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Effectiveness of hind hock rinsing as a process hygiene intervention in bovine slaughter

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Abstract

Bovine hind hocks represent a critical contamination site during slaughter due to their complex anatomical structure and exposure to organic contamination. Therefore, this pilot study assessed the effect of a targeted potable water rinsing treatment applied immediately after distal pre-skinning on microbiological indicators and macroscopic contamination under commercial slaughterhouse conditions. Ten carcasses were assigned to the treated (T) group (manual potable water rinse at 4-6 bar for 5 seconds per limb), while 10 were assigned to the control (C) group. For each group, the same carcasses were sampled at the beginning of the slaughter line (BSL) immediately after distal pre-skinning and at the end of the slaughter line (ESL) by sponge swabbing 6 predefined hock sites to quantify total viable count (TVC), *Escherichia coli* and Enterobacteriaceae, and to detect the presence of *Salmonella* spp. and Shiga toxin-producing *E. coli*. A visual assessment of macroscopic defects was performed on 800 hocks (400 per group).

The T group showed significantly greater reductions than the C group for TVC (-1.16 vs. -0.60 log₁₀ CFU/cm²), *E. coli* (-0.79 vs. -0.37 log₁₀ CFU/cm²), and Enterobacteriaceae (-0.49 vs. -0.01 log₁₀ CFU/cm²). Pathogens detected at BSL were not detected at ESL in either group. Visual non-compliances were lower in the T than the C group (9.8% vs. 24.8%; $p < 0.001$).

Within the limitations of this single-plant pilot study, localized hind hock rinsing appears to be a promising supplementary process hygiene intervention associated with reduced microbiological indicators and visible contamination. Further multi-site validation is warranted.

Introduction

Process hygiene criteria (PHC) are key regulatory and operational tools used in cattle slaughterhouses to assess carcass cleanliness and verify the effectiveness of preventive food safety systems, such as the Hazard Analysis Critical Control Point (HACCP) framework (Barco *et al.*, 2015; Blagoyevic *et al.*, 2014; Hochreutener *et al.*, 2017; Alvseike *et al.*, 2019; Barhoum *et al.*, 2026). Official controls performed throughout the food chain are regulated under Regulation (EU) 2017/625, which establishes the framework for official controls and other official activities carried out to ensure compliance with food safety legislation (European Parliament and Council of the European Union, 2017). In compliance with Regulation (EC) No 2073/2005 (Commission of the European Communities, 2005), these criteria act as indicators of process acceptability, establishing microbiological limits for parameters such as total viable count (TVC) and Enterobacteriaceae (Alvseike *et al.*, 2019; Barhoum *et al.*, 2026). For bovine carcasses, satisfactory process hygiene is generally associated with mean values below 3.5 log₁₀ CFU/cm² for TVC and 1.5 log₁₀ CFU/cm² for Enterobacteriaceae (Barhoum *et al.*, 2026). Exceeding these thresholds does not imply product rejection but requires food business operators to review hygiene procedures and implement corrective actions to restore process control (Barco *et al.*, 2015; Barhoum *et al.*, 2026). Since microbiological testing cannot guarantee absolute safety, current strategies focus on verifying that hygiene procedures maintain contamination levels within acceptable limits (Commission of the European Communities, 2005).

During complex processing stages, the hide is widely recognized as the main reservoir and vehicle for the transfer of spoilage and pathogenic microorganisms, including *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* (STEC), onto newly exposed carcass surfaces (Barco *et al.*, 2015; Barhoum *et al.*, 2026). Monitoring these indicators is particularly relevant considering the continued importance of STEC infections in Europe (EFSA and ECDC, 2025). Microbial transfer may occur through direct skin-to-meat contact, contaminated hands and equipment (such as knives), or aerosols generated during slaughter operations (Barhoum *et al.*, 2026). Although Regulation (EC) No 853/2004 (European Parliament and Council of the European Union, 2004) requires animals to be presented to slaughterhouses in a clean condition, the relationship between visible hide cleanliness and actual microbial contamination remains debated. While some studies report a direct association (Barco *et al.*, 2015), recent evidence indicates that effective hygiene management during skinning may mitigate contamination even in visibly dirty animals (Barhoum *et al.*, 2026).

Within this framework, the hind hock region represents a particularly vulnerable anatomical site (Gill *et al.*, 1998; Alvseike *et al.*, 2019). Due to the frequent accumulation of hair, mud, and faecal material on distal limbs, initial opening cuts and skinning in this area may facilitate the transfer of high microbiological and macroscopic contamination onto the underlying carcass surface (Bell, 1997; Gill *et al.*, 1998; Alvseike *et al.*, 2019). Furthermore, the irregular anatomical structure of the hock, characterized by tendons, folds, and bony prominences, may limit the effectiveness of conventional corrective interventions (Hochreutener *et al.*, 2017). Standard procedures such as trimming or steam-vacuum treatment, although effective on flatter surfaces, may show reduced efficiency in these complex anatomical niches where organic material can remain entrapped (Hochreutener *et al.*, 2017). While food safety interventions often focus on whole-carcass decontamination at the end of the slaughter line (ESL), interventions applied at this stage may require careful evaluation because water flow dynamics could influence the redistribution of microorganisms from contaminated areas to adjacent carcass surfaces (Bell, 1997). Therefore, targeted interventions applied immediately after high-risk operations may represent an alternative strategy to reduce contamination before its potential spread to other carcass areas. Recent research has further highlighted that targeted hygiene management at critical process stages may contribute to improved microbial control (Barhoum *et al.*, 2026).

A further element emerging from recent studies is that hygiene interventions applied close to the contamination event may provide advantages over corrective measures performed later in the process. Therefore, implementing localized measures immediately after distal pre-skinning could represent a practical approach to reduce contamination before its potential redistribution across the carcass surface.

To the authors' knowledge, no previous study has specifically evaluated the effect of a localized potable water rinse applied to bovine hind hocks immediately after distal pre-skinning under commercial slaughterhouse conditions.

The present study was conducted exclusively on bovine hindquarters and aimed to evaluate the effect of a localized potable water rinse intervention applied to bovine hind hocks immediately after distal pre-skinning. The intervention was examined under real commercial slaughterhouse operating conditions, focusing on its quantitative impact on process hygiene microbiological indicators (TVC, Enterobacteriaceae, and *E. coli*) and its association with changes in visible macroscopic non-compliances.

Materials and Methods

Experimental design and carcass sampling

The study was conducted in a bovine slaughterhouse located in Northern Italy between October 2024 and March 2025. Slaughter activities involved young bulls and heifers at a line speed ranging from 120 to 140 animals per hour, where, following sticking, the carcasses were suspended by their hind limbs and underwent partial skinning of the distal portion of the hocks. A schematic representation of the experimental design and sampling protocol along the slaughter line is illustrated in Figure 1. The sample size (10 carcasses per group for microbiological analysis and 400 carcasses per group for visual assessment) was defined considering statistical requirements and the operational constraints of a commercial high-speed slaughter line, in accordance with industrial-scale experimental conditions. Two experimental groups, treated (T) and control (C) group, were evaluated during non-consecutive sampling days distributed throughout the study period to account for potential variability related to animal origin and production conditions.

On each sampling day, carcasses were randomly allocated to the T or C group and processed contemporaneously on the same slaughter line. Carcasses in the T group received a manual potable water rinsing treatment, applied exclusively to the hind hocks immediately after distal pre-skinning. The treatment was applied for 5 seconds per limb using potable water at room temperature (18-20°C) and at 4-6 bar operating pressure, directed perpendicularly toward the hind hock region to limit water

runoff onto exposed skinless areas. Carcasses in the C group were processed according to the standard commercial slaughter procedure without any additional water intervention.

Microbiological evaluations followed a longitudinal design, with the same individual carcasses sampled at two distinct sampling points: at the beginning of the slaughter line (BSL, immediately after distal pre-skinning and prior to treatment application in T group) and at ESL. While Regulation (EC) No 2073/2005 establishes PHC based on four standard carcass sites selected according to general slaughter procedures, the present study was not designed to reproduce routine regulatory sampling. Instead, a six-site sampling design was specifically developed for experimental validation of the targeted intervention, including the internal and external surfaces of the hind hocks and the adjacent round areas (Figure 2) to monitor contamination changes in the treated region and surrounding anatomical sites (Commission of the European Communities, 2005).

Macroscopic defects were visually assessed at ESL on a total of 800 bovine hind hocks (400 T and 400 C group), with one hind hock evaluated per carcass to ensure independence among observations. Samples were collected by trained personnel from the research team in collaboration with plant quality control staff. Immediately after collection, samples were stored in refrigerated containers under controlled cold-chain conditions, transported to the laboratory, and processed within the time limits specified in ISO 17604:2015 (ISO, 2015). Operating staff, hygiene procedures, and environmental conditions were maintained as consistent as possible throughout the study period.

Microbiological analysis

Microbiological sampling was performed according to ISO 17604:2015 using sponges pre-moistened with 10 mL of Buffered Peptone Water (BPW). A surface area of 100 cm² (10×10) per anatomical site was sampled using a sterile single-use plastic template to ensure sampling area standardization and reproducibility. The template was positioned on the accessible surface of each predefined anatomical region, including the internal and external surfaces of the hind hocks and the adjacent round areas. The six sampling sites (Figure 2) were analyzed individually and not pooled. Each site was sampled using a separate sponge swab, in order to characterize the distribution of contamination across different anatomical areas of the hindquarters. Consequently, six swabs were collected from each carcass at BSL and a further six at ESL, resulting in a total of 12 samples per carcass per group. A total of 240 swab samples were collected and analyzed throughout the study.

TVC was determined according to ISO 4833-1:2013/Amd 1:2022 (ISO, 2022). Enterobacteriaceae and *E. coli* were enumerated using the TEMPO[®] EB and TEMPO[®] EC systems (bioMérieux, France), validated according to ISO 16140-2:2016 (ISO, 2016). Microbiological results were expressed as log₁₀ CFU/cm² based on the sampled surface area. Laboratory analyses were conducted in an accredited facility under standard quality assurance and quality control (QA/QC) procedures, in compliance with the validation requirements of the applied analytical methods.

Qualitative detection of *Salmonella* spp. was performed using the Thermo Scientific[™] SureTect[™] Salmonella Species PCR Assay according to ISO 6579-1:2017 (ISO, 2017). Detection of STEC was performed according to ISO/TS 13136:2012 (ISO, 2012). Qualitative results were expressed as presence/absence per 100 cm² of sampled surface.

Visual assessment of macroscopic defects

Macroscopic defects were assessed at ESL by trained on-site quality control personnel using a standardized internal scoring grid developed according to the company carcass quality procedure and aligned with the hygiene requirements established by Regulation (EU) 2019/627 (European Commission, 2019). To improve consistency among evaluators, inspectors underwent preliminary calibration sessions before data collection, based on predefined visual descriptions and examples of classified defects. Periodic supervisory checks were performed during the study period to verify consistent application of the scoring criteria. Complete blinding of evaluators to treatment allocation was not feasible under commercial operating conditions because the intervention was visible during

processing; however, predefined criteria based on the extent and distribution of visible contamination were consistently applied throughout the assessment period.

The evaluated defects included hair contamination, slaughter residues, and faecal contamination. Hocks without visible defects were classified as compliant. Non-compliances related to hair contamination and slaughter residues were classified into three severity categories (minor, major, and critical) according to the extent and distribution of contamination:

- Hair contamination: minor defects included localized tufts or scattered hairs; major defects included numerous localized or scattered hairs or the presence of a piece of skin; critical defects included excessive hair contamination or multiple pieces of skin.
- Slaughter residues (defined as visible materials of uncertain origin generated during slaughter operations and not identifiable as faeces or ingesta): minor defects corresponded to few small residues over an area of approximately 100 cm²; major defects included numerous small residues over the same area or few larger residues in localized areas; critical defects included multiple residues distributed over a large surface area or concentrated in multiple locations.
- Faecal contamination: any visible faecal contamination, regardless of extent, was classified as a critical defect.

For effect size calculations (absolute risk reduction, ARR; relative risk reduction, RRR; and relative risk, RR), severity categories were subsequently aggregated into a binary outcome (compliance vs. non-compliance). This approach was adopted because, from a regulatory perspective, the presence of visible contamination represents a non-compliance regardless of its severity (European Commission, 2019).

Statistical analysis

Quantitative microbiological data were transformed into log₁₀ CFU/cm² before statistical analysis. Microbial counts at or below the analytical threshold were treated as censored observations. For these values, the analytical threshold value (0.70 log₁₀ CFU/cm², corresponding to <10 CFU/cm²) was assigned before log₁₀ transformation and statistical analysis. This approach was applied to allow statistical evaluation while avoiding overestimation of microbial reductions. Given the limited sample size and the occurrence of censored observations, the potential influence of these assigned values on estimated reductions was considered during interpretation of the results.

Considering the experimental design, the individual carcass was defined as the statistical unit. The study followed a paired longitudinal design because the same carcasses were sampled at BSL and at ESL. The six anatomical sampling sites collected from each carcass at each sampling time point were aggregated by calculating the mean log₁₀ CFU/cm² value per carcass. This approach avoided pseudoreplication and allowed calculation of within-carcass microbial variation:

$$\Delta \log_{10} \text{CFU/cm}^2 = \text{ESL} - \text{BSL}$$

Although BSL and ESL observations were paired within individual carcasses, treatment effects were evaluated by comparing Δ values between the independent T and C groups. Data distribution was assessed using the Shapiro–Wilk test. Based on distributional characteristics, parametric or non-parametric analyses were applied. An independent samples t-test was used for TVC, while Mann–Whitney *U* tests were applied for *E. coli* and Enterobacteriaceae due to non-normal distribution of the Δ values.

One-tailed tests were applied because the study hypothesis was directional, with the intervention expected to result in lower microbial levels compared with the control procedure. Statistical significance was defined as $p < 0.05$. To complement hypothesis testing and provide an estimate of uncertainty, 95% confidence intervals (CIs) were calculated for microbiological comparisons.

Due to the low number of positive samples, results for *Salmonella* spp. and STEC were analysed descriptively.

BSL microbiological values were compared between groups to evaluate potential initial differences. Considering the limited sample size ($n = 10$ carcasses per group), absence of statistically significant differences was interpreted as absence of detectable differences rather than evidence of statistical equivalence.

Macroscopic defect data were analysed using a chi-square (χ^2) test of independence on a 2×4 contingency table based on severity categories. For effect size estimation, defect outcomes were subsequently aggregated into compliant and non-compliant categories, and ARR, RRR, and RR, with corresponding 95% CIs, were calculated.

Results

For microbiological analyses, values detected at the two sampling stages (BSL and ESL) are reported in *Supplementary Table 1*. BSL contamination levels showed no statistically detectable differences between the T and C groups; however, given the limited sample size ($n=10$ carcasses per group), these findings should not be interpreted as evidence of formal equivalence. At BSL, mean TVC values were 3.43 ± 0.41 and $3.74 \pm 0.52 \log_{10}$ CFU/cm² in the T and C groups, respectively; mean *E. coli* counts were 1.53 ± 0.47 and $1.11 \pm 0.38 \log_{10}$ CFU/cm², while Enterobacteriaceae averaged 1.26 ± 0.52 and $1.27 \pm 0.63 \log_{10}$ CFU/cm² in the T and C groups, respectively. At ESL, most *E. coli* and Enterobacteriaceae values in the T group were at the analytical threshold value ($0.70 \log_{10}$ CFU/cm², corresponding to <10 CFU/cm²), whereas the C group showed higher residual contamination, particularly for Enterobacteriaceae, which remained detectable in several samples.

Table 1 reports the log reductions ($\Delta \log_{10}$ CFU/cm²) between BSL and ESL in the two experimental groups. In the T group, significant reductions were observed for all microbiological parameters: TVC ($\Delta = -1.16 \pm 0.51 \log_{10}$ CFU/cm²; $p < 0.001$), *E. coli* ($\Delta = -0.79 \pm 0.45 \log_{10}$ CFU/cm²; $p < 0.001$), and Enterobacteriaceae ($\Delta = -0.49 \pm 0.49 \log_{10}$ CFU/cm²; $p = 0.012$). In the C group, reductions were observed for TVC ($\Delta = -0.60 \pm 0.31 \log_{10}$ CFU/cm²; $p < 0.001$) and *E. coli* ($\Delta = -0.37 \pm 0.37 \log_{10}$ CFU/cm²; $p = 0.011$), whereas Enterobacteriaceae showed no significant change ($\Delta = -0.01 \pm 0.55 \log_{10}$ CFU/cm²; $p = 0.935$).

Between-group comparisons showed greater reductions in the T group for all evaluated parameters. The difference in reduction was $0.55 \log_{10}$ CFU/cm² for TVC ($p = 0.009$), $0.41 \log_{10}$ CFU/cm² for *E. coli* ($p = 0.038$), and $0.47 \log_{10}$ CFU/cm² for Enterobacteriaceae ($p = 0.032$).

For qualitative analyses, STEC was detected at BSL in two T samples and one C sample, while one C sample was positive for *Salmonella* spp. All ESL samples were negative for both pathogens.

Macroscopic defects were observed in 99/400 C units (24.75%) and 39/400 T units (9.75%) (Table 2). The χ^2 test showed a significant association between treatment and defect classification ($\chi^2 = 35.01$; $p < 0.001$). The proportional differences between groups were 68.4%, 23.5%, and 66.7% for minor, major, and critical defect categories, respectively. The ARR was 15.0% (95% CI: 9.9-20.1), corresponding to 15 fewer non-compliant hocks per 100 evaluated units. The RRR was 60.6%, while the RR was 0.394 (95% CI: 0.279-0.555).

Discussion and Conclusions

This study evaluated the effectiveness of a localized potable water rinsing intervention applied to bovine hind hocks immediately after distal pre-skinning. The hind hock region is recognized as being particularly susceptible to contamination during slaughter due to the frequent presence of hair, dirt, and faecal material on distal limbs (Bell, 1997; Gill *et al.*, 1998). Hide removal represents a critical stage for potential microbial transfer from the hide to exposed carcass surfaces (Bell, 1997). In addition, the anatomical conformation of the hock may reduce the effectiveness of conventional interventions, such as knife trimming or steam-vacuum treatment, on irregular surfaces (Hochreutener *et al.*, 2017).

The collected data indicate that localized water rinsing was associated with greater reductions in microbiological indicators compared with the standard procedure under the conditions evaluated. Although reductions were also observed in the C group, significantly greater decreases were

consistently detected in the T group, particularly for Enterobacteriaceae, which showed limited reduction in controls. Since Enterobacteriaceae are commonly used as indicators of faecal contamination, their greater reduction in the T group may suggest improved removal of superficial organic contamination from the hock surface rather than a direct antimicrobial effect. This interpretation is consistent with the expected mechanical action of potable water rinsing, which primarily removes adherent material rather than inactivating microorganisms. However, literature on ambient or cold water washing on beef carcasses shows conflicting evidence: while some studies note that water washing without physical/chemical decontamination or thermal pasteurization is generally ineffective at inactivating bacteria (Mies *et al.*, 2004; Barboza de Martinez *et al.*, 2002), its efficacy can depend heavily on initial microbial loads, showing greater reductions when initial surface contamination is relatively high (Gill and Landers, 2003).

The visual assessment showed a pattern consistent with the microbiological findings, as treated hocks exhibited a lower prevalence of visible defects, including hair contamination, slaughter residues, and fecal contamination. This aligns with wider observations that targeted intervention protocols on visually contaminated animals or areas can effectively mitigate microbial loads to levels comparable with clean carcasses (Gill and Landers, 2004; McCleery *et al.*, 2008; Hauge *et al.*, 2012;). However, interpretation of these results should consider that the scoring system was based on an internal classification procedure and that some severity categories, particularly critical defects, included a limited number of observations.

From an operational perspective, the intervention required only potable water and a short application time, characteristics that may facilitate implementation within commercial slaughter environments without major modifications to existing processing lines or the use of chemical decontamination agents. This aspect is particularly relevant considering the legislative constraints on chemical treatments in many States, where non-chemical physical interventions are preferred to improve carcass hygiene without affecting organoleptic properties. Nevertheless, effect estimates and percentage reductions, especially those related to rare defect categories, should be interpreted cautiously. The low absolute number of critical defects makes percentage variations particularly sensitive to small numerical changes, whereas the overall binary classification of compliant versus non-compliant hocks provides a more stable representation of the observed operational effect.

The qualitative pathogen findings should also be interpreted cautiously because of the limited number of positive samples. Although STEC and *Salmonella* spp. were detected at BSL but not at ESL, the present study was not designed to investigate microbial redistribution pathways. Therefore, under the evaluated conditions, no increase in pathogen detection was observed after processing; however, potential redistribution through runoff, aerosols, equipment, or carcass contact cannot be excluded and requires further targeted investigation. Indeed, unheated water washing without sanitizing agents carries a recognized risk of transferring or redistributing bacteria across carcass surfaces rather than eliminating them, as highlighted by Barboza de Martinez *et al.* (2002) and Madden *et al.* (2004), who observed localized bacterial redistribution or even increases in overall counts following simple water washing steps. At this point, it is helpful to clarify that the treatment is applied locally to the hindquarters during the pre-skimming stage at the distal end, when only the hindquarters are flayed and the rest of the carcass remains hide-on. This is an important aspect of the treatment configuration, as maintaining the rest of the carcass hide-on helps to prevent contamination from migrating and spreading towards the anterior regions of the carcass.

Several limitations must be acknowledged. The study was conducted in a single industrial plant, and results may therefore reflect specific plant characteristics, equipment, operational practices, and animal cleanliness conditions at BSL. The microbiological evaluation included a limited number of carcasses (n=10 per group), reducing the ability to detect differences involving low-prevalence microorganisms. Furthermore, the study period did not include evaluation of seasonal variability, which is known to influence the BSL prevalence and load of indicator bacteria such as *E. coli* and Enterobacteriaceae on cattle hides and carcasses, with higher counts often reported during warmer or rainy seasons (Ruby *et al.*, 2007; Rigobelo *et al.*, 2006, 2008). Long-term repeatability across

different operational shifts was not assessed. Operator variability, both during rinsing application and visual evaluation, was not quantified and may represent an additional source of variation.

Additionally, direct comparisons with broader slaughterhouse literature remain challenging due to the high variability across studies in terms of carcass sampling sites, analytical techniques, and plant-specific operational layouts (McEvoy *et al.*, 2004). Finally, visual defect assessment relied on an internal scoring procedure rather than an externally validated visual classification system. Future studies should evaluate reproducibility across different slaughterhouses, seasonal conditions, animal cleanliness levels, and standardized external scoring frameworks.

In conclusion, within the scope of this single-plant pilot study, localized hind hock rinsing appears to be a promising supplementary intervention warranting validation in larger, multi-plant studies to confirm the reproducibility and broader applicability of the observed effects. While reductions in indicator microorganisms and visible carcass defects were observed following the intervention, the potential for microbial redistribution through pathways such as aerosols, runoff, equipment, or carcass-to-carcass contact remains an open question requiring further dedicated investigation.

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Online supplementary material

Supplementary Table 1. Carcass-level mean microbiological values ($\log_{10}$ CFU/cm² $\pm$ standard deviation) of total viable count, Enterobacteriaceae, and *Escherichia coli*, calculated by averaging six anatomical sampling sites for each carcass at the beginning of the slaughter line and at the end of the slaughter line in treated and control groups.

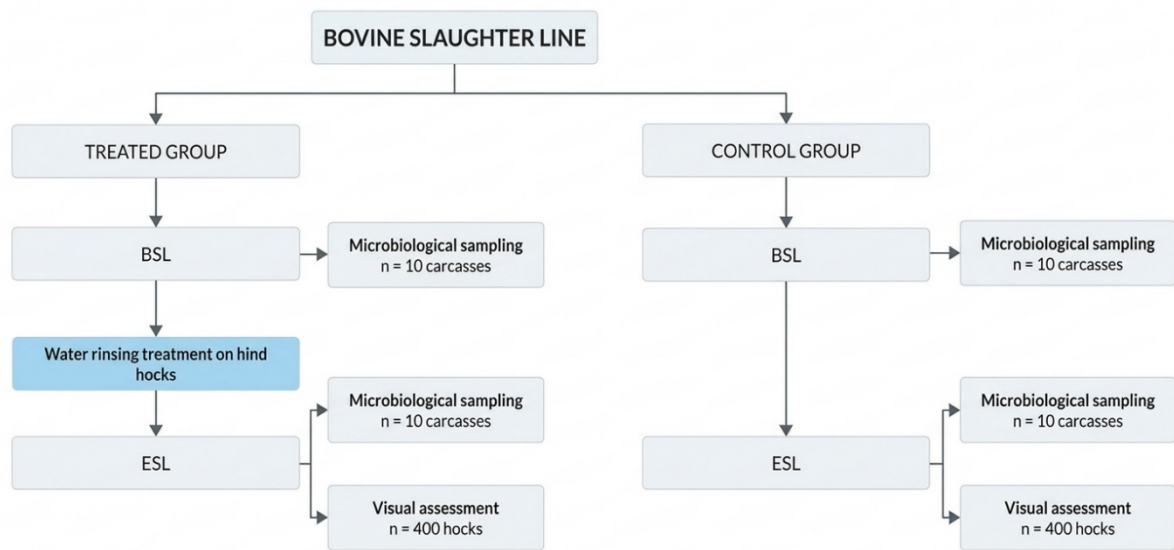


Figure 1. Schematic diagram of the experimental design, sampling points (beginning of the slaughter line, BSL; end of the slaughter line, ESL), and water treatment application on carcasses processed in a commercial slaughterhouse. Note: The water rinsing treatment on hind hocks is depicted in light blue.



Figure 2. Anatomical sites used for microbiological sampling on bovine carcasses. (1) External surface of the right hock; (2) internal surface of the right hock; (3) right round; (4) external surface of the left hock; (5) internal surface of the left hock; (6) left round. Sampling procedure was systematically performed from the right to the left limb.

Table 1. Log₁₀ reductions ($\Delta \log_{10}$ CFU/cm² $\pm$ standard deviation) of total viable count (TVC), Enterobacteriaceae, and *Escherichia coli* (*E. coli*) between the beginning of the slaughter line (BSL) and the end of the slaughter line (ESL) sampling in treated (T) and control (C) groups.

Parameter	Δ T group	p-value (Δ vs. 0)	Δ C group	p-value (Δ vs. 0)	Between-group difference (T-C)	p-value	95% CI of difference
TVC	-1.16 $\pm$ 0.51	<0.001	-0.60 $\pm$ 0.31	<0.001	-0.55	0.009	-0.94 to -0.16
<i>E. coli</i>	-0.79 $\pm$ 0.45	<0.001	-0.37 $\pm$ 0.37	0.011	-0.41	0.038	-0.79 to -0.03
Enterobacteriaceae	-0.49 $\pm$ 0.49	0.012	-0.01 $\pm$ 0.55	0.935	-0.47	0.032	-0.93 to -0.01

Negative Δ values indicate reductions in microbial counts between BSL and ESL. The *p* values in the within-group comparisons refer to testing whether Δ values differed from zero. Between-group comparisons evaluated differences in microbial reductions between T and C groups.

Table 2. Results of visual assessment of macroscopic non-compliances in treated (T) and control (C) bovine hind hocks.

Visual classification	T group (n = 400)	C group (n = 400)
Compliances	361 (90.25%)	301 (75.25%)
Minor non-compliances	25 (6.25%)	79 (19.75%)
Major non-compliances	13 (3.25%)	17 (4.25%)
Critical non-compliances	1 (0.25%)	3 (0.75%)
Total non-compliances	39 (9.75%)	99 (24.75%)