because the moist teats, when removing the first strips of milk, facilitate the release of dirt more easily, thus aiding in teat cleanliness.

Regarding bacterial counts, we evaluated 60 samples, 20 for each treatment for *Streptococcus* spp. and TBC, from both cows' teats and milkers' gloves. For *Staphylococcus* spp., coliforms, and Gram-negative noncoliform bacteria, we evaluated 59, 49, and 52 samples, respectively, as we did not consider samples from cows with no counts before treatment. Previous studies have reported lower or null counts of coliforms and Gram-negative bacteria before disinfection, which are associated with minimal bedding

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Table 1. Mean values and residual standard deviations (RSD) for teat cleanliness score (TCS), teat bacterial count, and *P* values for the contrasts between double disinfection (DD) and conventional disinfection (CONV) and between double disinfection with one disinfectant (DD1D) and with two disinfectants (DD2D)

		Treatment				Contrasts (*P* value)	
Variables	*N*	CONV	DD1D	DD2D	RSD	DD × CONV	DD1D × DD2D
Teat cleanliness score							
TCS_PRE	340	3.35	3.28	3.42	0.64	0.99	0.14
TCS_POST	340	1.13	0.98	0.96	0.66	0.04	0.92
Dif_TCS	340	−2.22	−2.32	−2.44	0.80	0.09	0.27
Teat bacterial count[a]							
COLC_PRE	49	2.66	2.93	3.07	0.87	0.12	0.62
COLC_POST	49	0.25	0.79	0.63	0.99	0.07	0.54
Dif_COLC	49	−2.42	−2.14	−2.45	1.25	0.73	0.41
GNNCOLC_PRE	52	2.68	3.11	2.98	0.89	0.27	0.91
GNNCOLC_POST	52	0.41	0.50	0.63	1.16	0.78	0.98
Dif_GNNCOLC	52	−2.27	−2.61	−2.35	1.23	0.59	0.92
STEC_PRE	60	5.14	5.06	4.91	0.75	0.75	0.44
STEC_POST	60	3.46	2.98	3.21	0.78	0.16	0.49
Dif_STEC	60	−1.68	−2.08	−1.70	0.86	0.31	0.19
STAC_PRE	59	4.48	4.50	4.83	0.63	0.19	0.21
STAC_POST	59	3.48	3.39	3.30	0.47	0.28	0.43
Dif_STAC	59	−0.99	−1.12	−1.53	0.63	0.04	0.07
TBC_PRE	60	4.72	5.13	5.10	0.53	0.01	0.74
TBC_POST	60	3.87	3.69	3.77	0.56	0.29	0.77
Dif_TBC	60	−0.85	−1.43	−1.33	0.68	0.01	0.65

COLC, coliforms count; GNNCOLC, Gram-negative noncoliform count; STEC, *Streptococcus* spp. count; STAC, *Staphylococcus* spp. count; TBC, total bacterial count; Dif, difference between count before and after treatment.
[a]All values are expressed as colony-forming units (CFU) per ml in logarithm base 10.

contaminations by these microorganisms (Gleeson *et al.*, 2009; Baumberger *et al.*, 2016; Fitzpatrick *et al.*, 2021).

Coliform count POST tended to be lower for CONV than DD ($P = 0.07$). However, the reduction in coliforms and Gram-negative noncoliform bacteria did not differ between the treatments ($P > 0.05$; Table 1). Compared with other bacterial groups, the great reduction in coliforms and Gram-negative noncoliform counts (more than 2.00 $\log_{10}$ CFU/ml) indicates good premilking disinfection efficacy in reducing these groups of microorganisms in both CONV and DD treatments. Baumberger *et al.* (2016) also observed a greater reduction in Gran-negative bacteria after treatment than for *Streptococcus* spp. and *Staphylococcus* spp. ($P < 0.01$).

We did not observe differences between treatments in the *Streptococcus* spp. count PRE and POST, but the mean values for all treatments were high, consistent with the findings of Baumberger *et al.* (2016). This could be due to the elevated *Streptococcus* spp. count on sand, sawdust, and organic non-manure bedding, which enhances teat contamination (Zdanowicz *et al.*, 2004; Rowe *et al.*, 2019). The reduction of *Streptococcus* spp. on the teats was similar between CONV and DD ($P = 0.31$), higher than −1.6 $\log_{10}$ CFU/ml (Table 1). However, the reduction was lower than reported by Baumberger *et al.* (2016). The authors claimed the reduction may vary depending on farm conditions, additional management practices, and initial teat contamination.

The reduction of *Staphylococcus* spp. on the teats was more effective in DD than in the CONV treatment ($P = 0.04$; Table 1). This could be attributed to the extended period when the teats were exposed to the disinfectant, thereby reducing the number of these microorganisms more effectively. When using two different disinfectants, the reduction of *Staphylococcus* spp. tended to be greater than when using one disinfectant ($P = 0.07$; Table 1). The combination of two different disinfectants (lactic acid and chlorine-based disinfectants) has a broad spectrum of action against a higher number of microorganisms. Fitzpatrick *et al.* (2021) found that iodine associated with lactic acid in single disinfection was the most effective in reducing *Staphylococcus* spp. on the teats compared to eight other active principles.

The TBC PRE was lower ($P = 0.01$) for cows subjected to the CONV treatment compared to DD, 4.72 and 5.00 $\log_{10}$ CFU/ml, respectively (Table 1). However, the TBC reduction was higher in the DD treatments ($P = 0.01$), with no difference between DD1D and DD2D ($P = 0.65$). The greater reduction in TBC in the DD treatment was associated with a reduction in all other groups of bacteria. This practice can ensure a higher microbiological quality of milk.

The lower TBC on the milkers' gloves in the DD treatments for both PRE and POST ($P < 0.05$) compared to CONV (Table 2) was due to continuous contact with the disinfectant, while the first strips of milk were removed. Bach *et al.* (2008) and Dufour *et al.*

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Table 2. Mean values and residual standard deviations (RSD) for teat cleanliness score (TCS), teat bacterial count before (PRE) and after (POST) treatment, and *P* values for the contrasts between double disinfection (DD) and conventional disinfection (CONV) and between double disinfection with one disinfectant (DD1D) and with two disinfectants (DD2D)

		Treatment				Contrasts (*P* value)	
Variable[a]	*N*	CONV	DD1D	DD2D	RSD	DD × CONV	DD1D × DD2D
TBCGloves_PRE	30	4.69	4.21	4.09	0.51	<0.01	0.29
TBCGloves_POST	30	4.40	4.09	3.90	0.57	0.01	0.24

[a]All values are in colony-forming units (CFU) per ml in logarithm base 10.

(2011) showed an association between wearing gloves and lower SCC on the bulk tank milk. With the lower adherence of contagious bacteria on the gloves, the transmission of these microorganisms decreases among the cows *via* the milkers' hands, reducing intramammary infections (Plozza *et al.*, 2011). Combining wearing gloves and a double premilking disinfection can be an alternative to reduce the transmission of contagious microorganisms such as *Staphylococcus aureus* and *Streptococcus agalactiae*.

In conclusion, double teat disinfection (DD) is more effective in cleaning the cows' teat skin, reducing Staphylococcus spp. and total bacterial count, and reducing the TBC on the milkers' gloves compared to CONV. DD is an alternative for reducing some bacterial populations on cows' teats and preventing the transmission of microorganisms between cows *via* milkers' hands. This study is the first to investigate the efficacy of double-teat disinfection in reducing teat bacterial counts. Therefore, we encourage future research to evaluate different combinations of active principles and to examine the effect of double premilking disinfection protocols on the incidence of clinical and subclinical mastitis caused by environmental and contagious microorganisms over extended durations, and the economic viability of using DD.

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