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Electrolyzed Oxidizing Anode Water as a Sanitizer for Use in Abattoirs

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ABSTRACT

The effectiveness of electrolyzed oxidizing anode (EOA) water (oxidation-reduction potential, 1,120 mV; pH 2.0) as a sanitizer for use in abattoirs was compared with the iodophor (IOD) Mikroklene (25 ppm), a sanitizer approved for use by regulatory authorities in Canada and the United States. A total of 240 swab (100 cm^2) samples were obtained from 4 sites on the kill floor and 16 sites in the secondary processing areas, during two visits within a 4-week period to each of three meat packing plants, processing ≤50 animals per week. Swabs were obtained 12 h after the application of IOD and EOA and were analyzed for the presence of total aerobic bacteria, total coliforms, and total *Escherichia coli.* Total aerobic bacteria (log CFU/100 cm^2) recovered from the 20 sample sites were lower ($P < 0.0001$) in EOA as compared with IOD (2.94 ± 0.12 versus 3.75 ± 0.12, respectively). Plant A was 1.5 times more likely ($P < 0.0001$) to have a sampling site positive for the presence of coliforms and *E. coli* than plants B and C. There was no difference ($P > 0.05$) between treatment IOD or EOA in the likelihood of obtaining a positive sample for the presence of total coliforms or *E. coli* among the three plants. When the kill floor and secondary processing areas are compared, the likelihood of obtaining a sample positive for coliforms or *E. coli* was similar ($P \geq 0.05$). Results indicate that EOA was more effective than IOD in reducing populations of total aerobic bacteria on equipment surfaces in the three meat packing plants studied. Because the likelihood of obtaining a positive sample for coliforms or *E. coli* in EOA as compared with IOD was similar, EOA may be a suitable alternative or complement to IOD as a sanitizer in small- to medium-sized abattoirs. Additional research is required to further evaluate the effectiveness of EOA to sanitize processing equipment on the basis of subsequent isolation of aerobes, coliforms, and *E. coli* from meat products.

Regulatory authorities in the United States require the implementation of hazard analysis and critical control point (HACCP) systems in abattoirs to ensure that controls are in place to prevent and reduce food safety hazards *(35).* The HACCP system is a science-based system recognized under Codex Alimentarius as an internationally accepted standard for food safety *(4).* In order to enhance the safety of meat produced in and imported into Canada, the Food Safety Enhancement Program is moving toward the mandatory implementation of HACCP in the federally regulated meat sector. The ability of industry to demonstrate adherence to HACCP will allow for continued access to foreign markets and effective, uniform conformance verification and mutual recognition of HACCP systems mandated within the United States and other countries *(4, 35).*

The contamination of beef carcasses during slaughter and processing is undesirable but unavoidable in the conversion of live animals to meat for consumption *(16).* Live animal contamination and transference during the carcass-breaking and dressing processes contribute to the microbial contamination of beef *(1, 5, 10).* Inadequately cleaned equipment in meat-processing facilities has been identified in many studies as contributing to the transfer of microorganisms to beef carcass surfaces, thus potentially having serious implications for food safety *(1, 10, 12, 13).*

As a prerequisite for the development of a HACCP system, all plants must identify and implement written standard operating procedures for cleaning and sanitation *(4, 10, 35).* Proper sanitation is important in ensuring that product contact surfaces are adequately disinfected and the proliferation of undesirable microorganisms is controlled. Sanitizers commonly used in the food industry include chlorine-based sanitizers, iodophors, quaternary ammonium compounds, acid-anionic surfactants, and peracetic acid *(24).*

Chlorine-based sanitizers are the most frequently used sanitizers in the food processing plants because of their effectiveness, low cost, and availability *(6).* They are available in solid, liquid, and gas injection forms and target a wide range of bacteria. In appropriately mixed solutions, chlorine-based sanitizers are colorless, relatively nontoxic, nonstaining, and easy to prepare and apply *(6).* The major

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disadvantage of chlorine is that it is rendered ineffective in the presence of organic material, requiring its constant replenishment *(8).*

Iodophors are a combination of elemental iodine and a nonionic surfactant or carrier *(15).* They are effective against most gram-positive and gram-negative bacteria, fungi, and some viruses. Additionally, these compounds are nontoxic, nonirritating, and stable at working concentrations and are less corrosive than chlorine at low temperatures. As with chlorine, iodophors are readily rendered inactive by organic matter *(15).* Despite this limitation, iodophors are commonly used as sanitizers for food contact surfaces and equipment in the meat-processing industry.

Electrolyzed oxidizing (EO) water has been extensively used in Japan and Russia in a variety of dental and medical antimicrobial applications *(17, 27, 34, 38).* The production of EO water involves the electrolysis of a dilute salt solution in a chamber where the anode and cathode electrodes are separated by a charged bipolar membrane. Upon application of a current across the electrodes, two characteristic types of water are produced: the cathode produces basic EO water containing sodium hydroxide (pH $>$ 11) and having an oxidation reduction potential (ORP) of approximately -800 mV, whereas the anode produces acidic EO water containing hypochlorous acid (pH $\leq$ 2.7) and having an ORP of $>$1,100 mV and 10 to 100 ppm free chlorine *(8, 20).* EO acidic water has gained the attention of the food industry because of its strong bactericidal effects against many human pathogens, including *Escherichia coli* O157:H7, *Salmonella, Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Enterobacter aerogenes,* and *Campylobacter jejuni (8, 20, 21, 28, 29, 31, 33, 37).* Many studies have documented the effectiveness of EO acidic water in reducing human pathogens attached to cutting boards *(36),* poultry carcasses *(9, 28),* and vegetables *(2, 22, 23, 30),* as well as against spoilage organisms *(18).* Because sodium chloride is the only chemical used in the production of EO water, its use does not involve the handling of hazardous chemicals, and adverse effects on the environment may be minimized *(9, 20).*

Park et al. *(31)* recently evaluated the effectiveness of EO water as a sanitizer for decontaminating surfaces such as glass, stainless steel, ceramic tile, and vitreous china. The effectiveness in reducing microbial contamination on such surfaces suggests that EO water may be an effective and appropriate sanitizer for use in the meat-processing industry. The objective of this study was to compare the efficacy of EO anode water to the iodophor Mikroklene as a sanitizer for reducing the presence of microorganisms in abattoirs.

MATERIALS AND METHODS

EO water. EO water was generated with a Biostel Schweiz AG Agrostel (Biostel Schweiz AG, Schufelistraße 8, CH-8863 Buttikon) at a current setting of 12.0 A. Two types of water with different characteristics were generated. Basic EO water (pH 12.7) containing dilute NaOH and having an ORP of -800 mV was produced by the cathode; and acidic EO water (pH 2.0) containing dilute hypochlorous acid (HOCl) and having an ORP of 1,120 mV resulting in a targeted Cl concentration of 10 to 90 ppm *(25),* was produced by the anode. After a stable amperage reading was achieved, EO water was collected from the anode side of the generator for use in each of the three plants. The water used to prepare the EO water was drawn from a common well source, and 100 liters of EO anode water was collected in a sealed container and transported to each of the meat packing plants to use in the disinfection process. The pH and ORP values of the anode water were taken immediately after preparation with a pH/redox meter (Aqua/Lytic MeBgerat, Langen, Germany) in order to ensure values were consistent among batches of EO water used in each plant.

Abattoirs. Three abattoirs located in central Alberta, Canada, inspected by Alberta Agriculture, Food & Rural Development, Regulatory Services Branch, Food Safety Division, participated in the study. Abattoirs were chosen on the basis of previous contact with the researchers, geographic location, and willingness to participate. The selection of abattoirs was also based on the wide range in hygiene standards exhibited that was reflective of the overall range of hygiene observed in the meat processing industry. Plant A processed fewer than 20 beef cattle per week, and plants B and C processed 21 to 50 beef cattle per week. All plants had been in operation for at least 5 years. The degree of mechanization was higher in plant C as compared with plants A and B.

Cleaning and sanitizing process at plants A, B, and C. Standard operating procedures for the cleaning and sanitizing of the processing plants were documented and included the following. (i) Dry clean up—all floor scraps were collected with a floor squeegee, and large pieces of meat scraps were removed from the equipment. (ii) Prewash—equipment was dismantled and washed with warm water; meat scraps were then collected and the floor rinsed. (iii) Foaming—all equipment, walls, and floors were foamed with 5% e-Chem Q-Foam (Ecolab Inc., Mississauga, Ontario, Canada), a highly alkaline self-foaming liquid cleaner and degreaser specifically designed to remove heavy fat and grease deposits. The cleaning agent was applied to all surfaces, allowing for a minimum contact time of 10 min. (iv) Washing—all surfaces, including hard-to-reach areas such as undersides of tables and corners, were scrubbed with a brush, and all equipment was washed with hot (90°C) water. (v) Sanitizing—the standard sanitizing treatment consisted of a 25-ppm solution of the IOD detergent disinfectant, Mikroklene (Ecolab). Sanitizer was applied to equipment in the kill floor and secondary processing areas with a pressure bottle containing the appropriate concentration of Mikroklene. Solution was applied in a manner that ensured even coverage over all exposed surfaces. A final rinsing of the equipment was not required with the concentration of sanitizer used.

Treatments. The efficacy of the standard sanitizing treatment (IOD) used by each of the three processing plants was compared with 5% (vol/vol) electrolyzed oxidizing anode (EOA) water. The temperature of EOA upon application was 20°C. Seventy liters of IOD or EOA water was applied to equipment and plant surfaces at 0.75 liter/min with a pressurized spray bottle, ensuring even coverage of all exposed surfaces. No rinsing of the product occurred, and no admittance was allowed to treated areas of the plant until the next morning, allowing both IOD and EOA to remain on the processing equipment and operational areas for 12 h. The sanitizing process and subsequent inspection of sanitized equipment was performed by the same members of the project team to ensure consistency. Sanitizing treatments were administered after the daily end of production cleaning.

TABLE 1. *Total aerobic bacteria recovered from the kill floor and secondary processing areas of three abattoirs after sanitizing with IOD (25 ppm of the iodophor Mikroklene) or EOA (5% electrolyzed oxidizing anode water)*

Site	Population (log CFU/100 cm^2)[a]					
	Plant A		Plant B		Plant C	
	IOD	EOA	IOD	EOA	IOD	EOA
Kill floor						
Apron	5.1 ± 0.9	2.8 ± 1.0	2.7 ± 0.7	2.9 ± 1.3	3.3 ± 0.3	2.3 ± 0.2
Inedible cart	5.9 ± 1.7	3.0 ± 1.5	3.9 ± 0.9	2.6 ± 0.8	4.9 ± 0.6	2.9 ± 0.9
Inspection table	6.1 ± 1.2	4.1 ± 1.5	2.7 ± 0.5	2.7 ± 0.2	3.0 ± 2.4	2.4 ± 0.6
Splitting saw guide	5.4 ± 1.8	3.0 ± 2.6	2.9 ± 0.2	2.9 ± 0.6	4.1 ± 0.9	2.1 ± 0.1
Secondary processing area						
Bone barrel	4.9 ± 2.5	4.1 ± 2.5	3.7 ± 0.5	3.7 ± 0.9	3.5 ± 1.5	2.7 ± 0.7
Cut table frame	1.6 ± 0.1	3.1 ± 1.7	1.9 ± 0.2	2.7 ± 1.0	3.7 ± 0.8	4.2 ± 2.3
Cut table top	3.7 ± 1.5	3.3 ± 0.0	3.5 ± 0.5	1.7 ± 1.1	4.2 ± 0.4	3.1 ± 0.2
Drip cooler floor	5.5 ± 0.4	4.3 ± 0.9	3.3 ± 1.1	3.2 ± 0.6	5.5 ± 1.3	3.9 ± 0.4
Grinder hopper	2.8 ± 0.1	4.1 ± 0.0	1.8 ± 0.1	1.7 ± 0.3	4.0 ± 0.5	2.8 ± 0.1
Handsaw blade[b]	4.1 ± 3.2	3.7 ± 2.8	1.7 ± 0.0	2.0 ± 0.0	3.4 ± 1.2	2.9 ± 0.0
Hose handles	5.7 ± 0.8	4.0 ± 1.6	3.6 ± 0.4	3.7 ± 0.4	4.2 ± 1.2	3.1 ± 0.3
Meat tub	5.1 ± 2.2	3.9 ± 0.9	4.3 ± 1.9	4.3 ± 2.4	4.1 ± 1.1	3.5 ± 1.3
Saw slider	4.3 ± 0.3	2.8 ± 2.3	4.8 ± 0.5	2.0 ± 1.5	4.1 ± 0.6	2.0 ± 1.1
Saw guide	4.4 ± 3.6	2.6 ± 2.8	2.2 ± 2.4	3.0 ± 1.8	4.5 ± 0.4	2.2 ± 0.7
Silent cutter	3.5 ± 3.8	3.4 ± 2.1	2.0 ± 0.4	2.2 ± 0.7	3.8 ± 1.9	4.0 ± 1.5
Sink	2.6 ± 2.2	3.5 ± 1.0	2.7 ± 0.4	2.3 ± 0.1	4.4 ± 2.2	2.0 ± 0.7
Slicer blade[b]	1.8 ± 0.1	2.6 ± 1.4	2.5 ± 1.1	2.5 ± 0.1	4.2 ± 1.0	1.9 ± 0.3
Stuffer horn	3.7 ± 3.0	2.3 ± 1.2	1.7 ± 0.0	2.6 ± 1.9	4.6 ± 2.5	2.6 ± 0.5
Tenderizer blade[b]	3.9 ± 0.9	3.2 ± 1.7	2.6 ± 1.2	1.7 ± 0.1	4.1 ± 0.4	3.2 ± 1.9
Utensils[c]	3.3 ± 1.9	2.9 ± 1.4	5.0 ± 1.9	2.7 ± 1.5	4.3 ± 0.2	3.0 ± 1.4

[a] Mean ± standard deviation of two determinations; 100 cm^2 of each item was sampled where possible.
[b] The blade of each item was sampled.
[c] Utensils sampled included knives and steels.

Sample collection. At each visit to the plant, samples were obtained from each treatment on two consecutive days. On days 1 and 2, samples were taken after end-of-production cleaning and sanitizing with IOD or EOA, respectively. The number of cattle slaughtered per day remained constant over the 2-day sampling period. A total of 20 sites, 16 in the secondary processing area and 4 in the kill floor area, were sampled (Table 1). The surfaces of processing equipment and sites were sampled by swabbing an area of 100 cm^2 (where possible) with a Nu-gauze sterile swab (5 by 5 cm; CDMV Inc., St. Hyacinthe, Quebec, Canada) moistened with 5 ml of 0.1% (wt/vol) peptone water (Difco, Ottawa, Ontario, Canada). Care was taken to ensure that the same area on the equipment was sampled over the 2-day study. Each swab was placed in a separate stomacher bag, which was immersed in ice before being processed in the laboratory within 18 h. In order to comply with standard operating procedures for cleaning and sanitizing approved by Alberta Agriculture, Food & Rural Development, Regulatory Services Branch, the standard sanitizing treatment (IOD) was implemented after EOA application and sampling, before the processing of meat products. Each plant was visited and sampled on two consecutive days on two occasions during a 4-week period; a total of 240 swabs were collected.

Microbiological analysis. Microbiological analysis of the swabs was performed according to the methods described by Gill et al. *(10).* Briefly, each swab was stomached for 2 min in 10 ml 0.1% peptone water (Difco) in a Stomacher 400 laboratory blender (Seward Medical, London, UK). Serial dilutions were prepared in 0.1% peptone water with a 1-ml portion of the homogenate. Portions (100 μl) of each dilution were spread onto duplicate plates of plate count agar (Difco) and incubated for 48 h at 25°C. Where possible, determinations of total aerobic bacteria were made from plates containing between 20 and 200 colonies.

The remaining homogenate was filtered through a 5-μm stainless steel prefilter of an Iso-Grid filtration unit (Neogen, Lanceen, Mich.), in order to eliminate any large particles that might interfere with microbial analysis. The sample was then filtered through a hydrophobic-grid membrane filter (Neogen), and the membrane was removed and placed onto a plate of lactose monensin glucuronate agar (Dalynn, Calgary, Alberta, Canada) and incubated for 24 h at 35°C. After incubation, the membrane filter was examined for the presence of blue colonies. Squares containing blue colonies were counted and converted to a most probable number (MPN) of coliforms by the formula $MPN = N \ln[N/(N - X)]$, where N is the total number of squares on the filter (1,600) and X is the number of squares containing blue colonies *(7).* Membrane filters were transferred to buffered 4-methylumbelliferyl-β-D-glucuronide agar (Dalynn) plates and incubated for 2 h at 35°C, and subsequently examined under UV light with a UVGL-58 multiband shortwave–long-wave handheld UV lamp (Entela, Upland, Calif.). Blue-white colonies that fluoresced under long-wave UV light were counted, and values (MPN) for *E. coli* were calculated as in the determination of the MPN of coliforms. The MPN values for both *E. coli* and coliforms were then converted to log CFU.

Statistical analysis. The MIXED procedure of SAS *(32)* was used to compare numbers (log CFU per 100 cm^2) of total aerobic bacteria recovered at each plant and to compare IOD and EOA

TABLE 2. *Total aerobic bacteria recovered at three abattoirs after sanitizing with IOD (25 ppm of the iodophor Mikroklene) or EOA (5% electrolyzed oxidizing anode water)*[a]

Abattoir	Treatment		SEM[b]	*P* value[c]
	IOD	EOA		
A	4.21 A	3.31 A[d]	0.20	0.003
B	2.97 B	2.67 B	0.20	0.306
C	4.09 A	2.84 AB	0.20	<0.001
Overall	3.75	2.94	0.12	<0.001

[a] Values shown (log CFU per 100 cm^2) are means of duplicate assay of 20 sampling sites in the kill floor and secondary processing areas of each abattoir.
[b] SEM, standard error of the mean ($n = 20$).
[c] Treatment effect (IOD versus EOA).
[d] Effect of abattoir. Within a column, means followed by different letters differ ($P < 0.05$).

treatments. Orthogonal contrasts and odds ratios within the GENMOD procedure of SAS were used to compare the utility of the treatments and to determine the impact of plant (A, B, C), treatment (IOD, EOA) and sampling area (kill floor or secondary processing area) on the presence of total coliforms and total *E. coli.*

RESULTS AND DISCUSSION

Populations of total aerobic bacteria (log CFU per 100 cm^2) recovered from the 20 sampling sites on the kill floor and secondary processing areas of the three abattoirs after sanitizing with IOD and EOA are presented in Table 1. In plants A and C, counts for total aerobic bacteria were lower ($P < 0.05$) in treatment EOA as compared with IOD; similar ($P > 0.05$) counts were obtained between treatments in plant B (Table 2). Overall, populations of aerobes recovered from plants A, B, and C were lower ($P < 0.001$) in treatment EOA as compared with IOD. Numerous studies have evaluated the effectiveness of EO water against human pathogens in cell suspensions and on various surfaces in vitro; however, the effectiveness of EO water as a sanitizer in operational abattoirs has not been previously examined *(8, 20, 31, 33, 37, 38).* Results of the present study indicate that treatment EOA was more effective than IOD in reducing total aerobes on surfaces of equipment in the three plants studied.

EO water has been proven to be highly effective against *E. coli* O157:H7, *Salmonella, S. aureus, E. aerogenes,* and *L. monocytogenes* in pure culture *(8, 31, 33, 36).* Studies evaluating the effectiveness of EO water to reduce pathogens on surfaces such as glass, stainless steel, glazed and unglazed ceramic tile, and vitreous china found that immersion of inoculated test surfaces in EO water reduced populations of *E. aerogenes* and *S. aureus* by 2.2 to 2.4 and 1.7 to 1.9 log CFU/cm^2, respectively *(31).* In these studies, viable cells were not recovered from the wash water, indicating the potential of EO water in minimizing cross-contamination. Cutting boards inoculated with 10^{10} CFU of *E. coli* O157:H7 and soaked in EO water for 5 min at 55°C reduced *E. coli* populations by ≥5 log CFU/100 cm^2. The use of EO water at higher temperatures was found to reduce the exposure time required to achieve reductions in microbial counts that were similar to those obtained with prolonged exposure at a lower temperature *(36).* The recovery of lower ($P < 0.001$) populations of total aerobes in plants A, B, and C in EOA as compared with IOD, in the present study (Table 2), suggests that although EOA was effective in reducing aerobic populations, EOA used at a higher concentration and applied at a temperature higher than ambient, may result in an even more significant reduction in microorganisms in abattoirs. Additional research is required to evaluate this hypothesis.

Improperly cleaned equipment has clearly been identified as a significant source of microorganisms for the contamination of meat *(1, 10–12).* In a study conducted by Aslam et al. *(1),* genotypic analysis of *E. coli* recovered from carcasses and equipment at a beef packing plant revealed unique types of *E. coli* isolated from conveyer belts as characterized by random amplification of polymorphic DNA analysis. Results suggested that *E. coli* may form resident populations at these sites that serve as a source of contamination of ground beef. In an evaluation of the sources of contamination for beef trimmings in the carcass-breaking process, conveyor belts, steel mesh gloves, and detritus persisting on saws and conveyors were identified as sources contributing to the increase in the numbers of aerobes, coliforms, and *E. coli* on product *(10, 11, 12).*

Our findings identified numerous sources of coliforms and *E. coli* in the three abattoirs after sanitizing (Table 3). When evaluating the recovery in the two operational areas of the abattoirs, the likelihood of obtaining a sample positive for coliforms or *E. coli* from sampling sites in the kill floor or secondary processing areas was similar ($P > 0.05$). Plant A was 1.5 times more likely ($P < 0.0001$) to have a sampling site positive for the presence of coliforms and *E. coli* than plants B and C. However, among the three plants, there was no difference ($P > 0.05$) between treatment with EOA or IOD in the likelihood of obtaining a positive sample for the presence of coliforms and *E. coli.* The absence of coliforms and *E. coli* from samples obtained from aprons, splitting saw guide, cut table top, meat tub, and silent cutter may indicate that these items can be effectively cleaned and sanitized and are unlikely to be major sources of contamination in the three plants evaluated (Table 3).

Large populations (>5.0 log CFU) of coliforms and *E. coli* have been found to be associated with detritus persisting on pieces of equipment after they were deemed to be adequately cleaned and sanitized *(10, 12).* Coliform populations recovered from both moist and drier detritus associated with the conveying equipment were found to be composed of no more than 10% *E. coli.* The recovery of large populations of coliforms containing less than 10% *E. coli* from processing equipment was expected because the predominance of *E. coli* in coliform populations is more indicative of fecal contamination than the microflora that grows in detritus over time *(10, 12).*

In the present study, coliform populations recovered from plant A were 100% *E. coli* in 5 of 6 and 8 of 10 samples from IOD and EOA treatments, respectively (Table 3). In plant A, results suggest that coliforms were likely derived from fecal material as opposed to detritus persisting

TABLE 3. *Total coliforms and* E. coli *recovered from swab samples obtained from kill floor and secondary processing areas of three abattoirs after treatment with IOD (25 ppm of the iodophor Mikroklene) or EOA (5% electrolyzed oxidizing anode water)*

Site	Total coliforms (log CFU)[a]						Total *E. coli* (log CFU)[a]					
	Plant A		Plant B		Plant C		Plant A		Plant B		Plant C	
	IOD	EOA	IOD	EOA	IOD	EOA	IOD	EOA	IOD	EOA	IOD	EOA
Kill floor												
Apron	—[b]	—	—	—	—	—	—	—	—	—	—	—
Inedible cart	—	0.70	—	—	—	—	—	0.70	—	—	—	—
Inspection table	—	0.0003	—	—	—	—	—	0.0003	—	—	—	—
Splitting saw guide	—	—	—	—	—	—	—	—	—	—	—	—
Secondary processing area												
Bone barrel	3.57	—	—	—	—	—	3.57	—	—	—	—	—
Cut table frame	1.39	3.00	—	—	—	—	1.39	3.00	—	—	—	—
Cut table top	—	—	—	—	—	—	—	—	—	—	—	—
Drip cooler floor	—	0.70	—	—	—	0.0003	—	0.70	—	—	—	—
Grinder hopper	—	0.0003	—	—	—	—	—	0.0003	—	—	—	—
Handsaw blade[c]	5.23	4.00	—	—	—	—	4.21	3.23	—	—	—	—
Hose handles	—	—	—	0.0003	—	—	—	—	—	—	—	—
Meat tub	—	—	—	—	—	—	—	—	—	—	—	—
Saw slider	0.0003	1.10	—	2.90	—	—	0.0003	1.10	—	0.00003	—	—
Saw guide	—	2.78	—	—	—	—	—	1.61	—	—	—	—
Silent cutter	—	—	—	—	—	—	—	—	—	—	—	—
Sink	—	0.70	—	—	—	—	—	0.70	—	—	—	—
Slicer blade[c]	—	—	—	—	0.0003	—	—	—	—	—	—	—
Stuffer horn	2.84	—	—	—	—	—	2.84	—	—	—	—	—
Tenderizer blade[c]	2.57	1.90	—	—	—	—	2.57	1.90	—	—	—	—
Utensils[d]	—	—	—	—	—	0.0003	—	—	—	—	—	—

[a] Values represent the total of two determinations; 100 cm^2 of each item was sampled where possible.
[b] None detected.
[c] The blade of each item was sampled.
[d] Utensils sampled included knives and steels.

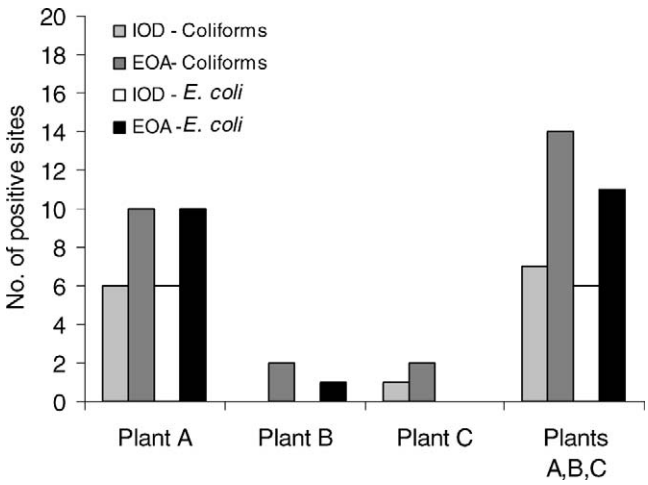

FIGURE 1. *Number of sampling sites positive for the presence of coliforms and* E. coli *in three abattoirs after sanitizing with IOD (25 ppm of the iodophor Mikroklene) or EOA (5% electrolyzed anode water). A total of 20 sites were sampled on two separate visits to each plant, 4 on the kill floor and 16 in the secondary processing area.*

on equipment, indicating inadequate cleaning of the kill floor and secondary processing areas before sanitation. Although an increased number of samples was positive for *E. coli* and coliforms in EOA as compared with IOD in plant A, populations of coliforms and *E. coli* recovered from EOA were lower as compared with IOD (Fig. 1 and Table 3). In 5 of 10 EOA samples, $\leq$0.70 log CFU/100 cm^2 *E. coli* and coliforms were recovered, but only 1 of 6 samples in IOD contained $\leq$0.70 log CFU/100 cm^2. For plant A, EOA more effectively reduced populations of total aerobic bacteria as compared with IOD. In plants B and C, small populations of coliforms were recovered from equipment in the secondary processing area, with *E. coli* populations composing $<$10% of coliform populations recovered. Gill and McGinnis *(12)* reported that the recovery of large numbers of coliforms from processing equipment is generally due to their association with detritus. No difference ($P >$ 0.05) in the likelihood of obtaining a positive sample for coliforms or *E. coli* among plants in IOD or EOA indicates the similar effectiveness of both sanitizers in controlling coliform and *E. coli* populations. The effectiveness of any sanitizer in disinfecting equipment is dependent on the adequate cleaning of the equipment before sanitizing. It is essential to ensure that cleaning is performed thoroughly and effectively before disinfection. The effectiveness of

sanitizers has been known to decrease in the presence of organic material, increasing water hardness and the presence of residual cleaning chemicals *(24).* EO water has also been shown to less bactericidal in the presence of organic matter *(33),* emphasizing the importance of adequate cleaning before sanitation.

The oxidation-reduction potential (ORP) of a substrate has been defined as its ability to gain or lose electrons *(19, 26).* A specific range of ORPs is required for the growth of aerobic (200 to 800 mV) and anaerobic (−200 to −400 mV) bacteria, with positive and negative ORPs being indicative of oxidizing and reducing ability, respectively *(19).* When bacteria are subjected to a solution with an extremely high oxidizing capability (high ORP), such as was present in EOA (ORP, 1,120 mV), ions are sequestered and the cellular membrane becomes unstable, facilitating the entry of antimicrobial agents *(19).* The primary antimicrobial agent in EOA is hypochlorous acid (HOCl). The neutral charge of hypochlorous acid enables it to readily enter bacterial cells and inhibit growth by halting ATP production by oxidative and fermentative pathways *(3).* In acidic EO water, the maximum concentration of HOCl occurs at pH 4. Consequently, as the pH of a chlorine-containing solution increases, the HOCl dissociates into hydrogen ions (H^+) and hypochlorite ions (OCl^-) *(25, 26).* The bactericidal effect of OCl^- is 20 times less than HOCl *(25).* Although high ORP values are related to the concentration of HOCl, it has been reported that the ORP of a treatment solution may be a greater determinant of microbial inactivation because it is an indication of oxidation capability, irrespective of pH and chlorine concentration *(20, 26).* An ORP of 650 mV has been reported to result in the immediate destruction of *E. coli* regardless of pH or chlorine concentration *(26).* The high ORP (1,120 mV) of EOA used in the present experiment likely played an integral part in its bactericidal activity.

The use of *E. coli* or coliform data (indicative of fecal contamination) is appropriate for assessing the hygienic characteristics of a beef carcass dressing process in terms of HACCP, whereas counts of total aerobic bacteria are an appropriate measure of cleanliness in Quality Management systems *(14).* These conclusions were arrived at through the microbiological monitoring of carcass surfaces as they passed though a beef dressing process. Gill et al. *(10, 11)* subsequently reported that the adequacy of a cleaning process for meat-fabricating equipment cannot be reliably assessed by the visual inspection of cleaned equipment and microbiological sampling of meat contacting surfaces, but that the enumeration of an indicator organism or organisms on product entering and leaving the process is required. Only in this manner can it be determined whether microorganisms from improperly cleaned and sanitized equipment are being transferred to the meat. We recognize that our study has limitation in defining the risk of meat contamination because microbial samples were only collected from potential contact surfaces in the plants. However, operational requirements at the plants participating in the study dictated the extent and frequency of sampling. Future studies to evaluate the effectiveness of EOA to sanitize processing equipment, based on subsequent appearance of aerobes, coliforms, and *E. coli* on meat products, are in the planning stages.

In summary, treatment with EOA was more effective than treatment with IOD in reducing populations of total aerobic bacteria on surfaces of equipment in the three abattoirs studied. Because there was no difference in the likelihood of a positive sample for coliforms or *E. coli* in treatment EOA as compared with IOD, EOA may be a potential alternative or complement to the use of existing sanitizers in meat processing plants. In addition to possessing strong antimicrobial properties, EO water is easy to prepare, involves the use of only sodium chloride in its preparation, and minimizes adverse effects on the environment *(20).* Given that hot water is already extensively used in the plant cleaning process, the introduction of EO water into existing cleaning protocols would require minimal changes in plant operations. Additional research is required in order to optimize the application (concentration and time) of EOA and to further evaluate the effectiveness of EOA in terms of subsequent product contamination.

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REFERENCES

1. Aslam, M., G. G. Greer, F. M. Nattress, C. O. Gill, and L. M. McMullen. 2004. Genotypic analysis of *Escherichia coli* recovered from product and equipment at a beef-packing plant. *J. Appl. Microbiol.* 97:78–86.
2. Bari, M. L., Y. Sabina, S. Isobe, T. Uemura, and K. Isshiki. 2003. Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella* Enteriditis, and *Listeria monocytogenes* on the surface of tomatoes. *J. Food Prot.* 66:542–548.
3. Barrette, W. C., D. M. Hannum, W. D. Wheeler, and J. K. Hurst. 1989. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. *Biochemistry* 28:9172–9178.
4. Canadian Food Inspection Agency. 1996. Food safety enhancement program implementation manual. Available at: http://www.inspection.gc.ca/english/fssa/polstrat/haccp/manu/manue.shtml. Accessed 21 March 2006.
5. Cassin, M. H., A. M. Lammerding, E. C. D. Todd, W. Ross, and R. S. McColl. 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int. J. Food Microbiol.* 41:21–44.
6. Dychdala, G. R. 1983. Chlorine and chlorine compounds, p. 157–181. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 3rd ed. Lea & Febiger, Philadelphia.
7. Entis, P., and P. Boleszczuk. 1990. Direct enumeration of coliforms and *Escherichia coli* by hydrophobic grid membrane filter in 24 hours using MUG. *J. Food Prot.* 53:948–952.
8. Fabrizio, K. A., and C. N. Cutter. 2003. Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella* Typhimurium and *Listeria monocytogenes. J. Food Prot.* 66:1379–1384.
9. Fabrizio, K. A., R. R. Sharma, A. Demirici, and C. N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. *Poult. Sci.* 81:1598–1605.
10. Gill, C. O., M. Badoni, and J. C. McGinnis. 1999. Assessment of the adequacy of cleaning equipment used for breaking beef carcasses. *Int. J. Food Microbiol.* 46:1–8.

11. Gill, C. O., L. P. Baker, and T. Jones. 1999. Identification of inadequately cleaned equipment used in a sheep carcass–breaking process. *J. Food Prot.* 62:637–643.
12. Gill, C. O., and J. C. McGinnis. 2000. Contamination of beef trimmings with *Escherichia coli* during a carcass breaking process. *Food Res. Int.* 23:474–479.
13. Gill, C. O., and J. C. McGinnis. 2003. Decontamination of cleaned personal equipment used during beef carcass processing. *Food Prot. Trends* 33:125–130.
14. Gill, C. O., J. C. McGinnis, and M. Badoni. 1996. Assessment of the hygiene characteristics of a beef carcass dressing process. *J. Food Prot.* 59:136–140.
15. Gottardi, W. 1983. Iodine and iodine compounds, p. 183–196. *In* S. S. Block (ed.), Disinfection, sterilization, and preservation, 3rd ed. Lea & Febiger, Philadelphia.
16. Grau, F. H. 1986. Microbial ecology of meat and poultry, p. 1–36. *In* A. M. Pearson and T. R. Dutson (ed.), Advances in meat research. AVI Publishing Company Inc., Westport, Conn.
17. Horiba, N., K. Hiratsuka, T. Onoe, T. Yoshida, K. Suzuki, T. Matsumoto, and H. Nakamura. 1999. Bacterial effect of electrolyzed neutral water on bacteria isolated from infected root canals. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 87:83–87.
18. Izumi, H. 1999. Electrolyzed water as a disinfectant for fresh-cut vegetables. *J. Food Sci.* 64:536–539.
19. Jay, J. M. 2000. Modern food microbiology, 6th ed. Aspen Publishers, Gaithersburg, Md.
20. Kim, C., Y. C. Hung, and R. E. Brackett. 2000. Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of food borne pathogens. *Int. J. Food Microbiol.* 61:199–207.
21. Kim, C., Y. C. Hung, and R. E. Brackett. 2000. Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *J. Food Prot.* 63:19–24.
22. Kim, C., Y. C. Hung, R. E. Brackett, and C.-S. Lin. 2003. Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts. *J. Food. Prot.* 66:201–214.
23. Koseki S., K. Yoshida, S. Isobe, and K. Itoh. 2001. Decontamination of lettuce using acidic electrolyzed water. *J. Food Prot.* 64:652–658.
24. Lelieveld, H. L. M., M. A. Mostert, J. Holah, and B. White (ed.). 2003. Hygiene in food processing. CRC Press, Boca Raton, Fla.
25. Len, S.-V., Y.-C. Hung, M. Erickson, and C. Kim. 2000. Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. *J. Food. Prot.* 63:1534–1537.
26. McPherson, L. L. 1993. Understanding ORP's role in the disinfection process. *Water Eng. Manage.* 140:29–31.
27. Middleton, A. M., M. V. Chadwick, J. L. Sanderson, and H. Gaya. 2000. Comparison of a solution of super-oxidized water (Sterilox) with glutaraldehyde for the disinfection of bronchoscopes, contaminated in vitro with *Mycobacterium tuberculosis* and *Mycobacterium avium-intrallulare* in sputum. *J. Hosp. Infect.* 45:278–282.
28. Park, H., Y.- C. Hung, and T. R. E. Brackett. 2002. Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. *Int. J. Food Microbiol.* 72:77–83.
29. Park, H., Y.-C. Hung, and D. Chung. 2004. Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes. Int. J. Food Microbiol.* 91: 13–18.
30. Park, H., Y.-C. Hung, M. P. Doyle, G. O. I. Ezeike, and C. Kim. 2001. Pathogen reduction and quality of lettuce treated with electrolyzed oxidizing and acidified chlorinated water. *J. Food Sci.* 66: 1368–1372.
31. Park, H., Y.-C. Hung, and C. Kim. 2002. Effectiveness of electrolyzed water as a sanitizer for treating different surfaces. *J. Food Prot.* 65:1276–1280.
32. SAS Institute Inc. 1999. SAS user's guide: statistics, version 8.2. SAS Institute Inc., Cary, N.C.
33. Stevenson, S. M. L., S. R. Cook, S. J. Bach, and T. A. McAllister. 2004. Effects of water source, dilution, storage, and bacterial and fecal loads on the efficacy of electrolyzed oxidizing water for the control of *Escherichia coli* O157:H7. *J. Food Prot.* 67:1377–1383.
34. Tanaka, N., N. Tanaka, T. Fujisawa, T. Daimon, K. Fujiwara, M. Yamamoto, and T. Abe. 2000. The use of electrolyzed solutions for the cleaning and disinfecting of dialyzers. *Artif. Organs* 24:921–928.
35. U.S. Department of Agriculture, Food Safety and Inspection Service. 1996. Pathogen reduction: hazard analysis and critical control point (HACCP) systems: final rule. *Fed. Regist.* 61:38805–38989.
36. Venkitanarayanan, K. S., G. O. Ezeke, Y. C. Hung, and M. P. Doyle. 1999. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water. *J. Food Prot.* 62:857–860.
37. Venkitanarayanan, K. S., G. O. Ezeke, Y. C. Hung, and M. P. Doyle. 1999. Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis,* and *Listeria monocytogenes. Appl. Environ. Microbiol.* 65:4276–4279.
38. Vorobjeva, N. V., L. I. Vorobjeva, and E. Y. Khodjaev. 2004. The bactericidal effects of electrolyzed oxidizing water on bacterial strains involved in hospital infections. *Artif. Organs* 28:590–599.