Research Note

Efficacy of a Peroxyacetic Acid Formulation as an Antimicrobial Intervention To Reduce Levels of Inoculated Escherichia coli O157:H7 on External Carcass Surfaces of Hot-Boned Beef and Veal

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ABSTRACT

The efficacy of a peroxyacetic acid formulation (POAA) at reducing Escherichia coli O157:H7 contamination on external carcass surfaces of hot-boned beef and veal with a commercial spray apparatus was determined. Hot-boned external carcass surfaces were inoculated with either a high dose (10⁶ CFU/cm²) in fresh bovine feces or with a low dose (10³ CFU/cm²) in diluent of laboratory-cultured E. coli O157:H7. Treatments included a water wash, a POAA (180 ppm) wash, or a water plus POAA wash. Samples were extracted from the external carcass surface with a cork borer to determine the numbers of viable E. coli O157:H7 remaining on the carcass surface after treatment. Although a water wash alone resulted in a 1.25 (94.4%) and a 1.31 (95.1%) mean log reduction on veal and beef inoculated with a high dose of E. coli O157:H7, the POAA treatment resulted in a substantially greater mean log reduction of 3.56 and 3.59 (>99.9%). The water wash only resulted in a 33.9% reduction on veal and 62.8% on beef inoculated with a low dose of E. coli O157:H7, whereas POAA treatment greatly improved pathogen reduction to 98.9 and 97.4% on veal and beef, respectively. The combination of a water wash followed by a POAA treatment resulted in a similar E. coli O157:H7 reduction to that achieved by POAA treatment alone. In conclusion, POAA treatment significantly reduced viable E. coli O157:H7 numbers on experimentally contaminated beef and veal carcasses, which justifies its use as a chemical intervention for the removal of this human pathogen.

Studies carried out by Cobbold and Desmarchelier (4, 5) identified calves as the cattle group most likely to be shedding Escherichia coli O157. In particular, they showed that around the time of weaning, dairy calves pose the highest risk for potential transmission. Microbial contamination of meat occurs when the overlying hide carrying feces, dust, and dirt is removed from the animal carcass (dressing). The New Zealand red meat industry achieves very high standards of hygiene when dressing red meat, which has facilitated the export of New Zealand meat to international markets. However, the microbiological indicator profile for bobby veal (meat from bovine calves younger than 2 weeks of age) can be two times (E. coli) and 10 times (aerobic plate counts) that achieved for beef, so the New Zealand meat industry has implemented interventions to reduce the overall level of microbial contamination of veal.

Most New Zealand bobby veal producers have installed POAA sprays, marketed by EcoLab (Hamilton, New Zealand) as Inspxex 200, which is used as an aqueous solution according to manufacturer’s recommendations. POAA has been used as a sanitizer of fruit and salad vegetables and has antimicrobial activity against viruses (11) and bacterial pathogens such as E. coli O157:H7 (17). A few studies have monitored the benefits of POAA at reducing the E. coli O157:H7 load on meat carcasses (10, 13, 16). However, results of some studies of the efficacy of POAA on brisket beef have been inconclusive (10), or have showed little or no effect on inoculated surfaces of chilled meat (6, 13). To our knowledge, there have been no studies assessing the efficacy of POAA on veal produced from calves younger than 2 weeks old.

Although Salmonella and E. coli O157:H7 are considered to be reasonably unlikely to occur on New Zealand beef meat (12), the aim of this study was to determine the potency of a POAA spray at removing E. coli O157:H7 on experimentally contaminated hot-boned beef and bobby veal.

MATERIALS AND METHODS

Culture preparation. A nonpathogenic laboratory strain of E. coli O157:H7 (NZRCC 3614) was grown in tryptone soy broth for 12 to 14 h at 35°C. A total of 100 μl of the culture was used to inoculate 0.9 ml of sterile 0.1% peptone water and stored at approximately 4°C until required later the same day. Cultures were mixed vigorously to reduce agglutination before inoculation into
feces or into maximum recovery media (Difco, Becton Dickinson, Sparks, Md.).

**Experimental meat.** Hot-boned bobby calf (5 to 7 days old) flaps (flank and navel end brisket) and beef (5 years old) flaps were obtained fresh from local export slaughter plants and transported to the laboratory within 30 min of slaughter. Each bobby calf or beef flap was attached to a polystyrene board with stainless steel pins, and a template was used to mark a 400-cm² area onto the original external carcass surface of the meat.

**Inoculation procedure.** The experimental procedure was based on the methods described by Castillo et al. (2, 3). Fresh bovine feces were collected immediately after defeecation from dairy cows on a farm adjacent to the laboratory. Feces were kneaded to ensure consistency, dispensed as 10-g portions into fresh sterile stomacher bags, and stored at 4°C. An appropriate volume of *E. coli* O157:H7 culture was added to the feces to give an approximate concentration of 10⁶ CFU/cm² for a high-dose inoculum or was separately diluted in maximum recovery media to produce a low-dose inoculum (with an approximate concentration of between 10⁵ to 10⁶ CFU/cm²). A sterile spatula was used to evenly spread the inocula over the marked 400-cm² area on the external flap surfaces and left for 10 min to allow the bacteria to attach, and also to mimic worst-case time frames on the slaughter chain, before proceeding with the decontamination procedure.

**Decontamination procedure.** Inspexx 200 is a mixture of hydrogen peroxide, acetic acid, octanoic acid, peroxyoctanoic acid, and hydroxyethylidene-1,1-diphosphoric acid (7). The peroxyacetic and peroxyoctanoic acid are formed by the reaction of hydrogen peroxide with acetic acid or octanoic acid, respectively. The formulation (POAA) was diluted in potable water sprayed onto red meat carcasses at the recommended concentrations of 180 ppm total peroxyacids after slaughter (7). A spray cabinet was set up to EcoLab's specifications as described below to simulate the decontamination procedures used by the New Zealand meat industry. Three fan spray nozzles were attached to a delivery tube 28 cm apart to give a full and even vertical coverage of the inoculated surface of the meat. The tube was able to rotate so the spray could be directed evenly over the inoculated surface of the meat. A Wilden pump with a pulse dampener was set to deliver the Inspexx 200 at 180 ppm with an even pressure of 6 bar at the spray nozzle. The meat was placed 30 cm from the spray nozzles to give repeatable coverage.

**Treatments.** Treatments were conducted on five inoculated hot-boned bobby calf and five hot-boned beef flaps. Treatments included a wash with diluted POAA at 180 ppm, or a wash in potable water followed by a wash in diluted POAA at 180 ppm. The duration of all washes was for 5 s at approximately 20°C.

**Posttreatment sampling.** Samples were collected from flaps immediately after inoculation and after water wash or POAA treatments. A sterile cork borer with a 7-cm² surface area was used to take four identically positioned cork borer samples (total 28 cm²) from each flap, and these were composited in a sterile stomacher bag with 100 ml of sterile maximum recovery media and pumped for 2 min in a Seward Stomacher 400 (Lab System, Seward, Thetford, Norfolk, UK). Three 0.1-ml aliquots of serial dilutions were plated in duplicate onto CHROMagar 157 plates (CHROMagar, Paris, France) according to the manufacturer’s instructions. *E. coli* O157:H7 was enumerated as a distinctive pink colony (1).

**Controls.** Uninoculated flaps were washed with potable water with the EcoLab spray system, and four cork borer samples were taken from the middle third of the flap. The flaps were then washed with POAA at 180 ppm for 5 s, and a final set of four cork borer samples were taken from the bottom third of the flap. Background levels of *E. coli* O157:H7 in feces were determined in cork borer samples collected from five hot-boned bobby calf and five hot-boned beef flaps on which 10 g of fresh bovine feces had been spread over a 400-cm² area.

**Data analysis.** The treatments form a 2 × 2 factorial of “with and without water wash” × “with and without POAA wash”; the nil treatment (inoculation) was doubly replicated. The data were transformed to log scale for statistical analysis by the REML directive of GenStat (9). Those values below the minimum level of detection were estimated by the CENSOR procedure of GenStat. Treatment effects were assessed by the Wald tests. An observed difference in the control values for the two high-dose treatment groups was accounted for in the analysis. The analyses suggested that the animal component of variance was not statistically significant and was excluded.

**RESULTS AND DISCUSSION**

This study evaluated the use of POAA spray washing of carcasses as an intervention step to reduce the level of *E. coli* O157:H7 contamination before refrigeration. *E. coli* O157:H7 was not detected on un inoculated beef or veal meat surfaces. The inoculation of veal or beef flaps with a high dose of *E. coli* O157:H7 followed by a water wash resulted in a modest reduction (~1.25 log) in numbers of bacteria (P < 0.01 and P < 0.001, respectively). The high-dose contamination in feces was not expected under New Zealand processing conditions (14) but was adopted to reflect on other published studies. Castillo et al. (2, 3) achieved a 1- to 3-log reduction by water washing hot-boned beef inoculated with a high dose of *E. coli* O157:H7. However, an undesirable consequence of washing without any specific decontamination agent is the spread of contaminants over a greater proportion of the carcass surface (2).

Treatment of inoculated meat with a water wash followed by a POAA wash (Fig. 1a) significantly reduced the numbers of *E. coli* O157:H7 recovered (P < 0.001). This treatment resulted in a mean reduction of 2.73 log CFU/cm² on veal and 3.21 log CFU/cm² on beef. The direct treatment of inoculated meat with POAA at 20°C (Fig. 1a) without a previous water wash resulted in the largest reduction (>99.9%) of *E. coli* O157:H7 numbers on the sampled meat, a mean log reduction of 3.56 on veal and 3.59 on beef.

Our findings support the results of the study conducted by King et al. (13), in which the application of 200 ppm Inspexx 200 onto hot carcass surfaces at a higher temperature of 43°C was found to be capable of reducing a high dose of *E. coli* O157:H7 by 2.6 log CFU/cm². These authors have also found that the sanitizing effects of Inspexx 200 were reduced when used on chilled carcass surfaces (13), illustrating that the efficacy of this intervention is dependent on exact processing parameters. In another study by the same group with Inspexx 200 on preground beef trimmings, only a modest reduction (<1 log) of *E. coli* O157:H7 was achieved (6).
FIGURE 1. Effect of different wash treatments at reducing contamination of fresh veal and beef flaps inoculated (I) with high-dose E. coli O157:H7 in feces (a) or with low dose of E. coli O157:H7 bacteria in diluent (b). Decontamination treatments included washing with potable water (IW), washing with water followed by POAA solution (IWT), and washing with POAA solution alone (IT). Each treatment shown was mean obtained from sample of n = 5, except for untreated sample I (n = 10).

To represent realistic levels of microbial contamination, a low level dose of E. coli O157:H7 was applied in diluent to beef and veal (~10^2 CFU/cm^2 on beef and ~10^3 CFU/cm^2 on veal) (Fig. 1b). A water wash resulted in a modest mean log reduction of 0.18 (33.9%) on veal (P > 0.05) and 0.43 (62.8%) on beef (P < 0.001) (Fig. 1b). Treatment with POAA resulted in a 1.97 (98.9%) and 1.58 (97.4%) mean log reduction of E. coli O157:H7 on veal and beef, respectively (P < 0.001). The treatment of carcasses with POAA, with or without a previous water wash, resulted in similar levels of reduction in E. coli O157:H7 numbers (Fig. 1b). Bacteria in a low-dose inoculum and a liquid matrix have a greater opportunity to attach to the carcass surface and hence can be more likely to resist removal. The ability of POAA treatment to remove >1.5 log CFU/cm^2 of E. coli O157:H7 (97.4%) from a carcass contaminated with >2 log CFU/cm^2 demonstrates the potency of this intervention over a water wash alone. Reductions of 1.2 log CFU/cm^2 have also been reported for POAA treatment of fresh beef trim inoculated with 2 log CFU/cm^2 in diluent (6, 15).

This study found that the decontamination procedures for E. coli O157:H7 were consistently less effective on veal than on beef (Fig. 1). This might reflect a compositional difference of veal that facilitates greater or more rigid attachment of pathogens. A study that used electron microscopy revealed that E. coli O157:H7 predominantly associates with collagen fibers (8), but greater amounts of collagen are normally associated with older meat. The veal samples taken from a smaller-sized animal perhaps contain more lean-fat junctions where crevices and crypts might protect bacteria (2, 3), and perhaps this contributed to the higher numbers of E. coli O157:H7 retained on veal.

In conclusion, we have established that a commercially produced POAA (Inspexx 200) treatment is an effective surface decontamination with the potential to reduce E. coli O157:H7 CFUs by 97.4 to >99.9% from freshly slaughtered beef and veal carcasses. The potency of this intervention under these experimental conditions exceeds the levels of all E. coli contamination monitored on veal and beef carcasses in New Zealand. Variation of the processing parameters can alter the intervention efficacy, and so valida-
tion of decontamination is recommended for different meat processes.

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REFERENCES