Journal of Food Protection, Vol. 67, No. 2, 2004, Pages 303–309

Protocol for Evaluating the Efficacy of Cetylpyridinium Chloride as a Beef Hide Intervention†

JOSEPH M. BOSILEVAC,[1]* TOMMY L. WHEELER,[1] MILDRED RIVERA-BETANCOURT,[1] XIANGWU NOU,[1] TERRANCE M. ARTHUR,[1] STEVEN D. SHACKELFORD,[1] MATTHEW P. KENT,[1] DIVYA JARONI,[1]‡ MATTHEW S. OSBORN,[2] MICHELLE ROSSMAN,[3] JAMES O. REAGAN,[3] AND MOHAMMAD KOOHMARAIE[1]

[1]*U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166;* [2]*Excel Corporation, 151 North Main Street, Wichita, Kansas 67202; and* [3]*National Cattlemen's Beef Association, 9110 East Nichols Avenue, Centennial, Colorado 80112, USA*

MS 03-204: Received 12 May 2003/Accepted 29 August 2003

ABSTRACT

The objective of this study was to establish the necessary protocols and assess the efficacy of cetylpyridinium chloride (CPC) as an antimicrobial intervention on beef cattle hides. Experiments using CPC were conducted to determine (i) the methods of neutralization needed to obtain valid efficacy measurements, (ii) the effect of concentration and dwell time after treatment, (iii) the effect of CPC on hide and carcass microbial populations when cattle were treated at a feedlot and then transported to a processing facility for harvest, and (iv) the effectiveness of spray pressure and two-spray combinations of CPC and water to reduce hide microbial populations. Residual CPC in hide sponge samples prevented bacterial growth. Dey-Engley neutralization media at 7.8% and a centrifugation step were necessary to overcome this problem. All dwell times, ranging from 30 s to 4 h, after 1% CPC application to cattle hides resulted in aerobic plate counts and *Enterobacteriaceae* counts 1.5 log CFU/100 cm^2 lower than controls. The most effective dose of CPC was 1%, which reduced aerobic plate counts and *Enterobacteriaceae* counts 2 and 1 log CFU/100 cm^2, respectively. Low-pressure application of 1% CPC at the feedlot, transport to the processing facility, and harvest within 5 h of application resulted in no effect on *Escherichia coli* O157 prevalence on hides or preevisceration carcasses. Two high-pressure CPC washes lowered aerobic plate counts and *Enterobacteriaceae* counts by 4 log CFU/100 cm^2, and two medium-pressure CPC washes were only slightly less effective. These results indicate that under the proper conditions, CPC may still be effective for reducing microbial populations on cattle hides. Further study is warranted to determine if this effect will result in reduction of hide-to-carcass contamination during processing.

Beef carcass contamination during processing has been shown to occur principally from hides *(5, 6, 14).* During the hide removal process, pathogens such as *Escherichia coli* O157:H7 and *Salmonella* are transferred from the hide, where they are high in prevalence, to the carcass *(5, 6, 14).* Numerous carcass interventions have been implemented by beef processing plants to remove carcass microbial contamination *(3, 15, 27).* However, occasional process failures occur that result in greater contamination than can be effectively removed with current carcass interventions. Processes that effectively clean the hides before hide removal may be effective interventions for preventing carcass microbial contamination. For instance, in an evaluation of chemical dehairing, the prevalence of *E. coli* O157:H7 on the carcass was almost eliminated if bacterial contamination of the hide was greatly reduced before removal *(21).*

Cetylpyridinium chloride (CPC), a common oral antimicrobial compound *(22),* may have potential for use in a hide intervention process. In a series of published studies, Kim and Slavik *(18),* Xiong et al. *(30),* and Yang et al. *(31)* demonstrated that CPC is efficacious for reducing populations of *Salmonella* on poultry carcasses. Cutter et al. *(11)* demonstrated the effectiveness of CPC for reducing microbial counts on beef carcasses, and Pohlman et al. *(23)* recently demonstrated the effectiveness of CPC for reducing *E. coli,* coliforms, and aerobic bacteria in ground beef when applied to beef trimmings before grinding. Before we could test CPC as a hide intervention, however, there were several protocol issues that needed to be addressed. For example, the residual CPC remaining in the sponge after sampling had to be neutralized to prevent artifactually low microbial counts. The optimal concentration of CPC had not been determined, and the most effective approach for CPC application to hides had not been studied. Thus, the objective of this study was to determine the necessary experimental protocols and application parameters to adequately assess the efficacy of CPC as a beef carcass hide intervention.

MATERIALS AND METHODS

Experiment 1. Effect of dwell time on CPC efficacy on cattle hides. Dwell time was tested using a complete block design with five treatments. Ten cattle were used, and five rectangular (16 by 31 cm) areas were marked on both sides of each animal. Each rectangle covered an approximately 500-cm^2 area. Dwell time was blocked by location (the same block on both sides of

* Author for correspondence. Tel: 402-762-4225; Fax: 402-762-4149; E-mail: bosilevac@email.marc.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

‡ Present address: Room 119, Veterinary Diagnostic Center, East Campus, University of Nebraska, Lincoln, NE 68583-0907, USA.

the same animal), and each dwell time was represented in each location four times for a total of 20 samples per dwell time. Treatments included control and 30-s, 30-min, 2-h, and 4-h dwell times. Control locations were sprayed briefly with low-pressure (LP) water from a hand sprayer so that all sampling locations were equally wet. All CPC locations were completely soaked using an LP hand-pump sprayer with 1% CPC for approximately 45 to 60 s. After spraying and sampling, cattle were penned individually until the next sampling time.

Experiment 2. Effect of CPC concentration and dwell time on bacteria levels on cattle hides. The effect of CPC concentration was determined in a 3 (CPC concentration) × 3 (dwell time) factorial arrangement of an incomplete block design. Thirty cattle were used and, to control for variability between animals and sites of sampling, three 500-cm^2 rectangles (16 by 31 cm) were marked on the right and left side of each animal. The CPC concentrations examined were 0.5, 1, and 3%, and each concentration was randomly assigned to a group of five cattle. Dwell times were control, 30 s, and 4 h after CPC treatment and were blocked by location on the right and left side of each animal. Spray treatments were applied as described for experiment 3, except that after spraying and sampling, cattle were penned in groups of three or four until the next sampling time. Each concentration × dwell time combination was replicated 20 times, and aerobic plate counts (APC) and *Enterobacteriaceae* counts (EBC) were obtained. Dey-Engley (DE) concentrations were varied for each CPC concentration to ensure effective neutralization: 1× DE for 0.5% CPC, 2× DE for 1% CPC, and 5× DE for 3% CPC.

Experiment 3. Effects of CPC application at the feedlot on hide and carcass levels of APC, EBC, and *E. coli* O157 prevalence at the processing plant. Eighty-six market-ready cattle were divided into two groups of 43. One group was loaded into a clean cattle truck and transported to the processing plant and sampled as controls. The other group was treated with 1% CPC applied with a modified hydraulic squeeze chute fitted with spray nozzles on the top and each side. Each animal was sprayed with 1% CPC at LP for 30 s. A hand-wand spray attachment was used to ensure the brisket, belly, crotch, and anal-hock areas were well soaked. The head and neck were not sprayed. Treatment of the 43 cattle required approximately 2 h. The treated cattle were loaded into a clean cattle truck and transported to the processing plant. At the processing plant, control cattle were processed and sampled first; then, treated cattle were processed and sampled. The treated cattle were sampled approximately 5 h after CPC treatment. During hide sampling, samples were processed immediately after collection. It took 8 min to collect the 43 samples and an additional 2 min to transport the samples to a mobile processing laboratory. Aliquots (1.5 ml) were taken from the sample bags for centrifugal removal of residual CPC. After centrifugation (8 min, 8,000 × *g*), the samples were resuspended and serially diluted in buffered peptone water (BPW). The samples were plated for APC and EBC within 20 to 30 min from the time of collection. Sample bags were enriched with 75 ml of tryptic soy broth (TSB) immediately after the centrifugation aliquot for APC and EBC was taken. TSB-enriched sample bags were then transported at 25°C back to the U.S. Meat Animal Research Center laboratory for incubation. *E. coli* O157 prevalence was determined by immunomagnetic separation of the enrichments the following day as described below.

Experiment 4. Evaluation of CPC application parameters on beef hides. At a beef processing plant, pulled hides were draped over barrels to simulate hide-on carcasses in order to evaluate CPC application parameters. LP water was sprayed briefly (3 s) on control sample sites to prevent controls from underrepresenting counts, because it has been shown that sponges do not pick up the same level of bacteria from dry versus wet hides *(24)*. After control samples were obtained, the appropriate treatment was applied to the hide. Nine treatments were evaluated: (i) control, (ii) high-pressure (HP) water/HP water, (iii) HP water/LP CPC, (iv) HP water/HP CPC, (v) medium-pressure (MP) water/MP CPC, (vi) LP CPC/LP CPC, (vii) MP CPC/MP CPC, (viii) HP CPC/LP CPC, and (ix) HP CPC/HP CPC. Each spray treatment lasted 20 s and consisted of four passes across the hide end to end with a 20-s delay between the two sprays. Samples were obtained after a 30-s dwell period following the second treatment for treatments including CPC in the prewash and 70 s after the second spray for treatments with CPC only in the second wash (to keep total dwell time following CPC exposure the same for all treatments). Each treatment was applied to one side of two hides. Each side of each hide was sampled once in the center for control and four times on either side of the control sample site for a total of eight samples per side and 16 samples per treatment. Spray 1 was considered a prewash, and spray 2 was considered a treatment. The hand-pump sprayer that was used in experiments 3 through 5 provided the LP application. HP and MP applications were made with a Karcher (Duluth, Ga.) pressure sprayer, set at either full (1,200 lb in^{-2}) for HP or minimum (500 lb in^{-2}) for MP. The distance between the nozzle and the hide was kept at 65 cm during HP and MP sprayings. Municipal water was used, or the sprayer was set to dispense 1% CPC from 40% stock. The sprayer heated the water to 60°C before spraying. APC and EBC data were collected on all samples.

Evaluation of neutralization media. Neutralization media were compared for ability to recover aerobic bacteria and *E. coli* O157:H7 in the presence of CPC. Neutralization of CPC was tested with a 4 (neutralization media) × 4 (CPC concentration) × 2 (media/CPC ratio) factorial arrangement of a completely randomized design. Neutralization media included BPW (Difco Laboratories, Detroit, Mich.), BPW containing 5 g/liter of sorbitol and 7 g/liter of lecithin (BPW+SL), and DE neutralization broth (Difco) at 1× (39 g/liter) and 2× (78 g/liter) concentrations. CPC concentrations included 0, 0.01, 0.1, and 1%. CPC was supplied by Safe Foods Corporation (North Little Rock, Ark.) as a 40% stock that was diluted with sterile distilled water. The neutralizing media were added at ratios of 1:1 and 1:10 to the CPC solutions 10 min prior to the addition of approximately 1×10^3 CFU of *E. coli* O157:H7 (ATCC 43895) or 1×10^3 CFU aerobic bacteria taken from an enriched cattle hide sponge sample that had been incubated at 37°C for 16 h. Each neutralized sample was plated for enumeration on 3M Microbiology (St. Paul, Minn.) Aerobic Count Plate (AC) Petrifilm.

The optimal removal of residual CPC was determined using various sample processing protocols that incorporated neutralization, centrifugation and resuspension, and serial dilutions in BPW or 1× DE. The source of bacteria again was an enriched cattle hide sponge sample. APC and EBC were performed on all samples using 3M Petrifilm according to the directions of the manufacturer (see below).

Sampling of hides and carcasses. All hide samples were collected using Speci-Sponge Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) containing 25 ml of 2× DE, unless otherwise stated (BPW or 1× DE used). Samples were collected from 500-cm^2 areas (experiments 1, 2, and 3) or 100-cm^2 areas (experiment 4) using a template on hides with 10 bidirectional strokes of sponge, turned over halfway through the process. Carcass samples were

collected from an area of 8,000 cm^2 along the anal-hock, brisket, and plate using Speci-Sponge bags containing 25 ml of BPW. Carcass samples were collected similarly to hide samples using bidirectional strokes and turning the sponge halfway through the process. Fresh latex gloves were used for each sample.

APC and EBC determinations. All sample bags were thoroughly mixed by hand massaging that consisted of firmly squeezing the sample bag and sponge a minimum of five times or until a uniform suspension was visible in the bag. Aliquots (1.5 ml) were then taken for serial 10-fold dilutions. One milliliter of each subsequent serial dilution was plated to AC Petrifilm and 3M *Enterobacteriaceae* (EB) Petrifilm. Petrifilms were incubated according to the specifications of the manufacturer and were counted manually.

***E. coli* O157 isolation.** Isolation of *E. coli* O157 consisted of enrichment, immunomagnetic separation, and plating as described previously *(7)*. The plates were incubated at 37°C for 16 h, and suspect colonies were confirmed to be *E. coli* O157 using Oxoid (Ogdensburg, N.Y.) DrySpot latex agglutination tests. The suspect colonies that tested positive with latex agglutination were considered positive *E. coli* O157:H7, as more than 90% of similar isolates had been confirmed to be *E. coli* O157:H7 in earlier studies *(5)*.

CPC concentration determination. The concentration of CPC was determined before and during applications. Aliquots (50 ml) were collected from the sprayer nozzle. A 1:100 dilution was prepared in warm (37°C) distilled water to facilitate a thorough dilution of the CPC sample. The absorbance at 260 nm was measured with a spectrophotometer using water as a blank. The absorbance at 260 nm of a 1:100 dilution was linear in the range of 0.100 to 1.500, and a 1% solution of CPC gave an absorbance of approximately 1.000. Optical density values at 260 nm between 0.850 and 1.150 were used to establish the concentration of CPC.

Statistical analyses. Data were analyzed by analysis of variance using the General Linear Models procedures of SAS (SAS Institute, Inc., Cary, N.C.). For significant ($P < 0.05$) main effects and interactions, least-squares means separation was accomplished by the PDIFF option (a pairwise *t* test). In experiment 1, dwell time was tested with a repeated-measures design. The model included the main effect of dwell time (control, 30 s, 30 min, 2 h, and 4 h). In experiment 2, a 3 (CPC concentration) × 3 (dwell time) factorial arrangement of a repeated-measures design was used. The model included the main effects of CPC concentration (0.5, 1, and 3%) and dwell time (0, 30 s, and 4 h). In experiment 3, a completely randomized design was used to test CPC treatment. The model included the main effect of CPC treatment (control and treated). Pairwise comparisons of frequencies were made using PROC FREQ and Mantel-Haenszel chi-square analysis (SAS). In experiment 4, a completely randomized design for the main effect of treatment was used to evaluate nine treatments. The model included the main effect of treatment (various combinations of CPC or water and spray pressure).

RESULTS

Validation of procedure to neutralize residual CPC. Two measures of CPC efficacy as a hide intervention are APC and EBC. Our protocol called for BPW-wetted sponges to sample hides that were treated with 1% CPC. However, preliminary measurements of APC and EBC on CPC-treated hides showed a very high efficacy of CPC (a 5-log reduction in bacterial counts). Volume measurements of sample sponges and bags determined that sponges absorb up to 3 ml of liquid CPC from hides during sampling. This is about a 1:10 dilution of the CPC in the 25-ml sample bag. It was hypothesized that this residual CPC in the sample was not properly neutralized and that it prevented viable cells in the sample from growing in the subsequent APC and EBC assays *(29)*. Therefore, neutralization studies were conducted to determine the optimal neutralization media for CPC concentrations ranging from 0.01 to 1%. We compared the neutralization efficacies of DE broth to BPW and to BPW+SL that act to inhibit quaternary ammonium compounds such as CPC *(16)*. The results (data not shown) demonstrated that BPW alone was unable to neutralize the CPC absorbed by the sponge during sampling; thus, artifactually high CPC efficacy would be implied from the use of BPW as the sponge-wetting media. The media containing neutralizing compounds all provided improved recovery, with 2× DE providing the best results. Use of 2× DE allowed the recovery of organisms in the presence of CPC at concentrations of up to 1% when used at a neutralization ratio of 1:10, whereas other neutralizing media (1× DE and BPW+SL) did not. While neutralization using 2× DE appeared to remove the effects caused by residual CPC on APC, EBC, and *E. coli* O157 isolation, the addition of a centrifugation/resuspension step to our sample processing protocol increased the reliability and reproducibility of the data. The effect of neutralization, centrifugation, and resuspension buffer on the removal of residual CPC in hide samples demonstrated a modest improvement in recovery (data not shown). Also, we observed that the standard error of the mean was decreased in centrifuged samples, indicating that results would be more reproducible when following this procedure.

During the above neutralization studies, we observed a greater level of *E. coli* O157:H7 recovery than APC, suggesting a potential difference in CPC sensitivity among bacterial species. To determine if this was the case, similar neutralizations were performed using gram-positive and gram-negative pools of bacteria. No differences in colony counts between the gram-positive and gram-negative pools were detected (data not shown), suggesting that the sensitivity to CPC among gram-positive and gram-negative bacterial species as we examined them is uniform and that, therefore, the APC values observed could be considered representative of those that remain after CPC treatment.

DE media contains thiosulfate, thioglycolate, and bisulfate *(12)*. The effects of these compounds as well as CPC were evaluated to rule out potential interference with *E. coli* O157 isolation by immunomagnetic separation. An experiment was performed in which it was observed that samples must be neutralized within the first 5 min after CPC exposure if *E. coli* O157 is to be isolated by immunomagnetic separation and that a 1-min interval before neutralization should be considered the maximum. Inocula of *E. coli* O157:H7 as low as 5 CFU per sample could be isolated from CPC-treated hides if properly sampled with sponges wetted with 2× DE. Therefore, the DE media and CPC did not interfere with the isolation protocols for *E. coli* O157.

TABLE 1. *Effect of dwell time after 1% cetylpyridinium chloride (CPC) treatment on bacteria levels on feedlot cattle hides*[a]

	0	30 s	30 min	2 h	4 h
APC[b]	6.5 A[c]	4.8 B	5.3 B	5.2 B	5.2 B
EBC[d]	2.8 A	1.0 C	1.9 AC	2.4 AB	1.1 BC

[a] All values were determined using 3M Petrifilm and are mean log CFU/100 cm^2 of 8 to 10 replicates. The standard error of the mean was 0.2 for APC and either 0.4 or 0.5 for EBC.
[b] Aerobic plate counts.
[c] Means within a bacteria type with a common letter are not different ($P > 0.05$).
[d] *Enterobacteriaceae* counts.

Experiment 1. Effect of dwell time after CPC treatment on bacteria levels on cattle hides. Having an effective sample processing protocol, we then examined the efficacy of CPC treatment on reducing the bacterial counts on feedlot cattle hides. Because it was not yet clear at what stage in processing a hide intervention might be applied, in this experiment, we tested the effectiveness of 1% CPC after various dwell times up to 4 h. The APC levels were reduced by 1.5 to 2 log CFU/100 cm^2 after CPC treatment, regardless of dwell time (Table 1). With a 30-s dwell time after treatment, APC levels were reduced by nearly 2 log CFU/100 cm^2, with a 1.5-log CFU/100 cm^2 reduction detected after 4 h. The levels of EBC were reduced by CPC treatment after 30-s and 4-h dwell times. A dwell time of 2 h resulted in an EBC that was not lower than that at 4 h or 30 min. It is not clear why EBC levels were inconsistent across dwell times, but it could have been because of sampling location variation. We concluded that the antibacterial effect of 1% CPC is immediate (30 s) and is maintained for at least 4 h.

Experiment 2. Effect of CPC concentration and dwell time on bacteria levels on hides. In the previous experiment, we used 1% CPC because Cutter et al. *(11)* had shown this concentration reduced bacteria counts on beef carcass surfaces. However, CPC has been shown effective in different settings at concentrations as low as 0.1% *(30)*. Our dwell time studies showed only a 1.5- to 2-log CFU/100 cm^2 reduction of APC values using 1% CPC. Thus, perhaps a higher concentration of CPC would be needed to be effective with the high load of organic matter on cattle hides. Therefore, we titrated CPC concentrations to determine the lowest concentration that would be effective on hides.

All CPC concentrations reduced APC levels relative to controls, regardless of dwell time (Table 2). CPC at 1 and 3% reduced APC levels more effectively than CPC at a concentration of 0.5%. The 4-h dwell time reduced APC relative to the 30-s dwell time, regardless of CPC concentration. All CPC concentrations reduced EBC levels relative to controls after a 4-h dwell time. Within the same dwell time, 3% CPC greatly reduced EBC when compared to 0.5 or 1% CPC. Closer analysis showed that collecting samples from hides treated with 3% CPC was problematic for determining EBC. DE at a 5× concentration was needed to neutralize 3% CPC, and DE at that concentration alone decreased the growth of EBC controls by 20% (data not shown). Therefore, we concluded that 1% CPC was the most effective concentration that would reduce APC and EBC while allowing reliable measurements to be made.

TABLE 2. *The effect of cetylpyridinium chloride (CPC) concentration and dwell time on bacteria levels on feedlot cattle hides*[a]

		Bacteria level at CPC concentration		
	Time[b]	0.5%	1%	3%
APC[c]	0	6.8 AB[d]	7.0 A	6.6 B
	30 s	5.6 C	5.2 D	5.2 DE
	4 h	4.9 EF	4.6 G	4.7 FG
EBC[e]	0	4.7 A	5.2 A	3.8 AB
	30 s	3.9 B	4.0 A	2.4 B
	4 h	2.5 D	2.0 B	0.4 C

[a] Values were determined using 3M petrifilm and are mean log CFU/100 cm^2 of 18 to 20 observations. The standard error of the mean was 0.1 for APC and either 0.2 or 0.9 for EBC.
[b] Samples were collected at 30 s and 4 h after application of CPC at time 0.
[c] Aerobic plate counts.
[d] Means within a bacteria type with a common letter are not different ($P > 0.05$).
[e] *Enterobacteriaceae* counts.

Experiment 3. Effect of 1% CPC treatment of cattle at the feedlot on bacterial load of hides and preevisceration carcasses (before processing interventions) at a commercial processing plant. Because CPC had not been approved for application in a federally inspected beef processing plant, the most feasible way to test the potential efficacy of CPC as a hide intervention was to treat the cattle hides at the feedlot immediately before transporting the cattle to the processing plant. The time between the CPC treatment and the harvesting at the processing plant was expected to be 3 to 4 h, which was consistent with earlier results indicating a similar or greater effect of CPC after 4 h. In this experiment, a decrease ($P < 0.05$) in hide APC and EBC was detected, but the reduction was only about 0.5 log CFU/100 cm^2 (Table 3). However, the prevalence of *E. coli* O157 on the hides was not affected by CPC treatment. Results from the preevisceration carcasses were similar, such that APC levels were significantly, but negligibly, decreased, and *E. coli* O157 prevalence was not different between control and treated cattle. We concluded from this experiment that the transport of treated cattle from the feedlot to the holding pen at the processing plant allows the introduction of many uncontrollable recontamination events and that the optimal CPC treatment should occur at the processing plant immediately before or after stunning. In addition, these data may indicate that a prewashing step is necessary for CPC treatment to be effective when applied under commercial operating conditions.

Experiment 4. Effect of two-spray treatments varying in spray type and pressure on bacterial counts on cattle hides. On the basis of experiment 4, we hypothesized that a prewashing step as suggested by Byrne et al. *(8)*

TABLE 3. *Effects of 1% cetylpyridinium chloride (CPC) treatment of cattle at feedlot on bacterial load of hides and preevisceration carcasses at the processing plant*

	Hides[a]		Carcasses[b]	
	Control	Treated	Control	Treated
n	43	43	43	43
APC[c]	6.6 A[d]	6.1 B	2.9 A	2.6 B
EBC[c]	5.2 A	4.7 B	1.2 B	1.1 B
Escherichia coli O157[e]	97.7 A	95.3 A	16.3 A	20.9 A

[a] Hides of stunned cattle were sampled before opening.
[b] Carcasses were sampled preevisceration (before processing interventions).
[c] APC (aerobic plate counts) and EBC (*Enterobacteriaceae* counts) values are the mean log CFU/100 cm^2. The standard error of the mean was 0.1 for APC and EBC.
[d] Means within a bacteria and sample type with a common letter are not different ($P > 0.05$).
[e] *E. coli* O157 values are the percent positive by culture.

TABLE 4. *Effects of two-spray treatments varying in spray type and pressure to reduce APC[a] and EBC[b] on cattle hides[c]*

Treatment[d]	APC	EBC
Control	8.2 A[e]	6.0 A
HP water/LP CPC	5.2 C	2.9 CD
HP water/HP CPC	4.9 C	3.2 C
MP water/MP CPC	4.9 C	3.0 C
LP CPC/LP CPC	6.3 B	4.7 B
MP CPC/MP CPC	4.7 CD	2.8 CD
HP CPC/LP CPC	4.3 DE	3.0 C
HP CPC/HP CPC	3.8 E	2.2 D

[a] Aerobic plate counts.
[b] *Enterobacteriaceae* counts.
[c] Samples were taken immediately (30 s) after spraying and processed. Values are the mean of the log CFU/100 cm^2 for 15 to 16 replications. The standard error of the mean was 0.2 for APC and 0.3 for EBC.
[d] HP, high pressure (~1,200 lb in^{-2}); MP, medium pressure (~500 lb in^{-2}); LP, low pressure (<50 lb in^{-2}); CPC, cetylpyridinium chloride.
[e] Means within a bacteria type with a common letter are not different ($P > 0.05$).

would decrease organic matter and bacterial levels and enhance CPC effectiveness. Two HP water washes, while resulting in remarkably cleaner hides visually, did not reduce APC and EBC relative to controls (data not shown). Two LP CPC washes reduced APC and EBC, but this treatment was the least effective of all those that included CPC (Table 4). When the first wash was water and the second wash was CPC, regardless of pressure, APC and EBC were reduced more than for the double water wash and were not different from the two MP CPC washes. The greatest reduction in APC resulted from a first wash of HP CPC that was followed by an LP or an HP CPC. The most effective treatment for reducing EBC was the two HP CPC washes. As application pressures for the CPC prewash and treatment were increased, greater efficacy was detected, likely because of the removal of a greater amount of organic matter from the hide. The limited effect of the two LP CPC treatments relative to the higher-pressure CPC treatments appears to confirm our hypothesis that a higher-pressure prewash is needed to remove organic matter and may partially explain the lack of greater effect in experiment 5. These data indicate that the greatest efficacy from the 1% CPC treatment would come from an HP CPC prewash followed by a second HP CPC wash on the stunned, shackled hide-on animal where HP would not be a problem. However, for treatment of live cattle immediately before stunning, we conclude that the MP CPC prewash and MP CPC second wash should be used because of concerns regarding the humane treatment of the animals.

DISCUSSION

The described experiments were intended as a prelude to a field study of CPC use as a hide intervention. Before proceeding with such a field study, a number of questions needed to be addressed with regard to the sampling of CPC-treated hides and the optimal concentration for and dwell time of CPC activity, as well as the optimal application method. These studies were initiated with those goals in mind.

Johnston et al. *(16)* and Kemp and Schneider *(17)* recently discussed the importance of arresting the activity of disinfectants at the moment of sampling in order to accurately assess the level of organisms surviving in the presence of the biocide. We initially observed an overestimation of CPC activity because of the inadequate neutralization of absorbed residual CPC in the sponge sample from the hide. Langsrud and Sundheim *(19)* and Russell et al. *(26)* describe early studies of disinfectant efficacies that were often overestimated because of similar circumstances. The U.S. Pharmacopoeia recommends that, during the evaluation of disinfectants as antimicrobials, the disinfectant under investigation be adequately neutralized to avoid exaggerated measures of microbicidal activity *(1, 29)*. Dey and Engley *(12, 13)* established the DE media formulation and described its use for situations such as this and its advantages for the full neutralization of disinfectant compounds. We chose to use 2× DE as the sampling buffer because of its broad effectiveness and potential use in the future with other chemical treatments. In addition to quaternary ammonium compounds such as CPC, DE media can neutralize phenols, iodines, and aldehydes *(12, 13)*. DE also appeared to provide better growth conditions than BPW, likely because of its richer formulation containing yeast extract. Even with the use of 2× DE, an effect of CPC residue in the 1% CPC samples was still detected. Therefore, a centrifugation and resuspension step was included to effectively remove the residual CPC in samples, ensuring that the most accurate data possible were collected *(1, 29)*. This process maintained sample integrity for APC, EBC, and *E. coli* O157 isolation and reduced the sample-to-sample variation that inevitably occurs during field sampling. One such variable is some samples absorbing more residual CPC than others. This was corrected through neutralization combined with

centrifugation and resuspension; thus, reproducibility among the samples increased.

In experiments 1 and 2, we evaluated the time course of CPC treatment for the effective reduction of bacteria on cattle hides. The dwell times examined represented relevant treatment intervals that would be expected if treatments were to occur at the feedlot prior to transport to a processing plant (2 to 4 h), at the processing plant preslaughter (30 min and 2 h), or during the slaughter process in a spray cabinet after stunning (30 s). Published observations of CPC efficacy have not measured the impact of the dwell time of CPC effects over a period of hours. Kim and Slavik *(18)* treated poultry carcasses for up to 3 min with CPC but did not discuss dwell time effects. In studies of CPC washes of beef surfaces or beef trim, Cutter et al. *(11)* and Pohlman et al. *(23)* monitored the efficacy of CPC for a number of days to weeks in the final vacuum-packed beef products. We, however, treated hides of live animals with the possibility of them becoming recontaminated. We measured immediate reductions in APC and EBC levels on hides and noted that the effect lasted for at least 4 h, even though the treated cattle of our study were released from the treatment chute and penned in small groups.

Effective CPC concentrations on produce and poultry carcasses have been described at 0.1 and 0.5% *(18, 30, 31)*. In the case of beef products, Pohlman et al. *(23)* treated beef trim with 0.5% CPC, and Cutter et al. *(11)* treated beef surfaces with 1% CPC. Because the average bioload on a carcass or on trim is substantially less than on a hide *(21)*, we examined CPC concentrations from 0.5 up to 3%. We found that concentrations of 1% were sufficient for CPC effectiveness and that the use of concentrations greater than 1% presented additional sample processing problems.

We tested the efficacy of a 1% CPC treatment applied to cattle at the feedlot before they were transported to the processing plant. There are a number of potential reasons for the limited effect of CPC in experiment 3. There was a potential for recontamination of the cattle hides while in transport *(4, 9, 10)*, in holding pens at the processing plant *(25, 28)*, and in the stunning box *(2)*. Although it has been described that contamination may likely occur during lairage *(4, 9, 28)*, we hypothesized that the antimicrobial activity of CPC that was shown to last for up to 4 h would still provide a measurable protective effect in our group of treated cattle relative to the control group. Additionally, this was the only option available to determine the effect of CPC treatment, as the compound was not approved for use in a processing plant. The application of CPC in pens directly outside the plant would have also required approval as well as logistical needs that could not be met at the time.

Another potential interference with the effectiveness of CPC treatment in experiment 3 was the level of cleanliness of the cattle of our study. Although CPC is rapidly neutralized by organic matter *(20)*, as are all quaternary ammonium compounds, we assumed that by completely soaking the hide, except for the head and neck, enough CPC would be present to overcome the bioload. However, the hides of the cattle were very dirty, so the levels of organic matter present could have inactivated the CPC. The use of an HP prewash spray that removes as much interfering organic matter as possible was suggested by Byrne et al. *(8)* to reduce *E. coli* O157:H7 transfer from hides to carcasses. In their study, Byrne et al. *(8)* did not observe a statistically significant reduction of *E. coli* O157:H7; however, by following such an HP water wash with an HP CPC treatment, it seems likely the reasons for the limited effectiveness of CPC observed in experiment 3 will be resolved.

CPC has been shown to be an effective antimicrobial and has been used as an intervention strategy in many poultry processes. However, CPC has never been examined for use as a cattle hide intervention. We have developed a protocol that allows us to accurately determine APC and EBC levels and to isolate *E. coli* O157 from hides treated with 1% CPC. Our results indicate that under the proper conditions, CPC treatment can be effective for reducing microbial populations on cattle hides. The results establish the parameters needed to develop a protocol to test whether a 1% CPC hide intervention process would reduce microbial contamination of the carcass by the hide during processing.

We assume that the most feasible application of CPC as a cattle hide intervention would be on the stunned, shackled animal in the processing plant. Our results indicate that effective intervention should be possible for that method of application. However, without approval to use CPC in the processing plant, further validation of its application will require testing under conditions that mimic the conditions of in-plant application while actually applying the CPC intervention outside the plant to live animals. Our data also indicate that an experiment could be designed to further test the potential of CPC as a hide intervention by applying the treatment to animals in the holding pens of the processing plant immediately before stunning.

ACKNOWLEDGMENTS

This project was funded in part by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen's Beef Board and state councils by the National Cattlemen's Beef Association. We gratefully acknowledge Safe Foods Corporation for generously supplying cetylpyridinium chloride for these studies. We thank the participating processing plants for their cooperation. We also acknowledge Patrick West, Ed Korth, Don Porter, and John Hill of the Excel beef processing plant for their assistance in the execution of experiments 1, 2, and 4. We thank Patty Beska, Julie Dyer, Bruce Jasch, Dee Kucera, Frank Reno, and Gregory Smith for technical support and Carol Grummert for secretarial assistance.

REFERENCES

1. Anonymous. 2002. Disinfectants and antiseptics (USP General Chapter 1072). *Pharmaocopoeial Forum* 28:143–152.
2. Avery, S. M., A. Small, C. A. Reid, and S. Buncic. 2002. Pulsed-field gel electrophoresis characterization of Shiga toxin-producing *Escherichia coli* O157 from hides of cattle at slaughter. *J. Food Prot.* 65:1172–1176.
3. Bacon, R. T., K. E. Belk, J. N. Sofos, R. P. Clayton, J. O. Reagan, and G. C. Smith. 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *J. Food Prot.* 63:1080–1086.
4. Barham, A. R., B. L. Barham, A. K. Johnson, D. M. Allen, J. R. Blanton, Jr., and M. F. Miller. 2002. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence

levels of *Escherichia coli* O157 and *Salmonella* spp. *J. Food Prot.* 65:280–283.

5. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* spp. in commercial beef cattle processing plants. *J. Food Prot.* 66:1978–1986.
6. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the Midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
7. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
8. Byrne, C. M., D. J. Bolton, J. J. Sheridan, D. A. McDowell, and I. S. Blair. 2000. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Lett. Appl. Microbiol.* 30:142–145.
9. Corrier, D. E., C. W. Purdy, and J. R. DeLoach. 1990. Effects of marketing stress on fecal excretion of *Salmonella* spp. in feeder calves. *Am. J. Vet. Res.* 51:866–869.
10. Cray, W. C., T. A. Casey, B. T. Bosworth, and M. A. Rasmussen. 1998. Effect of dietary stress on fecal shedding of *Escherichia coli* O157:H7 in calves. *Appl. Environ. Microbiol.* 64:1975–1979.
11. Cutter, C. N., W. J. Dorsa, A. Handie, S. Rodriguez-Morales, X. Zhou, P. J. Breen, and C. M. Compadre. 2000. Antimicrobial activity of cetylpyridinium chloride washes against pathogenic bacteria on beef surfaces. *J. Food Prot.* 63:593–600.
12. Dey, B. P., and F. B. Engley, Jr. 1994. Neutralization of antimicrobial chemicals by recovery media. *J. Microbiol. Methods* 19:51–58.
13. Dey, B. P., and F. B. Engley, Jr. 1995. Comparison of Dey and Engley (D/E) neutralizing medium to Letheen medium and standard methods medium for recovery of *Staphylococcus aureus* from sanitized surfaces. *J. Ind. Microbiol.* 14:21–25.
14. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
15. Galland, J. C. 1997. Risks and prevention of contamination of beef carcasses during the slaughter process in the United States of America. *Rev. Sci. Tech.* 16:395–404.
16. Johnston, M. D., R. J. Lambert, G. W. Hanlon, and S. P. Denyer. 2002. A rapid method for assessing the suitability of quenching agents for individual biocides as well as combinations. *J. Appl. Microbiol.* 92:784–789.
17. Kemp, G. K., and K. R. Schneider. 2000. Validation of thiosulfate for neutralization of acidified sodium chlorite in microbiological testing. *Poult. Sci.* 79:1857–1860.
18. Kim, J. W., and M. F. Slavik. 1996. Cetylpyridinium chloride (CPC) treatment on poultry skin to reduce attached *Salmonella. J. Food Prot.* 59:322–326.
19. Langsrud, S., and G. Sundheim. 1998. Factors influencing a suspension test method for antimicrobial activity of disinfectants. *J. Appl. Microbiol.* 85:1006–1012.
20. McDonnell, G., and A. D. Russell. 1999. Antiseptics and disinfectants: activity, action, and resistance. *Clin. Microbiol. Rev.* 12:147–179.
21. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
22. Pitten, F. A., and A. Kramer. 2001. Efficacy of cetylpyridinium chloride used as oropharyngeal antiseptic. *Arzneimittelforschung* 51:588–595.
23. Pohlman, F. W., M. R. Stivarius, K. S. McElyea, and A. L. Waldroup. 2002. Reduction of *E. coli, Salmonella* typhimurium, coliforms, aerobic bacteria, and improvement of ground beef color using trisodium phosphate or cetylpyridinium chloride before grinding. *Meat Sci.* 60:349–356.
24. Reid, C.-A., S. M. Avery, M. L. Hutchison, and S. Buncic. 2002. Evaluation of sampling methods to assess the microbiological status of cattle hides. *Food Control* 13:405–410.
25. Rivera-Betancourt, M. 2003. Personal communication.
26. Russell, A. D., I. Ahonkhai, and D. T. Rogers. 1979. Microbiological applications of the inactivation of antibiotics and other antimicrobial agents. *J. Appl. Bacteriol.* 46:207–245.
27. Sheridan, J. J. 1998. Sources of contamination during slaughter and measures for control. *J. Food Saf.* 18:321–339.
28. Small, A., C. A. Reid, S. M. Avery, N. Karabasil, C. Crowley, and S. Buncic. 2002. Potential for the spread of *Escherichia coli* O157, *Salmonella,* and *Campylobacter* in the lairage environment at abattoirs. *J. Food Prot.* 65:931–936.
29. Sutton, S. V., D. W. Proud, S. Rachui, and D. K. Brannan. 2002. Validation of microbial recovery from disinfectants. *PDA J. Pharm. Sci. Technol.* 56:255–266.
30. Xiong, H., Y. Li, M. F. Slavik, and J. T. Walker. 1998. Spraying chicken skin with selected chemicals to reduce attached *Salmonella* typhimurium. *J. Food Prot.* 61:272–275.
31. Yang, Z., Y. Li, and M. F. Slavik. 1998. Use of antimicrobial spray applied with an inside-outside bird washer to reduce bacterial contamination of prechilled chicken carcasses. *J. Food Prot.* 61:829–832.