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Research Note

Efficacy of Hypobromous Acid as a Hide-On Carcass Antimicrobial Intervention†

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ABSTRACT

Escherichia coli O157:H7 and *Salmonella* on cattle hides at slaughter are the main source of beef carcass contamination by these foodborne pathogens during processing. Hypobromous acid (HOBr) has been approved for various applications in meat processing, but the efficacy of HOBr as a hide antimicrobial has not been determined. In this study, the antimicrobial properties of HOBr were determined by spraying cattle hides at either of two concentrations, 220 or 500 ppm. Treatment of hides with 220 ppm of HOBr reduced the prevalence of *E. coli* O157:H7 on hides from 25.3 to 10.1% ($P < 0.05$) and reduced the prevalence of *Salmonella* from 28.3 to 7.1% ($P < 0.05$). Treatment of hides with 500 ppm of HOBr reduced ($P < 0.05$) the prevalence of *E. coli* O157:H7 on hides from 21.2 to 10.1% and the prevalence of *Salmonella* from 33.3 to 8.1%. The application of 220 ppm of HOBr reduced ($P < 0.05$) aerobic plate counts, total coliform counts, and *E. coli* counts on hides by 2.2 log CFU/100 cm^2. The use of 500 ppm of HOBr resulted in reductions ($P < 0.05$) of aerobic plate counts, total coliform counts, and *E. coli* counts by 3.3, 3.7, and 3.8 log CFU/100 cm^2, respectively, demonstrating that the use of higher concentrations of HOBr on hides resulted in additional antimicrobial activity. These results indicate that the adoption of a HOBr hide wash will reduce hide concentrations of spoilage bacteria and pathogen prevalence, resulting in a lower risk of carcass contamination.

The presence of *Escherichia coli* O157:H7 and *Salmonella* on cattle hides has been recognized as the principle source of carcass contamination at commercial beef processing facilities *(2, 4, 5, 7, 8, 13, 20)*. Hide interventions proven to significantly reduce carcass contamination in processing facilities include chemical dehairing *(20),* cetylpyridinium chloride washing *(8),* and a 65°C sodium hydroxide wash followed by a water rinse *(10)*. Ozonated water, electrolyzed oxidative water, and a minimal hide water wash followed by chlorine spray have also been demonstrated to significantly reduce hide contamination *(3, 11)*. Hide-washing systems have been adopted by several beef processing plants, but space limitations, waste disposal issues, and costs have prevented wider adoption. Recently, the U.S. Food Safety and Inspection Service of the U.S. Department of Agriculture approved the use of hypobromous acid (HOBr) prepared from hydrogen bromide in aqueous solution without a subsequent water rinse in the production of meat and poultry products without a labeling requirement *(24)*.

Bromine-containing compounds have been widely used as disinfectants in water treatment systems. HOBr shares several disinfectant properties with hypochlorous acid, including high reactivity with most biological molecules *(14)*. The use of HOBr in hide wash systems may have advantages over the use of hypochlorous acid since the bromamines formed by the reaction of HOBr with organic compounds are more reactive with biological molecules than the chloramines formed by hypochlorous acid reaction with organic compounds *(14)*. Since the antimicrobial effects of HOBr when applied to cattle hides are unknown, the goals of this experiment were to determine the ability of HOBr to reduce the concentrations of indicator bacteria and the prevalence of *E. coli* O157:H7 and *Salmonella* on cattle hides at a processing plant.

MATERIALS AND METHODS

Experimental protocol. HOBr was prepared using 24% (wt/vol) hydrogen bromide in aqueous solution (HB2 HOBr precursor, EnviroTech Chemical Services Inc., Modesto, CA) according to the manufacturers' instructions. The concentration of HOBr was determined using a colorimeter (Hach Co., Loveland, CO).

Treatment with either 220 ppm ($n = 99$) or 500 ppm ($n = 99$) was evaluated. At a beef processing plant, hides were selected randomly from the processing line. The hides were collected

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immediately after removal from the carcasses and before hide processing steps occurred. To evaluate the treatment of hides with HOBr, whole pulled hides were draped over barrels to simulate hide-on carcasses. Prior to HOBr treatment, a pretreatment sample was obtained from a 500-cm^2 section of hide surface using a sterile sponge (Whirl-Pak, Nasco, Fort Atkinson, WI) prewetted with 20 ml of Dey-Engley neutralizing broth (BD, Franklin Lakes, NJ). A second pretreatment sample was obtained from a separate 500-cm^2 section of hide surface using a sterile sponge prewetted with 20 ml of buffered peptone water (BD) containing 0.002% (wt/vol) calcium carbonate (buffered peptone water plus $CaCO_3$). HOBr was applied to the hide using a sprayer at 45 psi at the source for 15 s, delivering 6 gal per min. The sprayer nozzle was maintained at a distance of 40 cm from the hide surface during spraying. Following a 2-min dwell period, two posttreatment samples were obtained, each from separate 500-cm^2 hide surface sections. One section was sampled with a sterile sponge prewetted with 20 ml of Dey-Engley neutralizing broth, while the other was sampled with a sterile sponge prewetted with 20 ml of buffered peptone water plus $CaCO_3$. Each sponge was placed into a sterile filter barrier bag (Whirl-Pak, Nasco) and massaged to ensure neutralization of samples prior to closing the bag. All samples were transported to the laboratory on ice and processed within 24 h. The sponge samples were homogenized by hand massage, and aliquots of the homogenate were transferred to microfuge tubes for the microbial analyses described below.

Aerobic plate count (APC), total coliform count (TCC), and *E. coli* count (ECC). Sample homogenates from sponges presoaked in buffered peptone water plus $CaCO_3$ were serially diluted, and 1-ml aliquots of the dilutions were plated onto PetriFilm aerobic count plates and PetriFilm *E. coli*/coliform count plates (3M Microbiology, St. Paul, MN). Plates were incubated and colonies counted according to the manufacturers' instructions.

***E. coli* O157:H7 and *Salmonella* enumeration.** *E. coli* O157:H7 and *Salmonella* bacteria were enumerated from hide samples using previously described methods *(12)*. After being vortexed, homogenate samples from sponges presoaked in Dey-Engley neutralizing broth were held for 3 min to allow particulates to settle, and then 50-μl amounts were spiral plated (Spiral Biotech, Norwood, MA) on Chromagar O157 plates (DRG International, Mountainside, NJ) supplemented with 5 mg of novobiocin per liter (Sigma, St. Louis, MO) and 2.5 mg of potassium tellurite per liter (nt-Chromagar; Sigma) and xylose lysine deoxycholate plates supplemented with 4.6 ml of Tergitol per liter, 15 mg of novobiocin per liter, and 5 mg of cefsulodin per liter (XLD_{tnc}; Sigma). nt-Chromagar plates were incubated overnight at 42°C. XLD_{tnc} plates were incubated overnight at 37°C and then at 25°C for 24 h. Suspected *E. coli* O157:H7 colonies were enumerated and screened using latex agglutination tests for the O157 antigen (DrySpot O157, Oxoid, Ltd., Basingstoke, UK), and up to six suspected colonies per sample were confirmed by multiplex PCR *(15)*. Up to six suspected *Salmonella* colonies per XLD_{tnc} plate were confirmed by PCR for the *Salmonella*-specific portion of the *invA* gene *(21, 22)*. The lower limit of detection for enumeration of *E. coli* O157:H7 and *Salmonella* was 80 CFU/100 cm^2.

***E. coli* O157:H7 and *Salmonella* prevalence.** The prevalences of *E. coli* O157:H7 and *Salmonella* in hide samples were determined using previously described methods *(6, 9, 19)*. Following the removal of aliquots for enumeration, the sponge samples were enriched in 80 ml of tryptic soy broth (BD) and incubated at 25°C for 2 h, 42°C for 6 h, and then 4°C overnight. *Salmonella* cells were concentrated by immunomagnetic separation, and the immunomagnetic separation beads were then enriched by incubation in Rappaport-Vassiliadis-soy broth (Oxoid) at 42°C for 18 h. Cultures were then swabbed onto Hektoen enteric medium (BD) supplemented with 5 mg of novobiocin per liter and brilliant green agar supplemented with 80 mg of sulfadiazine per liter (BD). Suspected colonies were isolated and confirmed to be *Salmonella* by PCR *(21, 22)*. *E. coli* O157:H7 was concentrated by immunomagnetic separation, and the immunomagnetic separation beads were plated onto nt-Chromagar. Suspected *E. coli* O157:H7 colonies were screened using latex agglutination tests for the O157 antigen (DrySpot O157, Oxoid) and confirmed by multiplex PCR *(15)*.

Statistical analysis. APC, TCC, and ECC were log transformed, and the geometric means determined. Counts before and after HOBr treatment (220 ppm or 500 ppm) were compared using the two-tailed unpaired *t* test with Welch's correction for unequal variances performed with the Prism 5.0 program (GraphPad Software, La Jolla, CA); comparisons with *P* values of <0.05 were considered significant. Differences in the proportions of prevalence-positive samples and enumerable samples were examined by a two-tailed Fisher exact chi-square test performed with the WINPEPI Compare2 program *(1)*. Comparisons with *P* values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Both concentrations of HOBr tested resulted in significant reductions ($P < 0.05$) of indicator organism concentrations, *E. coli* O157:H7 prevalence, and *Salmonella* prevalence. The geometric means of the indicator organism counts on the 99 samples treated with 220 ppm of HOBr were 8.4 log APC/100 cm^2, 6.5 log TCC/100 cm^2, and 6.4 log ECC/100 cm^2 prior to the HOBr treatment (Table 1). The 220-ppm HOBr treatment reduced ($P < 0.05$) APC, TCC, and ECC counts by 2.2 log. Increasing the concentration of HOBr to 500 ppm resulted in greater reductions ($P < 0.05$) of indicator organism counts. Following the 500-ppm HOBr treatment, APC was reduced 3.3 log, TCC was reduced 3.7 log, and ECC was reduced 3.8 log (Table 1). The use of 500 ppm of HOBr resulted in a log reduction of TCC on hides similar to that observed for 4% chlorofoam and a greater log reduction of TCC than was observed when 4% phosphoric acid, 1.6% sodium hydroxide, or 4% trisodium phosphate was used *(10)*. Treatment of hides with 500 ppm of HOBr resulted in a log reduction of APC that was similar to that observed with electrolyzed water treatment of hides and was greater than that observed with treatment of hides with 60°C water or ozonated water *(11)*.

The prevalence of *E. coli* O157:H7 on hides was reduced at both concentrations of HOBr tested (Table 2). Treatment with 220 ppm of HOBr reduced ($P < 0.05$) the prevalence of *E. coli* O157:H7 on hides from 25.3 to 10.1%. Washing hides with 500 ppm of HOBr reduced ($P < 0.05$) the prevalence of *E. coli* O157:H7 from 21.2 to 10.1%. These reductions in the prevalence of *E. coli* O157:H7 on cattle hides were similar to those observed when cattle hides were treated with cetylpyridinium chloride, 1.6% sodium hydroxide, ozonated water, and electrolyzed water *(8, 10, 11)*. The reduction of *E. coli* O157:H7 concentration on

TABLE 1. *Levels of indicator bacteria pre- and posttreatment with HOBr*

Treatment, sample	No. of samples	Geometric mean log count/100 cm^2 or log reduction as indicated (95% CI)[a]		
		Aerobic plate count	Total coliform count	*E. coli* count
220 ppm of HOBr				
Pretreatment	99	8.4 (8.3–8.5) A	6.5 (6.3–6.7) A	6.4 (6.2–6.5) A
Posttreatment	99	6.2 (5.9–6.5) B	4.3 (4.0–4.6) B	4.1 (3.8–4.4) B
500 ppm of HOBr				
Pretreatment	99	8.6 (8.5–8.7) C	6.5 (6.3–6.6) C	6.3 (6.1–6.5) C
Posttreatment	99	5.3 (5.1–5.5) D	2.7 (2.5–3.0) D	2.5 (2.2–2.8) D
Log reduction				
220 ppm of HOBr	99	2.2 (2.0–2.4) E	2.2 (2.0–2.4) E	2.2 (2.0–2.5) E
500 ppm of HOBr	99	3.3 (3.3–3.5) F	3.7 (3.5–4.0) F	3.8 (3.6–4.1) F

[a] Within the same column, means from each treatment that do not have a common letter are significantly different ($P < 0.05$).

hides has been demonstrated to be an effective means of reducing the contamination of carcasses *(3, 10, 20)*. *E. coli* O157:H7 was not enumerable from any of the 396 samples obtained during this study, so we could not determine whether HOBr treatment was effective in reducing the concentration of *E. coli* O157:H7 on hides. However, our laboratory recently demonstrated that spray treatment of inoculated beef carcass surfaces and beef hearts with the antimicrobial 1,3-dibromo-5,5-dimethylhydantion, which hydrolyzes to its active HOBr form in aqueous solution *(23)*, was effective in reducing the levels of *E. coli* O157:H7 by 1.6 to 2.1 log CFU/100 cm^2 *(16)*. Since we were able to demonstrate that HOBr reduced *E. coli* concentrations (ECC/100 cm^2) by 2.2 to 3.8 log (Table 1), it is reasonable to assume that enumerable *E. coli* O157:H7 concentrations, when present on hides, would be reduced by a similar level *(4, 18)*.

TABLE 2. *Prevalence and enumeration of* E. coli *O157:H7 and* Salmonella *pre- and post-HOBr treatment*

Organism, treatment, sample	No. of samples	% prevalence[a]	No. of samples enumerated		
			80–99 CFU/100 cm^2	100–999 CFU/100 cm^2	>999 CFU/100 cm^2
E. coli O157:H7					
220 ppm of HOBr					
Pretreatment	99	25.3 A	0	0	0
Posttreatment	99	10.1 B	0	0	0
500 ppm of HOBr					
Pretreatment	99	21.2 C	0	0	0
Posttreatment	99	10.1 D	0	0	0
Salmonella					
220 ppm of HOBr					
Pretreatment	99	28.3 E	11	8	0
Posttreatment	99	7.1 F	4	0	0
500 ppm of HOBr					
Pretreatment	99	33.3 G	7	3	0
Posttreatment	99	8.1 H	1	6	0

[a] Results from each treatment that do not have a common letter are significantly different ($P < 0.05$).

Treatment of hides with 220 ppm of HOBr reduced ($P < 0.05$) the *Salmonella* prevalence on hides from 28.3 to 7.1% (Table 2). Prior to treatment with 220 ppm of HOBr, 19.2% of hides had enumerable concentrations of *Salmonella* that ranged from 80 to 400 CFU/100 cm^2. Following the 220-ppm HOBr treatment, only 4.0% of hides had enumerable concentrations of *Salmonella* and the concentration for all enumerated hides was 80 CFU/100 cm^2, the lower detection limit of enumeration (Table 2). The use of 500 ppm of HOBr reduced ($P < 0.05$) the prevalence of *Salmonella* on hides from 33.3 to 8.1%. Prior to treatment with 500 ppm of HOBr, *Salmonella* bacteria were enumerated from 10.1% of hides with concentrations ranging from 80 to 320 CFU/100 cm^2. Following the 500-ppm HOBr treatment, *Salmonella* bacteria were enumerated from 7.1% of hides with concentrations ranging from 80 to 880 CFU/100 cm^2 (Table 2). The presence of higher *Salmonella* concentrations on some of the 500-ppm-treated hides is most likely a product of unequal distribution of pathogens on hides, which our laboratory has demonstrated occurs *(17)*.

In summary, spraying HOBr on cattle hides significantly reduced the prevalences of *E. coli* O157:H7 and *Salmonella* and indicator organism concentrations (APC, TCC, and ECC) (Tables 1 and 2). The results of this study suggest that HOBr treatment of cattle hides is as effective or more effective as a hide intervention than treatment with hot water, chlorofoam, phosphoric acid, sodium hydroxide, trisodium phosphate, ozonated water, or electrolyzed water. Thus, the adoption of a HOBr hide wash by cattle processors should be effective in reducing carcass contamination. Additionally, we suggest that processers currently using HOBr in carcass washes could improve food safety with a minimal increase in cost by reusing the HOBr carcass wash to treat hides.

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