

## ORIGINAL ARTICLE

## Food Microbiology and Safety

# Comparison of peracetic acid and bacteriophage application by vascular rinsing on *Salmonella* reduction in lymph nodes of goat carcasses

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**Abstract:** The ability of carcass vascular rinsing supplemented with bacteriophage (BP) and peracetic acid (PAA) to reduce *Salmonella* in lymph nodes (LNs) from experimentally infected goats was determined. Cull dairy goats ( $n = 60$ ) were randomly assigned to a control (CN, nonrinsed) and two vascular rinse treatments: BP and PAA. Goats were inoculated intradermally with *Salmonella* Enteritidis and slaughtered after a 7-day incubation. Vascular rinsing was performed postexsanguination via a catheter in the heart. Carcasses were skinned, eviscerated, sprayed with 5% lactic acid, and chilled (2°C) overnight. The superficial cervical, popliteal, medial iliac, and subiliac LNs were collected aseptically for *Salmonella* enumeration and phage titer determination. The longissimus dorsi (LD) and semimembranosus muscles were also excised, and stored for 1, 4, and 7 days for meat surface color measurements. PAA-treated carcasses showed lower ( $p < 0.05$ ) temperatures and pH values within 8 h postmortem compared to CN and BP. The average counts of *Salmonella* in the LNs associated with PAA ( $3.4 \pm 1.3$  log CFU/g) were significantly lower compared to CN ( $3.8 \pm 1.1$  log CFU/g), with the lowest load observed in medial iliac LNs ( $2.7 \pm 1.5$  log CFU/g). Substantial phage titers were detected in LNs from BP-treated carcasses ( $7.0 \pm 0.91$  log PFU/g), and no differences were observed in *Salmonella* counts in BP compared to CN. The meat samples obtained from PAA-treated carcasses exhibited lower redness ( $a^*$  values) and deoxymyoglobin in the LD ( $p < 0.05$ ) but showed no differences in lightness or oxymyoglobin compared to BP and CN. Vascular rinsing has the potential to suppress *Salmonella* in the LNs with other antimicrobials and chemicals with different combinations and concentrations.

## KEYWORDS

bacteriophage, goat carcass vascular rinsing, lymph node, peracetic acid, *Salmonella*

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**Practical Application:** This study investigates a method to control *Salmonella* in goat meat by rinsing carcasses with peracetic acid (PAA) or bacteriophages through the bloodstream after slaughter. The findings suggest that PAA can mitigate *Salmonella* levels in lymph nodes, potentially improving meat safety. While bacteriophage treatment did not significantly affect the bacterial count to observe differences with the control group, vascular rinsing could still be promising with different antimicrobial combinations. This research would help meat processors enhance food safety measures, reducing the risk of *Salmonella* contamination in meat products.

## 1 | INTRODUCTION

Meat is one of the most essential parts of the daily intake of food as a primary source of protein for human consumption (Pereira & Vicente, 2013). Meat and meat production has increased due to the global demand that is driven by large population growth. According to the Organization for Economic Co-operation and Development (OECD), growth in global meat consumption is projected to increase by 14% by 2030 compared to the base period average of 2018–2020 (OECD/FAO, 2021). Meat safety and quality have become two critical factors affecting the buying decisions of consumers associated with the enlargement of the food markets and the improvement of living standards worldwide (Godfray et al., 2018).

The vascular rinsing process (Rinse & Chill<sup>®</sup>, RC) developed by MPSC Inc. is a carotid artery or intracardiac application of a chilled isotonic solution that is a source of metabolizable substrates (phosphates and saccharides) after exsanguination of meat animals to increase removal of the residual blood from the carcass (Yancey et al., 2002). The application was designed to improve the meat carcass quality which includes meat tenderness and color by providing an additional glucose source for anaerobic metabolism that facilitates more rapid pH reduction in applied carcasses (Kethavath et al., 2022). Vascular rinsing has also demonstrated potential for increasing meat safety. Feirtag and Pullen (2003) revealed that beef carcass surface aerobic counts were reduced by more than 41%, coliforms by 67%, and *Escherichia coli* by 83% in the RC process as compared to nonrinsed carcasses. Also, the total aerobic plate count of rinsed (RC processed) carcasses was determined as statistically lower than the nonrinsed carcasses in another surface swab sampling study (Moreira et al., 2018).

In carcasses from healthy livestock, deep tissues during the chilling process are typically considered aseptic (Gill, 1998). However, studies investigating the contamination route of *Salmonella* in ground beef have identified deep tissue lymph nodes (LNs) as a major reservoir of *Salmonella*

(Delgado-Suarez et al., 2024; Koohmaraie et al., 2012). Removal of LNs as a component of raw beef would effectively reduce the ground meat contamination although total removal of deep tissue LNs is not feasible. Most of the LNs located within adipose tissues of meat animal carcasses are not removed during processing. Routinely, these LNs located in the deep tissues are ground with lean and fat to produce ground beef and can lead to a possible risk of *Salmonella* contamination in meat. Hence, it is crucial to conduct further research to explore other interventions aimed at ensuring the safety of the meat with careful consideration and thorough investigation of the quality and integrity of the meat products.

The nontyphoidal *Salmonella enterica* serovars (*Salmonella* Enteritidis, Typhimurium, etc.) commonly cause self-limited gastroenteritis and rarely lead to extraintestinal infections in humans. But in certain conditions, nontyphoidal salmonellosis can become invasive (particularly in the bloodstream) in sensitive populations such as immunocompromised, very young, elderly, or pregnant (Gerba et al., 1996; Langridge et al., 2012). The nontyphoidal invasive salmonellosis can be an aggressive systemic infection with a high mortality rate, and it was estimated to cause 535,000 illnesses and 77,500 deaths in 2017. According to the World Health Organization (WHO), 52% of all cases of nontyphoidal salmonellosis are transmitted by food (Stanaway et al., 2019; WHO, 2015). Under these circumstances, infection models targeting deep tissue LNs in meat animals become an important research field for assuring meat carcass safety.

Different physical, chemical, and biological antimicrobial interventions have been applied to reduce the microbial risks from raw meat and meat products. Bio-control approaches based on bacteriophage application have great potential to enhance microbiological safety with certain features such as high antimicrobial activity, host specificity, safe use, and easy handling. Bacteriophages are distinctly suitable to decontaminate carcass surfaces and other raw meat products and extend the shelf life of

processed foods (Garcia et al., 2008). Also, another noteworthy advantage of phage application in the meat industry is that bacteriophages do not negatively affect meat quality parameters in beef (Ishaq et al., 2020). One of the important perspectives for enhancing meat quality is using safe antimicrobial chemicals such as organic acids. Peracetic acid (also known as peroxyacetic acid, PAA) is a strong bactericidal agent with high redox potential and is considered safe since it decomposes into harmless residuals such as water and oxygen (Dell'Erba et al., 2007; Viola et al., 2018). It has been known for a long time that consumers have demanded simple, unprocessed, and less chemical exposure foods. Health and food safety organizations have monitored food additives and their effects on health are constantly being studied (Zink, 1997). According to the US Department of Agriculture (USDA), bacteriophages and PAA are on the list of useful chemical substances, and they can be used in meat processing (USDA-FSIS, 2021).

In this study, the aim was to minimize deep tissue *Salmonella* contamination originating from LNs. This research presents a novel approach by integrating PAA and bacteriophage applications, which are well-known meat safety agents, with the vascular rinsing process (RC) to target deep tissue *Salmonella* contamination in LNs. Unlike conventional surface decontamination methods, this study explores an innovative internal intervention strategy, aiming to enhance microbial safety within the meat carcass. Additionally, the development of a cost-effective model for testing various antimicrobials in the RC system provides a valuable tool for prescreening interventions before conducting large-scale animal trials, ultimately accelerating the advancement of meat safety practices.

## 2 | MATERIALS AND METHODS

### 2.1 | Preparation of *Salmonella* Enteritidis inoculum

*Salmonella* Enteritidis C StR (SE13; isolated by Microes Food Safety) stock culture was cultivated in Tryptic Soy Broth (Merck) and incubated at 37°C for 24 h. SE13 was selected for this study, primarily because the bacteriophage studied routinely has been used commercially for titer determinations. This provided confidence in the assessment of the bacteriophage carcass treatment effects. In addition, this strain was used in bacteriophage studies associated with *Salmonella*-contaminated ground beef (Shebs-Maurine et al., 2021; Yeh et al., 2017). After incubation, the culture was streaked onto xylose lysine deoxycholate (XLD; Merck) agar for another consecutive overnight incubation to verify the purity of the strain and

produce a bacterial lawn. Following incubation, phenotypically confirmed colonies were collected and transferred to a fresh sterile saline solution (0.9%), and inoculum was adjusted to a 2.0 density with McFarland standard tube to achieve the target inoculum level ( $\sim 6.0 \times 10^8$  CFU/mL). *Salmonella* count was confirmed by plating on XLD and incubated at 37°C for 24 h after preparation at the desired concentration. Prior to administering to animals, the appropriate number of tubes containing the adjusted inoculum were pooled into 50 mL sterile falcon tubes and mixed thoroughly.

### 2.2 | Experimental *Salmonella* infection of goats

A total of 60 goats consisting of various breeds (Alpine, LaMancha, Nubian, Saanen, Crossbred) were obtained from a commercial dairy farm (Drumlin Dairy, LLC). Goats selected had the following characteristics: age (2–5 years), live weight ( $74.5 \pm 13.7$  kg), parity (kidded at least once), and milking status (recovered or withdrawn from milking and in rejuvenation mode). Selected goats were not treated with antibiotics (unless beyond the normal clearance period). The milking status of goats was either not totally dry or recovered. Goats with similar characteristics were grouped and divided homogeneously into treatment groups. Animal care was managed under the approved “Animal Care and Use Committee (IACUC A006428-A01)” protocol. Goats, upon delivery, were allowed 1 week acclimation period before *Salmonella* inoculation. Goats were housed in Animal Biosafety Level 2 (BSL2) of the Livestock Facility at the University of Wisconsin-Madison through an acclimation and incubation period under the care of a consulting veterinarian and the livestock staff of the facility. Infected animals were transported to the Meat Science & Animal Biologics Discovery Food Safety Facility (BSL2) with a live animal transportation cart for harvest and further analysis.

The *Salmonella* inoculation procedure used for the experimental infection model was developed and refined in part from research performed on beef animals that were inoculated (Edrington et al., 2016; Loneragan and Edrington, 2014). Briefly, 10 needle multiple skin test lancets (ComforTen) were utilized intact (10 surgical steel lancets with 1.2-mm tips) or reduced to 3-lancet tips for proper intradermal administration. Inoculation sites on the carcass were first sprayed with 70% ethanol which was allowed to evaporate. The lancets dipped into fresh inoculum ( $3.33 \pm 0.39 \times 10^8$  CFU/mL) were applied with light pressure to the inoculation sites (each lower leg, both anatomical sides of the caudal thorax near 12/13th thoracic vertebrae, and ventral abdomen) of healthy animals with

normal body temperature. Before inoculation, goats were anesthetized with xylazine and ketamine/midazolam combination by a consulting veterinarian. Atipamezole was administered for proper reanimation after the inoculation procedure. The rectal temperature of infected goats was monitored every day along with evidence of feed and water consumption during the 7-day incubation period (Hwang et al., 2024).

### 2.3 | Vascular rinsing treatments

After the incubation period, animals were mechanically stunned with a penetrating captive bolt stunner and bled on harvesting day. Twenty goats were conventionally slaughtered as a control group (CN;  $n = 20$ ) and the two different RC treatment groups (BP and PAA;  $n = 20$  each). The experiment was conducted at four independent trials (different times) with 16 goats per trial. Prior to applying the RC process, only the two jugular veins were severed, which was different than Hwang et al. (2024), in which a Halal stick (carotid arteries and jugular veins cut) was used. This change was made to increase the likelihood of delivering more of the rinse solution through the vasculature. After exsanguination, the RC process was applied by inserting an intracardiac catheter and vascular rinsing out the residual blood from the circulatory system using a chilled (3°C) isotonic substrate solution (consisting of 98.5% water balanced with glucose, phosphates, and maltose; Mickelson & Claus, 2020) supplemented with BP (PhageGuard S;  $\sim 2 \times 10^{10}$  PFU/mL) and PAA (2000 ppm) at an application rate of 10% of live weight of goats. After the RC process was applied, the carotid arteries and the vena cava were severed. Insta-Test high range PAA test strips (LaMotte) were used to confirm 2000 ppm concentration of PAA in RC solutions of PAA treatment. After treatment, rinsed and CN carcasses were skinned and eviscerated, and the exterior was sprayed with an antimicrobial solution (5% Lactic acid, Corbion) before being hung in the cooler overnight. After chilling overnight (2°C), the carcass superficial cervical (SC), medial iliac (MI), subiliac, and popliteal (POP) LNs, and the longissimus dorsi (LD) and semimembranosus (SM) muscles were collected from carcasses with aseptic conditions. Each LN at its specific location was combined from each anatomical side of the carcass to provide sufficient mass. The collected LNs were aseptically trimmed and mashed using a rubber mallet in a sterile bag. The mass of the mashed samples was split into half to assign a set for the *Salmonella* enumeration and the phage titer determination.

### 2.4 | Enumeration, confirmation, and detection of *Salmonella* in LN samples

Mashed LN samples were homogenized with a stomacher after being diluted with buffered peptone water (Merck). *Salmonella* counts were determined on XLD agar with decimal dilution after incubation at 37°C for 24–48 h. Representative *Salmonella* colonies grown on XLD agar (black center and a lightly transparent zone of reddish color) were subjected to biochemical confirmation. For this purpose, triple sugar/iron agar (Sigma), urea agar slants (Sigma), and L-lysine decarboxylation (Sigma) broth were used and results were interpreted according to the “Bacteriological Analytical Manual (BAM)” of US Food and Drug Administration. Enrichment-based *Salmonella* detection was also performed in no *Salmonella* seen LN samples to determine the presence of *Salmonella* below the detection limit of the enumeration process (FDA, 2007).

### 2.5 | Phage susceptibility testing by overlay spot assay

Phage susceptibility of the SE13 intradermally inoculated to animals was confirmed with overlay spot assay. After treatment, all *Salmonella* isolated from BP group LNs were also screened by the same method. Isolates were incubated at 37°C in Luria Bertani broth overnight (LB; Sigma). On the analysis day, LB top agar (0.4%) containing 100 µL *Salmonella* fresh suspension was poured onto the previously prepared LB agar (1.5%). After allowing agar solidification for 10–20 min, decimal dilutions of the bacteriophage suspension prepared with SM buffer were spotted on the LB agar as duplicates. All spots were allowed to fully absorb into the top agar and incubated at 22°C for 24 h for observation of plaque formation.

### 2.6 | Phage titer determination

The mashed LN samples were homogenized in SM buffer at 1000 rpm for 1 min using a Geno/Grinder® (Model 2010, SPEX SamplePrep LLC) with a 1 s/s ball, and 10 µL of chloramphenicol was added to each sample prior to homogenization. Prepared samples were sent to WBA Analytical Laboratories in an insulated box under the cold chain. Samples were filtered and serially 10-fold diluted subjected to the double-layer agar method. Analysis was performed in four repetitions and phage titer results were calculated as log PFU/g.



## 2.7 | Postmortem pH and temperature measurements

Postmortem pH and temperature data were collected with the deep tissue probe (Testo Model 205) in the SM of each goat carcass. Measurements were performed at 1, 4, 8, and 24 h postmortem. The pH meter and the standards (pH 4.0 and 7.0) were stored in the same cooler as the carcasses for calibration of measurements.

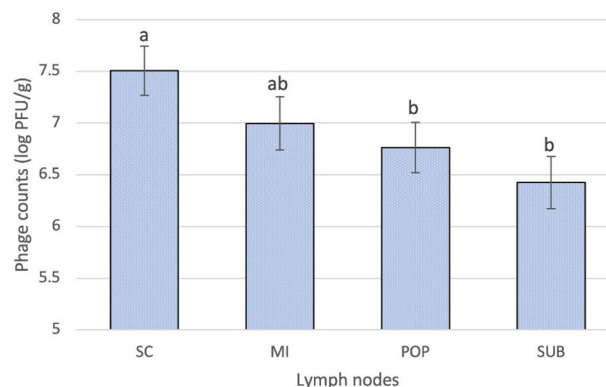
## 2.8 | Meat color determinations

At 24 h postmortem, the LD and SM were excised and cut perpendicular to the length into steaks (LD,  $n = 3$  and SM,  $n = 1$ ; about 2.0 cm thick). LD and SM were separately placed on Styrofoam trays, overwrapped with an oxygen permeable film (polyvinyl chloride, PVC; oxygen transmission rate: 22,480 cm<sup>3</sup>/m<sup>2</sup>/24 h at 23°C; water transmission rate = 496 g/m<sup>2</sup>/24 h at 37.8°C and 90% relative humidity; product code 75,003,815, AEP Industries Inc.), and stored in the dark (2°C) for 1, 4, and 7 days. Before measuring meat surface color, a spectrophotometric colorimeter (model CM-600d, 8-mm aperture, 10° observer angle; specular component included Konica Minolta Sensing Inc.) was standardized against a white calibration plate (No. 18133019: CIE L\* 97.06, a\* 0.17, b\* 1.91) overwrapped with the applicable PVC film. Six colorimeter measurements (CIE L\*a\*b\* and the chemical states of myoglobin) were obtained on each PVC-wrapped sample. Averages were determined for each measurement prior to statistical analysis.

The different myoglobin forms (deoxymyoglobin, DMb; oxymyoglobin, OMb; and metmyoglobin, MMb) were estimated using the equations from the AMSA Meat Color Measurement Guidelines (King et al., 2023). The reflectance at the isobestic points 474, 525, and 572 nm was converted to a reflex attenuance (Absorbance =  $\log 1/\text{Reflectance}$ ). The reflex attenuance at 730 nm was used as a reference to zero pigment-free meat. Then, the relative percentages of myoglobin forms were calculated as follows: DMb (%) =  $2.375 \times [1 - (A_{474} - A_{730}) / (A_{525} - A_{730})] \times 100$ , OMb (%) =  $100 - \text{DMb}(\%) - \text{MMb}(\%)$ , MMb (%) =  $[1.395 - (A_{572} - A_{730}) / (A_{525} - A_{730})] \times 100$ . Six repeated measurements taken on days 1, 4, and 7 of refrigerated (2°C) dark storage. The averages of the measurements were used for statistical analysis.

## 2.9 | Statistical analysis

For the statistical analysis, the animal served as the experimental unit. For pH and temperature decline, the data were analyzed as a split plot design with carcass chilling treatment as the whole plot factor and time postmortem as



**FIGURE 1** Bacteriophage counts of applied carcass lymph nodes in *Salmonella* infected goats. <sup>a,b</sup>Means with unlike letters are different ( $p < 0.05$ , s.e. = 0.25). MI, medial iliac; POP, popliteal; SUB, subiliac lymph nodes; SC, superficial cervical.

the split plot factor. For microbiological data, a  $3 \times 4$  factorial design (carcass chilling treatment  $\times$  LN) was used. For the color data, a split plot design was used with the main effects of carcass chilling treatment and muscle with the storage day, serving as the split plot factor. The main effects and their interactions were analyzed. The variable of the trial week was considered for use as a covariate in the analysis. The SAS procedure MIXED (SAS 9.1.3 Service Pack 3, SAS Institute Inc.) was used to determine significance ( $p < 0.05$ ) in the model and when significance was found, means were separated using the least significant difference method. Letter assignment to individual means to enable statistical comparisons was achieved using the pdmix800 macro (Saxton, 1998).

## 3 | RESULTS

*Salmonella* loads were successfully found in all LNs of infected goats and the difference in *Salmonella* levels was significant for each LN (Table 1,  $p < 0.0001$ ). It was determined that MI had lower *Salmonella* counts ( $2.68 \pm 0.29$  log CFU/g) while POP had higher counts ( $4.28 \pm 0.16$  log CFU/g) in the CN group without RC treatment (Table 2). Overall *Salmonella* loads of LNs were detected lower in the RC solution supplemented with the addition of PAA than in CN and BP groups ( $p < 0.05$ ). However, no statistical differences in *Salmonella* count in LNs compared to CN were observed in the RC process enhanced with BP supplementation under this experimental condition.

Phage titer determination results revealed that bacteriophage delivery with RC was successfully implemented. The highest phage delivery (Figure 1) was noticed in the SC ( $7.51 \pm 0.24$  log PFU/g) compared to the other collected LNs ( $p < 0.05$ ). The lowest level of phage accumulation was determined in the subiliac ( $6.46 \pm 1.10$  log PFU/g) while

**TABLE 1** Tests of fixed effects associated with *Salmonella* counts (log CFU/g) and bacteriophage (log PFU/g) titers of LNs, and pH and temperature declines of goat carcasses.

Independent variables	<i>Salmonella</i>	Bacteriophage	Independent variables	pH decline	Temperature decline
trt	0.0133		trt	<0.0001	<0.0001
LN	<0.0001	0.0019	time	<0.0001	<0.0001
trt*LN	0.2367		trt*time	<0.0001	0.0048

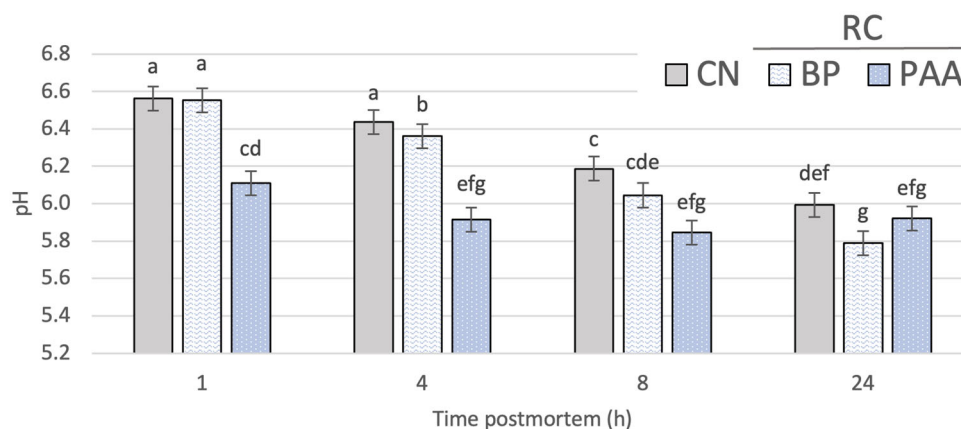
Note: trt: carcass chilling treatment [CN: nonrinsed as control ( $n = 20$ ); BP: bacteriophage ( $n = 20$ ); PAA: peracetic acid ( $n = 20$ )]; LN: lymph node; Time: 1, 4, 8, 24 h of measurement postmortem.

**TABLE 2** *Salmonella* counts in carcass lymph nodes (mean log CFU/g  $\pm$  s.e.) from *Salmonella*-infected goats.

Lymph nodes	CN	RC		LN average
		BP	PAA	
SC	4.07 $\pm$ 0.12	4.26 $\pm$ 0.13	4.00 $\pm$ 0.11	4.11 $\pm$ 0.07 <sup>x</sup>
MI	2.68 $\pm$ 0.29	3.14 $\pm$ 0.36	2.28 $\pm$ 0.32	2.70 $\pm$ 0.19 <sup>y</sup>
POP	4.28 $\pm$ 0.16	4.12 $\pm$ 0.28	3.42 $\pm$ 0.35	3.94 $\pm$ 0.16 <sup>x</sup>
SUB	4.07 $\pm$ 0.19	3.98 $\pm$ 0.16	4.03 $\pm$ 0.13	4.02 $\pm$ 0.09 <sup>x</sup>
trt average	3.77 $\pm$ 0.12 <sup>a</sup>	3.88 $\pm$ 0.13 <sup>a</sup>	3.43 $\pm$ 0.15 <sup>b</sup>	

Note: Means with unlike superscript letters are different ( $p < 0.05$ ).

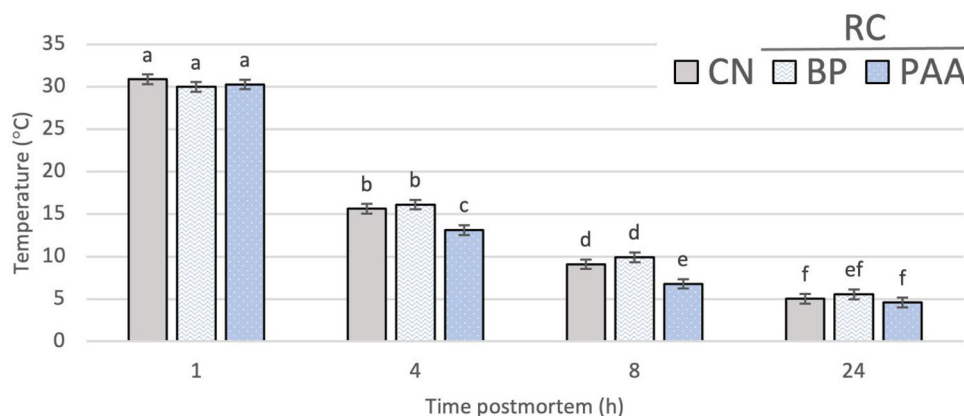
Abbreviations: BP, bacteriophages; CN, control (nonrinsed); LN, lymph node; MI, medial iliac; PAA, peracetic acid; POP, popliteal; RC, Rinse & Chill®; SC, superficial cervical; SUB, subiliac.

**FIGURE 2** Rinse & Chill® (RC) effects on postmortem pH decline of goat carcasses. <sup>a–g</sup>Means with unlike letters are different ( $p < 0.05$ ; s.e. = 0.06). BP, bacteriophages; CN, control (nonrinsed); PAA, peracetic acid.

the average counts of POP and MI were  $6.76 \pm 0.24$  and  $7.00 \pm 0.26$  log PFU/g, respectively.

RC supplemented with bacteriophage and PAA decreased the pH compared to the nonrinsed goat carcasses ( $p < 0.001$ ; Figure 2). RC supplemented with PAA resulted in lower ( $p < 0.001$ ; Figure 3) carcass temperatures than CN and BP. However, after 24 h chilling, no differences ( $p > 0.05$ ) in carcass temperature were found. Tests of fixed effects associated with *Salmonella* counts (log CFU/g), bacteriophage (log PFU/g) titers of LNs, and pH and temperature declines of goat carcasses are reported in Table 1.

Selected instrumental measures associated with meat color were affected by the independent variables (Table 3). However, Omb and CIE L\* were not affected ( $p > 0.05$ ) by TRT. Dmb was affected ( $p < 0.05$ ) by a TRT\* muscle interaction. In the LD, BP resulted in a greater ( $p < 0.05$ ) percentage Dmb than CN (Table 4). PAA resulted in the least ( $p < 0.05$ ) amount of Dmb in the LD. In contrast, no TRT differences ( $p > 0.05$ ) were found in the SM. PAA did result in an increase ( $p < 0.05$ ) in MMb compared to CN and BP which were not different ( $p > 0.05$ ) from one another. BP had greater ( $p < 0.05$ ) redness (CIE a\*) than CN while PAA was the least red (Table 5).



**FIGURE 3** Rinse & Chill® (RC) effects on postmortem temperature decline of goat carcasses. <sup>a–f</sup> Means with unlike letters are different ( $p < 0.05$ ; s.e. = 0.56). BP, bacteriophages; CN, control (nonrinsed); PAA, peracetic acid.

**TABLE 3** Tests of fixed effects associated with cull dairy goat carcass vascular carcass rinse treatments muscle, and display days on various meat color related dependent variables.

Effect	OMb	DMb	MMb	CIE L*	CIE a*
trt	0.7272	<0.0001	0.0067	0.4866	<0.0001
muscle	<0.0001	0.0062	<0.0001	<0.0001	0.0375
day	<0.0001	0.0207	<0.0001	<0.0001	<0.0001
trt*muscle	0.0851	<0.0001	0.8246	0.6338	0.1966
trt*day	0.8884	0.8246	0.8571	0.9057	0.501
muscle*day	0.0135	0.4588	0.0704	0.9879	0.1133

Note: Independent variables: Control (non-rinsed); RC with bacteriophages (BP); RC with peracetic acid (PAA); muscle; day = 1, 4, 7 days displayed refrigerated (2°C) dark storage.

Abbreviations: CIE a\*, redness; CIE L\*, lightness; DMb, deoxymyoglobin; LD, longissimus dorsi; MMb, metmyoglobin; OMb, oxymyoglobin; SM, semimembranosus.

Relative to muscle and display day, the OMb percentages on days 1, 4, and 7 in the LD were 65.8, 57.1, and 52.8, respectively, and in the SM were 61.2, 53.7, and 51.5, respectively. The LD had more ( $p < 0.05$ ) OMb than the SM, except on day 7, and OMb decreased ( $p < 0.05$ ) on each display day. DMb was greater ( $p < 0.05$ ) on day 7 than on day 1. SM had greater ( $p < 0.05$ ) MMb than LD, and MMb increased with display days (Table 4). LD was lighter (CIE L\*) than the SM, and muscles became darker after day 1 (Table 5). The LD had greater ( $p < 0.05$ ) CIE a\* than SM, and muscles became less red with display days.

## 4 | DISCUSSION

*Salmonella* occurrence in deep tissue LNs of healthy meat animals has a potential public health danger concerning contamination due to LNs being embedded in adipose tissues commonly included in ground beef as a final product (Arthur et al., 2008). Webb et al. (2017) conducted a study for investigating *Salmonella* prevalence in LNs of healthy cattle and they found that 289 out of 5450 (5.3%) samples were positive for *Salmonella*. In another study, the mesen-

teric, subiliac, and mandibular LNs were collected from sheep, and *Salmonella* was detected in at least one LN of 19 out of 119 (16%) animals (Hanlon et al., 2016). Live-stock animals can asymptotically harbor *Salmonella* in their LNs, posing a risk of contamination during meat processing (Garrido et al., 2020). Although LNs function as a filter mechanism for pathogens due to elimination by lymphocytes, certain invasive foodborne pathogens can evade the immune responses and survive within host cells such as macrophages (Richter-Dahlfors et al., 1997). LNs can be incorporated into ground beef during processing and present a major hazard as a potential source of pathogenic foodborne enteric bacteria (Grispoli et al., 2020). For instance, Bosilevac et al. (2009) reported that 4.2% of the 4136 commercial ground beef samples were found *Salmonella* contaminated. It is concluded that *Salmonella* in ground beef primarily originates from contamination of deep tissue LNs, and preharvest interventions have been seen to be effective in safe ground beef production (Li et al., 2015).

Intradermal administration of *Salmonella* using a multilance to healthy goats and after one week incubation period, was successful in achieving a useful microbial

TABLE 4 Effect of vascular rinse antimicrobial treatment on the percentage myoglobin chemical states in goat meat.

	Chill			Muscle			Chill			Muscle		
	Average			LD			Average			LD		
	Omb (%)	1	4	7	1	4	7	1	4	7	1	4
CN	57.28	58.44	55.38	63.28	55.60	51.84	6.89	6.60 <sup>b</sup>	7.18 <sup>ab</sup>	6.65	6.90	7.11
BP	56.91	57.84	55.94	63.12	55.43	52.12	7.38	7.72 <sup>a</sup>	7.05 <sup>ab</sup>	6.69	7.37	8.08
PAA	56.89	59.47	55.09	64.06	55.24	52.55	5.18	3.94 <sup>c</sup>	6.42 <sup>b</sup>	4.51	5.41	5.62
s.e.	0.82	0.91	0.99	0.99	0.99	0.46	0.46	0.52	0.58	0.58	0.64	0.87
Overall	58.58	55.47	63.49	55.42	52.17			6.08	6.88	5.95 <sup>b</sup>	6.56 <sup>ab</sup>	6.94 <sup>a</sup>
s.e.	0.79	0.82						0.43	0.46			0.65

Note: Means with unlike letters within a chemical state of myoglobin and muscle or day are different ( $p < 0.05$ ). Chill average means with unlike letters are different ( $p < 0.05$ ). Overall means within a chemical state of myoglobin and muscle or day with unlike letters are different ( $p < 0.05$ ).

Abbreviations: BP, bacteriophages; CN, control (nonrinsed); DMB, deoxymyoglobin; LD, longissimus dorsi; MMb, metmyoglobin; Omb, oxymyoglobin; PAA, peracetic acid; s.e., standard error; SM, semimembranosus.

TABLE 5 Effect of vascular rinsing and antimicrobial application on CIE L\* and CIE a\* in goat meat.

	Chill			Muscle			Chill			Muscle		
	Average			LD			Average			LD		
	CIE L*	1	4	7	1	4	CIE a*	1	4	7	1	4
CN	41.26	42.36	40.17	42.28	41.10	40.40	12.07 <sup>b</sup>	12.46	11.68	13.99	11.69	10.53
BP	41.21	42.40	40.02	42.36	40.95	40.33	12.68 <sup>a</sup>	13.04	12.32	14.26	12.30	11.48
PAA	40.85	42.29	39.40	41.52	40.68	40.35	11.38 <sup>c</sup>	11.33	11.44	12.67	11.04	10.44
s.e.	0.27	0.38	0.47	0.47	0.47	0.27	0.27	0.33	0.38	0.38	0.38	0.38
Overall	42.35 <sup>a</sup>	39.86 <sup>b</sup>	42.05 <sup>a</sup>	40.91 <sup>b</sup>	40.36 <sup>b</sup>			12.28 <sup>a</sup>	11.81 <sup>b</sup>	13.64 <sup>a</sup>	11.67 <sup>b</sup>	10.82 <sup>c</sup>
s.e.	0.22		0.27					0.24				0.27

Note: Means with unlike letters within an instrumental color dependent variable and muscle or day are different ( $p < 0.05$ ). Chill average means with unlike letters are different ( $p < 0.05$ ). Overall means within an instrumental color dependent variable and muscle or day with unlike letters are different ( $p < 0.05$ ).

Abbreviations: BP, bacteriophages; CIE a\*, redness; CIE L\*, lightness; CN, control (nonrinsed); LD, longissimus dorsi; PAA, peracetic acid; s.e., standard error; SM, semimembranosus.



load of *Salmonella* in the LNs ( $3.77 \pm 0.12$  log CFU/g) to assess antimicrobial effects. Edrington et al. (2016) introduced *Salmonella* Montevideo via intradermal inoculation using the same administration location and LN samples were collected multiple times (6 h, 1, 2, 4, 7, 9, 11, 14, and 21 days) postinoculation to observe bacterial colonization within LNs. Across these incubation periods, *Salmonella* counts in the subiliac, popliteal, and SC (prescapular) LNs consistently ranged from 0.8 to 1.8 log CFU/g. These relatively low levels highlight the capacity of *Salmonella* for persistent presence over three weeks. The observed *Salmonella* levels align with findings from comparable studies, underscoring the importance of evaluating progressive colonization of lymphatic tissue, as these reservoirs contribute to the spread of *Salmonella* within host systems. In another study, Holstein steers were intradermally inoculated with *Salmonella* Montevideo using the same device (ComforTen) on days 1, 3, and 5. A phage cocktail was subsequently administered subcutaneously on trial days 10, 12, and 14. All steers were euthanized on day 15, and the results indicated no significant difference ( $p > 0.10$ ) in *Salmonella* prevalence between the control and phage treatments (Wottlin et al., 2022). In a recent study, the animal infection model and LN sampling method were validated to determine which animal species would exhibit *Salmonella* Enteritidis colonization in LNs. *Salmonella*-inoculated sheep and goats ( $n = 5$  each) showed that goat LNs had about 53% higher levels of *Salmonella* than sheep LNs, with more consistent recovery, leading to the selection of goats as an animal model for this study. Similar to this study, the RC system successfully delivered a mean of 7.0 log PFU/g to infected LNs in goats (Hwang et al., 2024). Similarly, bacteriophage delivered with RC did not significantly affect *Salmonella* counts in examined LNs in the goat model.

Lymphatic carriage initiated immune responses significantly depend on the dendritic cells (DCs) that capture antigens in tissue and migrate to local draining LNs (Alvarez et al., 2008). It was shown that DCs are responsible for 15% of bacterial transport and it is estimated that 5% of DCs in LNs originated from the skin (Bonneau et al., 2006; Tomura et al., 2008). Also, antigens travel as free elements in the lymphatic system due to subatmospheric adverse pressure of interstitial space (Moore & Bertram, 2018). The structure of the initial lymphatic vessels provides highly permeable channels that allow quick absorption of extracellular fluid and free access to antigens into the lymphatic system (Randolph et al., 2005). In a study, passive lymphatic delivery of *Salmonella* was visualized using real-time probing (Itano et al., 2003). A pseudo-afferent cervical lymph collection study to analyze the *Salmonella* lymphatic transportation from tissue to LN in a sheep model showed that free *Salmonella* was found

in the fluid of lymphatic tissue after 24 h of *Salmonella* administration (Bonneau et al., 2006).

The administration volume and the number of injected bacteria (CFU/mL) affect the proportion of culturable bacteria in LNs. In our study, a 1-week incubation period was carried out following the *Salmonella* administration. LNs are structurally complex, containing dense fibroreticular networks and microcompartments that could hinder phage diffusion, thereby limiting their direct access to bacterial cells (Dabrowska, 2019). Although the 7-day incubation was successful in establishing a significant load of *Salmonella* in the LNs, it may be that this period was such that the immune response enabled a majority of the *Salmonella* to be phagocytized by macrophages, which thereby physically blocked the ability of the bacteriophage to achieve pathogen elimination. As a facultative intracellular pathogen, *Salmonella* had successfully invaded and persisted within host cells, as often observed in systemic infections, the extracellular phage particles would have been ineffective (Yan et al., 2021). Unlike small molecule antimicrobials such as PAA, which can permeate and cause oxidative damage at a broad scale, phages require direct contact with extracellular bacteria to initiate infection. This would explain why PAA had a greater effect in lower *Salmonella* loads in LNs. Further detailed studies are needed to understand why bacteriophages were not effective in reducing *Salmonella* when vascularly delivered.

PAA and the other organic acids have been used in microbial intervention studies unremittingly. Particularly, postharvest carcass washing enhanced with organic acids has acclaimed beneficial impacts on reducing the fecal contamination of meat. The applications of 1000 ppm concentrations of PAA spray before chilling reduced *Salmonella* Typhimurium and *E. coli* O157:H7 on inoculated beef carcass surfaces (King et al., 2005). The reduction of total viable counts and coliforms on the surface of carcasses was reported because of spraying lactic acid and PAA before and during chilling of beef carcasses (Han et al., 2021). Similarly in our study, vascular rinsing enhanced with PAA reduced the *Salmonella* load in LNs compared to the nonrinsed carcass. PAA and other oxidative compounds affect microorganisms through multiple mechanisms including peroxidation of membrane layers, enzyme inhibition, oxidation of nucleosides, disrupting energy production, and protein synthesis (Wheeler et al., 2014). Another important advantage of organic acids is they have been found efficacious in large-scale commercial trials and can be implemented cost-effectively by the industry.

Vascular rinsing maintains efficient blood removal, pH decline, and carcass chilling, and its substrates are fully metabolized by the muscle tissue without detectable

residues (phosphorus and glucose) in meat (Hwang et al., 2022). Kethavath et al. (2022) revealed that pH reduction was substantial in RC-processed carcasses compared to control carcasses early postmortem. Compared to control beef steaks, they found that the RC steaks (semitendinosus muscle) had longer sarcomeres, lower shear force, and improvement in some meat color parameters. In another vascular rinsing study, researchers reported positive effects on the color and tenderness of bison meat (Mickelson & Claus, 2020). Fowler et al. (2017) applied vascular rinsing to 30 lamb carcasses and found more than a 50% reduction in shear force of the *M. longissimus thoracis et lumborum* in infused carcasses than CN. Of the studies cited, no evidence of elevated levels of metmyoglobin formation or lipid oxidation were found.

In the current study, the application of vascular rinsing enhanced with PAA and bacteriophage to meat carcasses was investigated for their antimicrobial efficacy and potential to improve food safety. However, it is important to consider its potential impact on meat color, a critical attribute that influences consumer perception and purchasing decisions. RC technology has the potential to affect various instrumental color measurement parameters of meat carcasses. Studies have reported changes in parameters such as myoglobin chemical states, redness, and lightness (Fowler et al., 2017; Hwang et al., 2022). In the study, while RC significantly strengthened ensuring meat safety with the reduction of *Salmonella* loads in LNs of goat carcasses, we also evaluated meat color stability. The Omb and CIE L\* were not influenced by the TRTs and this result suggests that these color characteristics can be considered as stable at the trial application conditions such as BP or PAA supplementation of RC. Omb relates to a desirable bright red pigment. However, PAA resulted in the least DMb in LD but did not affect DMb in SM. PAA did result in the most MMb compared to the other TRTs. Although DMb is a less red pigment than Omb it is more desirable than MMb. MMb is typically associated with brown discoloration in meat, which is generally undesirable from a consumer perspective (Neethling et al., 2017). The difference responses of the TRTs in the different muscles could suggest that variations may be due to specific factors related to muscle type, such as myoglobin levels, oxygen consumption rate, and oxidative rancidity (Poveda-Arteaga et al., 2023). Also, antioxidant protein ( $\beta$ -enolase and creatine kinase) levels in different muscles might be relevant to changes in myoglobin and lipid oxidation (Joseph et al., 2012). Redness (CIE a\*), a key indicator of meat color quality, was affected by BP resulted in a greater redness compared to CN in the LD, which supports the higher DMb content and potentially enhancing the appearance of the meat. On the other hand, the PAA-treated samples exhibited the lowest redness (CIE

a\*), aligning with the increased MMb formation suggesting that PAA may negatively impact the visual appeal of meat due to its stronger oxidizing ability. The differential effects of BP and PAA on meat color characteristics highlight the complexity of interactions between TRTs and muscle types. These findings emphasize the need for careful selection of decontamination or preservation strategies depending on the muscle type and desired meat quality attributes.

## 5 | CONCLUSION

Several studies have explored pre- and postharvest interventions to ensure meat safety and reduce foodborne pathogens. This study aimed to eliminate deep tissue *Salmonella* contamination using vascular rinsing technology. The findings demonstrate that the RC system, enhanced with PAA, effectively reduced *Salmonella* in LNs, highlighting its potential for improving meat safety. However, while bacteriophage delivery through the vascular system did not significantly impact *Salmonella* in LNs, further research is needed to better understand phage kinetics and localization. Overall, incorporating organic acids such as PAA into the RC system offers a promising strategy for enhancing meat safety.

## AUTHOR CONTRIBUTIONS

**Serhat Al:** Writing—original draft; methodology; investigation; formal analysis. **Adam J. Franzen:** Writing—review and editing; investigation; methodology. **Koeun Hwang:** Investigation; methodology; writing—review and editing. **Robert E. Campbell:** Project administration; writing—review and editing; investigation; funding acquisition. **Kathleen Glass:** Methodology; writing—review and editing. **Kurt D. Vogel:** Writing—review and editing; methodology. **James R. Claus:** Methodology; writing—review and editing; conceptualization; formal analysis; funding acquisition; investigation; supervision.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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