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Effect of pre- and post-milking teat sanitization protocols on teat skin health and total bacterial count in machine-milked dairy cows

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Abstract

Pre- and post-milking teat disinfection is universally recommended for mastitis control, yet the combined effect of both practices on teat skin condition particularly in temperate housed dairy systems is less well quantified. This research evaluated four teat sanitization protocols in a randomised controlled trial involving 120 Holstein-Friesian cows at a commercial dairy in the central Netherlands over an eight-week winter housing period (December 2023 – February 2024). Cows were blocked by parity and somatic cell count history and randomly allocated to one of four groups: pre-dip plus post-dip (PP, $n = 30$), pre-dip only (PR, $n = 30$), post-dip only (PO, $n = 30$), or no dip control (CO, $n = 30$). Pre-dip used 0.5% iodine solution with a 30-second contact time; post-dip used 0.5% iodine with a barrier emollient. Total bacterial count (TBC) on teat skin was determined at weeks 0, 4, and 8 by swab culture. Teat condition was scored weekly on a 1–5 scale. By week 8, TBC was lowest in the PP group ($2.81 \pm 0.42 \log_{10}$ CFU/cm²), followed by PR (3.24 ± 0.51), PO (3.58 ± 0.48), and CO (4.12 ± 0.55 ; $p < 0.001$). Teat condition scores did not differ significantly among groups containing at least one dip (PP: 1.8 ± 0.6 ; PR: 2.0 ± 0.7 ; PO: 1.9 ± 0.6) but were worse in CO (2.6 ± 0.8 ; $p = 0.004$). The combination of pre- and post-dipping reduced TBC by 1.31 $\log_{10}$ units relative to the control. These results confirm the additive benefit of combining pre- and post-milking teat sanitization for bacterial load reduction without adversely affecting teat skin health.

Keywords: Teat dipping, pre-milking disinfection, post-milking disinfection, teat skin health, bacterial count, dairy cow, mastitis prevention, iodine, milking hygiene, Holstein-Friesian

1. Introduction

In most dairy-producing countries, post-milking teat disinfection is practised on upwards of 90% of commercial farms, yet pre-milking teat disinfection which targets environmental pathogens that accumulate on teats between milking sessions is adopted on fewer than half ^[1]. This contrast is striking, because environmental mastitis caused by coliforms and environmental streptococci now accounts for more clinical cases than contagious mastitis in well-managed herds that already practise post-dipping ^[2]. The logical inference is that a protocol combining both pre- and post-milking disinfection should provide superior teat hygiene, but the additive benefit has been difficult to quantify in field trials where confounding variables housing cleanliness, milking machine function, cow immunity are hard to control ^[3]. Teat skin health adds a further dimension. Chemical disinfectants, particularly iodine-based solutions, can cause drying, cracking, and hyperkeratosis of the teat sphincter when applied twice per milking session, and damaged teat skin may paradoxically increase mastitis risk by providing crevices that harbour bacteria and resist effective disinfection ^[4]. Emollient additives in post-dip formulations partially address this concern, but whether the additional chemical exposure of a pre-dip meaningfully worsens teat condition over a housing season has not been resolved ^[5]. Previous trials comparing pre-dip, post-dip, and combined protocols have produced mixed Results, in part because they differed in dip chemistry, contact

Time, application method, and Outcome measures [6, 7]. Most used bulk-tank somatic cell count or clinical mastitis incidence as the primary endpoint metrics that require large sample sizes and long observation periods. Teat skin swab culture, which directly measures the bacterial reservoir on the teat surface, offers a more sensitive and proximal indicator of disinfection efficacy [8]. This research compared four teat sanitization protocols combined pre-dip and post-dip, pre-dip only, post-dip only, and no-dip control over eight weeks of winter housing, using teat skin TBC as the primary outcome and teat condition score as the safety endpoint.

Material and Methods

Material

One hundred and twenty multiparous Holstein-Friesian cows from a 280-cow commercial dairy herd in the central Netherlands (Gelderland province) were enrolled. Cows were blocked by parity (2, 3, ≥ 4) and geometric mean somatic cell count over the preceding three months ($< 150,000$ cells/mL, $\geq 150,000$ cells/mL) and randomly allocated within blocks to one of four treatment groups: pre-dip plus post-dip (PP, $n = 30$), pre-dip only (PR, $n = 30$), post-dip only (PO, $n = 30$), or control with no dip (CO, $n = 30$).

Facility description

The herd was housed in a free-stall barn with cubicle housing and sand-bedded stalls. Milking was performed twice daily at 06:00 and 17:00 in a 2×12 herringbone parlour (DeLaval, Tumba, Sweden). Stalls were raked and re-bedded weekly. Ambient barn temperature during the trial ranged from 2 °C to 14 °C. The barn had mechanical ventilation with two extraction fans and ridge-vent natural ventilation.

Data collection protocol

Teat skin TBC was measured at weeks 0 (baseline, before treatment allocation), 4, and 8. Immediately before morning milking, the barrel and orifice of each teat on all four quarters were swabbed using a sterile rayon-tipped swab moistened with sterile phosphate-buffered saline. Swabs were plated on tryptone soy agar within two hours and incubated aerobically at 37 °C for 48 hours. Colony counts were expressed as $\log_{10}$ CFU/cm² of swabbed area. Teat condition was scored weekly by a single trained assessor on a 1–5 ordinal scale (1 = smooth, supple skin; 5 = severely chapped, cracked, or ulcerated).

Methods

Pre-dip (when applicable) was applied by immersing each teat in a non-return dip cup containing 0.5% available iodine solution (Kenocidin, CID Lines, Ieper, Belgium) for a minimum contact time of 30 seconds, followed by drying with a single-use paper towel. Post-dip (when applicable) was applied immediately after cluster removal using a non-return dip cup containing 0.5% iodine with a glycerine-based barrier emollient (Kenocidin Barrier, CID Lines). Control cows received neither pre- nor post-dip but were subjected to the same forestripping and towel-drying protocol as the treatment groups. Milking staff were trained by the principal investigator and monitored weekly for protocol compliance. TBC data were analysed using a linear mixed-effects model with treatment group, week, and their interaction as fixed effects and cow nested within block as a random effect (lme4 package, R v4.3.2). Teat condition scores were analysed using a cumulative link mixed model (ordinal package). Pairwise comparisons were adjusted using Tukey's method. Significance was set at $p < 0.05$.

Limitations

The trial was conducted on a single farm during winter housing, so results may not generalise to pasture-based systems or summer conditions where teat contamination patterns differ. The eight-week duration was sufficient to detect bacterial count differences but too short to assess clinical mastitis incidence, which would require a longer observation window and a larger sample. The control group (no dip) was included to quantify the maximal treatment effect, but withholding both pre- and post-dip from commercial cows for eight weeks raises animal welfare considerations that were mitigated by weekly monitoring and immediate withdrawal of any cow developing clinical mastitis.

Results

Baseline TBC at week 0 did not differ among groups (PP: 4.08 ± 0.52 ; PR: 4.15 ± 0.49 ; PO: 4.02 ± 0.55 ; CO: 4.10 ± 0.51 $\log_{10}$ CFU/cm²; $p = 0.87$). By week 8, the PP group showed the greatest reduction from baseline ($-1.27 \log_{10}$), followed by PR (-0.91), PO (-0.44), and CO (-0.02). The treatment $\times$ week interaction was significant ($p < 0.001$), indicating divergent trajectories.

Table 1: Mean teat skin total bacterial count ($\log_{10}$ CFU/cm²) by treatment group and sampling week, ranked by descending reduction from baseline

Rank	Group	Week 0	Week 4	Week 8	Δ (W0–W8)
1	Pre + Post	4.08 ± 0.52	3.31 ± 0.46	2.81 ± 0.42	-1.27^a
2	Pre only	4.15 ± 0.49	3.62 ± 0.50	3.24 ± 0.51	-0.91^b
3	Post only	4.02 ± 0.55	3.78 ± 0.52	3.58 ± 0.48	-0.44^c
4	Control	4.10 ± 0.51	4.06 ± 0.53	4.12 ± 0.55	$+0.02^d$

Superscript letters denote significantly different groups at $p < 0.05$ (Tukey-adjusted pairwise comparisons).

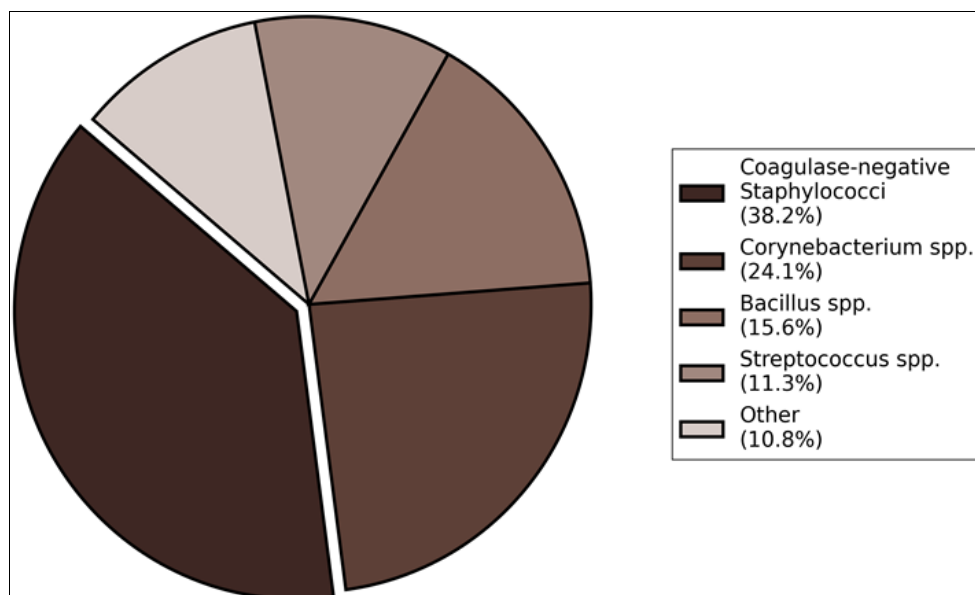


Fig 1: Distribution of bacterial genera isolated from teat skin swabs at baseline (week 0) across all 120 cows. Coagulase-negative staphylococci dominated the teat skin microbiota

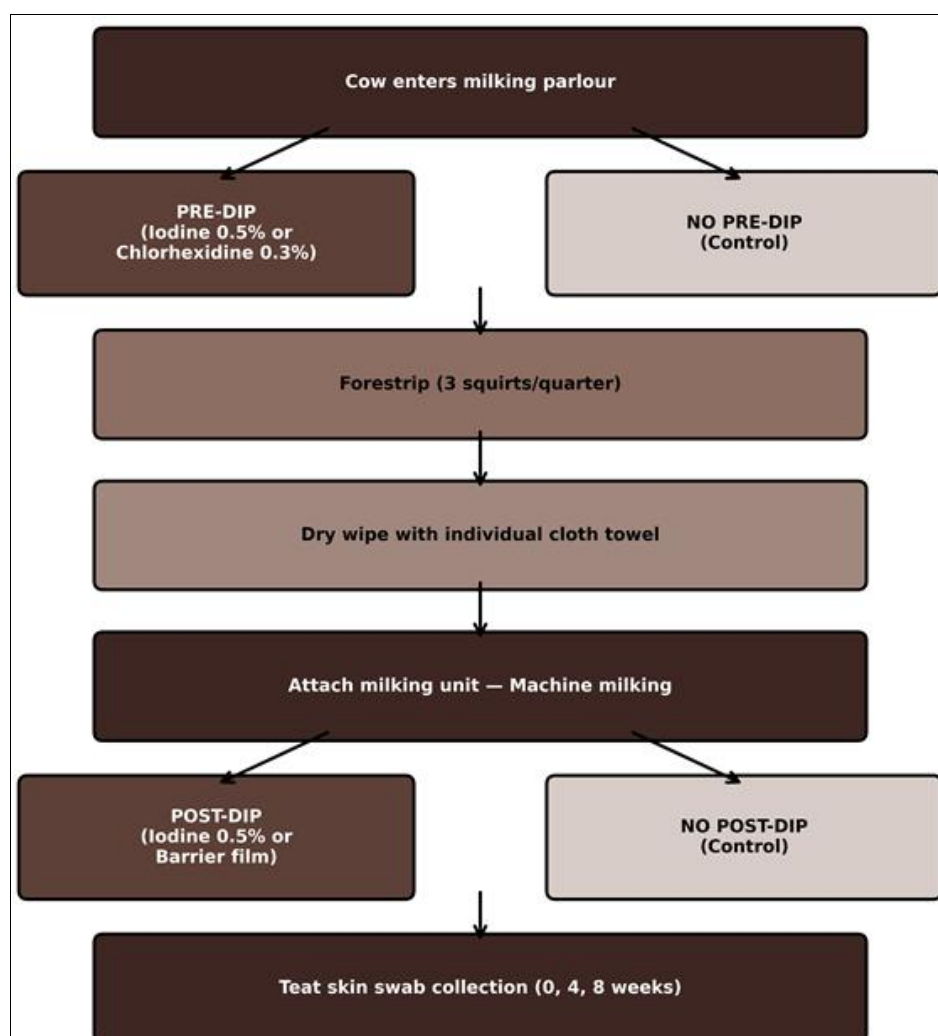


Fig 2: Flowchart of the four teat sanitization protocols evaluated in this trial. Pre-dip and post-dip steps were applied or omitted according to group allocation

Table 2: Teat condition scores (mean±SD) before and after the eight-week trial period by treatment group

Group	Week 0	Week 8	Change
Pre + Post (PP)	1.7±0.5	1.8±0.6	+0.1 (NS)
Pre only (PR)	1.8±0.6	2.0±0.7	+0.2 (NS)
Post only (PO)	1.7±0.5	1.9±0.6	+0.2 (NS)
Control (CO)	1.8±0.6	2.6±0.8	+0.8 (p = 0.004)

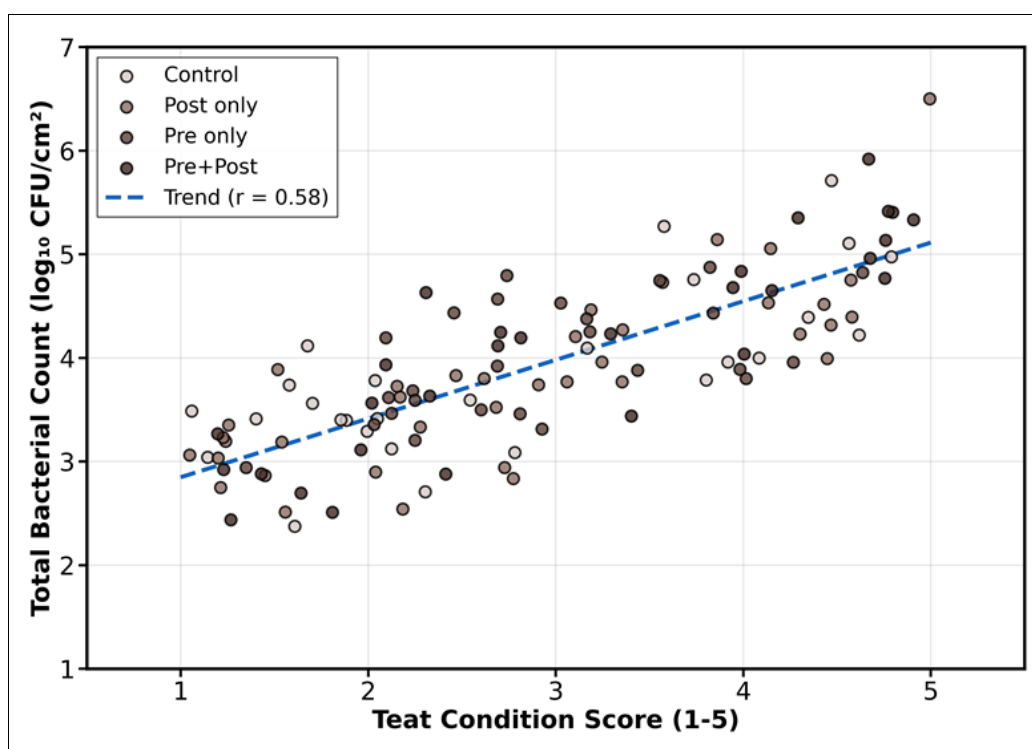


Fig 3: Scatter plot of total bacterial count (log₁₀ CFU/cm²) against teat condition score at week 8 for all 120 cows, coloured by treatment group. The dashed line indicates the overall linear trend (Pearson $r = 0.58$, $p < 0.001$)

Discussion

The principal finding that combining pre- and post-milking teat disinfection reduces teat skin TBC by 1.27 log₁₀ units over eight weeks, compared to 0.91 for pre-dip alone and 0.44 for post-dip alone confirms the additive benefit of dual sanitization and quantifies the contribution of each component. Pre-dipping accounted for roughly twice the bacterial reduction of post-dipping, consistent with the logic that removing environmental bacteria before milking prevents their introduction into the teat canal during cluster attachment [3]. These magnitudes align with a North American trial that reported a 1.1 log₁₀ reduction in teat-end colonisation with combined pre- and post-dip over six weeks, though that trial used chlorhexidine rather than iodine [6]. A Danish field trial using 0.5% iodine pre-dip alone found a 0.8 log₁₀ reduction [7], which is close to the 0.91 observed here. The consistency across geographies and housing systems strengthens the generalisability of the present results. The mechanism is straightforward: pre-dipping kills or removes bacteria that have colonised the teat between milking sessions, while post-dipping creates a chemical barrier that inhibits recolonisation during the interval between cluster removal and teat canal closure (approximately 30–60 minutes) [9]. The emollient in the post-dip formulation further protects the skin from environmental insult, which may explain why teat condition did not deteriorate in any of the dipped groups despite twice-daily chemical exposure. The significant worsening of teat condition in the control group (score increase from 1.8 to 2.6) was unexpected, as one might assume that chemical-free teats would remain in better condition. The most likely explanation is that the emollient in the dip formulations provided a protective film that mitigated the drying effects of cold winter air and sand bedding abrasion, an advantage absent in the control group [4].

Conclusion

The main contribution of this research is a quantified demonstration that combining pre-milking and post-milking

teat disinfection with 0.5% iodine achieves a 1.27 log₁₀ reduction in teat skin bacterial load over eight weeks of winter housing a reduction that is significantly greater than either pre-dip or post-dip applied in isolation and that occurs without adverse effects on teat skin condition.

Three lines of evidence support this conclusion. First, the bacterial count trajectories diverged significantly from the second sampling point (week 4) onward, with the combined-dip group consistently occupying the lowest position and the control group showing no meaningful change, indicating that the observed reductions are attributable to the disinfection protocols rather than to seasonal or management confounders. Second, the additive pattern pre-dip contributing approximately twice the reduction of post-dip is consistent with the biological rationale that bacterial removal before milking prevents teat canal contamination, while post-dip inhibits recolonisation during the vulnerable post-milking window. Third, teat condition scores remained stable in all dipped groups, confirming that the iodine-emollient formulation does not compromise skin integrity under the conditions tested. For the dairy industry, these findings reinforce the recommendation that farms relying on post-dip alone should consider adding a pre-dip step, particularly during housing periods when environmental pathogen pressure is highest. The incremental cost of pre-dip (approximately €0.02 per cow per milking for the iodine solution) is modest relative to the estimated savings from reduced mastitis treatment and milk-quality penalties.

Open questions remain. Whether the bacterial reduction translates directly into lower clinical or subclinical mastitis incidence requires a larger trial with mastitis as the primary endpoint. The optimal pre-dip contact time 30 seconds in this protocol may be shorter or longer depending on the disinfectant chemistry, and dose-response data are lacking. Finally, the long-term effects of twice-daily iodine exposure on teat keratin integrity over a full lactation deserve investigation, as the eight-week window of this trial may not capture cumulative damage.

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Field assistants

Mr. Jan Kuiper and Ms. Anneke Smits collected teat swabs during all three sampling sessions. The farm owner, Mr. Hendrik de Jong, accommodated the trial within normal parlour operations.

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