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ORIGINAL ARTICLE

A study on the needs to improve Korea abattoir's critical control point of HACCP system

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Abstract: These days abattoirs' hygiene of Korea is regulated by Hazzard Analysis Critical Control Point (HACCP). Although 20 years have been left since first HACCP was adjusted in Korea, 12% of abattoirs got inconsistence on evaluations. Food poisoning caused by bacteria feces like pathogenic *Escherichia coli* and *Salmonella* has not decreased. These bacteria on meat cross-contaminate at the abattoir. Therefore, field verification of abattoir's critical control point (CCP) and experiments to find alternative ways of the CCP were conducted. The aerobic bacteria were measured before and after high-pressure water based washing process set as CCP in most abattoirs. Four parts of cattle carcasses were selected to apply sponge-swab method. The effects were < 1 log reduction which is not significant. Lactic acid (LC), chlorine dioxide (ClO_2) and slightly acidic electrolyzed water (SAEW) were used to measure the effect of reducing bacteria on beef by the different time. LC has 1.24–2.02 log reduction for aerobic bacteria. ClO_2 has 1.44–1.96 log reduction for aerobic bacteria. SAEW has 1.1–1.91 log reduction for aerobic bacteria. There was significant difference according to concentrations ($p < 0.05$). This study presents legitimacy for hygiene improvement of CCP by field verification. In addition, chemical disinfectants that can be mechanically applied have better reduction effects of high-pressure washing.

Keywords: abattoirs, South Korea, Hazard Analysis and Critical Control Points, disinfectants

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Introduction

Meat is highly prone to contamination by various bacteria, such as *Salmonella*, *Listeria*, *Campylobacter*, *Clostridium perfringens*, *Escherichia coli* O157:H7, and *Yersinia*. Of these, the greatest threat to public health is *E. coli* O157:H7, which is derived from feces and is typically found in the intestine of cows [1]. So high-pressure water based washing method that is used at the majority of abattoirs in Korea is set as a critical control point (CCP). This method controls contamination in meat through water pressure and time. However, using only water pressure and time involves many disadvantages. Organic matters on the meat are often scattered due to the high-pressure, and these scattered organic matters can give way to secondary contamination. Moreover, if time, volume of water, pressure, temperature, and various other conditions are not met, the microorganism reduction effect drastically decreases, which means that contamination in one area of meat may quickly spread throughout other areas of meat [2-4]. Other various methods have been proposed to control meat contamination through results from previous studies. However, these methods cannot be used if they are not economically feasible. 82% of abattoirs in Korea replied that their business conditions are difficult or average, indicating that most abattoirs in Korea face financial issues [5].

Therefore, this study will evaluate the effectiveness of high-pressure water based washing that is used in most abattoirs. Also, the study of reduction effect of approved chemical disinfectants was conducted to replace CCP.

Materials and Methods

Verification of high-pressure water based washing method

Location

This study was performed a t Livestock Process Complex in Gyeonggi-do. The abattoir processed up to 10 cattles and 1,700 pigs on average per day.

Swab

Total 25 carcasses were collected in 5 days. Collecting site was high-pressure washing process defined as CCP on abattoir. Whirl-pak (Nasco, USA) was used to collect samples. Sterilized sponges soaked with 20 mL 0.1% (W/V) peptone water (PW; peptone, Difco, USA) were used to swab. Brisket, front-leg, flank and neck were collected (100 cm^2). To avoid overlapped sampling and accuracy, sampling was performed at the left side of carcasses. After collecting samples, samples were kept in the refrigerator until performing microbiological examination. Microbiological examinations were performed within 20 h after sampling.

Microbial testing

The 20 mL of 0.1% PW was inserted to Whirl paks sampling cattle carcass to make total volume as 40 mL. Whirl paks were homogenized for 30 sec using shaker. After homogenization, 1 mL was transported from whirl-pak to AC petri film (3M^{TM}, USA). All films were incubated at 36°C for 48 h.

Decontamination effects of chlorine dioxide (ClO_2), lactic acid (LC) and slightly acidic electrolyzed water (SAEW) for different concentration on bacteria inoculated beef

Bacteria culture

Aerobic bacteria were used. Aerobic bacteria were collected from carcass not taking high-pressure washing. Sampling points were brisket, flank and rump (100 cm^2). Samples were transported laboratory being chilled. The 1 mL was transported from whirl-pak to nutrient broth (NB; Oxoid, USA) to proliferate. After incubating at 37°C for 24 h, 1 mL of incubated NB was transferred to another NB. These cycles were repeated until performing experiment because of the cell activations.

Disinfectants

LC (Tokyo Chemical Industry Co., Ltd., Japan) was carried in powder form. LC powder was dissolved in water at 55°C before conduct experiment. Concentration of LC was 1%, 2%, 3% (W/V). Slightly acidic electrolyzed water (SAEW; Cosmic-Round Korea Co., Ltd., Korea) was carried in the aqueous form. SAEW was diluted in water at 55°C before conduct experiment. Concentration of SAEW was 10, 20 and 30 ppm. ClO_2 (Furgo-farm Co., Ltd., Korea) was carried in aqueous form. ClO_2 was diluted in water at 55°C before conducting experiment. Concentration of ClO_2 was 10, 20 and 40 ppm. Solutions were prepared using deionized water.

Application methods

To mimic abattoir's environment, the applied system was made. Beef was broken into pieces aseptically by 5 g in clean bench with sterilized scissor and forceps. Beef was transferred to small petri-dishes (35.00 × 10.00 mm) with a hole of the side. The hole at petri-dish was made for letting disinfectants get out. If disinfectant couldn't get out, it would affect the results of experiment. The 0.5 mL of NB which contains aerobic bacteria from the abattoir was inoculated. The inoculum density was adjusted to 0.010 absorbance units at 600 nm using a micro ELISA plate reader (SoftMax Pro, USA). The mean concentration of inoculated NB was 5.77 log colony forming unit (CFU)/mL. There was 10 min attaching bacteria to beef. Five petri-dishes put into the chamber obliquely. Spray (Marolex Sp. Z o.o., Poland) containing disinfectants was put in front of chamber. Length of spray to petri-dishes was 20 cm. Antimicrobial applications were sprayed for 5 or 10 sec. After being sprayed, wait 10 min. To stop activating disinfectants, beef on the dishes was transferred to neutralization broth which contains 20% yeast extract (BD DIFCO, USA). Then the beef was shaken for 30 min on shaker.

Microbial testing

1 mL was transported from neutralization broth to AC petri film (3M^{TM}). All films were incubated at 36°C for 48 h.

Statistical analysis

The data is expressed as mean and standard deviation of the $\log_{10}$ value. Statistical analysis was performed by GraphPad Prism 5.01(GraphPad Software Inc., USA). Two-way analysis of variance and Bonferroni post tests are used to analyze. Significant differences were defined as $p < 0.05$.

Results

Verification of high-pressure water based washing method

Before high-pressure washing, the contamination levels of flank, brisket, front leg and neck are 2.05, 3.63, 3.23 and 2.85 log CFU/cm^2, respectively. After high-pressure washing, the contamination levels of flank, brisket, front leg and neck are 2.18, 2.63, 2.38 and 2.16 log CFU/cm^2, respectively (Fig. 1). Average contamination level of carcasses at before high-pressure washing is 2.94 log CFU/cm^2. Average contamination level of carcasses at after high-pressure washing is 2.34 log CFU/cm^2. There is significant log reduction at brisket, front leg and neck. Contamination levels of brisket, front leg and neck after high-pressure washing were significantly reduced by 1, 0.86 and 0.68 log CFU/cm^2, respectively, compared with those before high-pressure washing ($p < 0.05$). However, in case of flank, there is no significant reduction between before and after high-pressure washing ($p > 0.05$). On the contrary, contamination level of after high-pressure washing is slightly increased than that of before high-pressure washing.

Decontamination effects of ClO_2, LC and SAEW for different concentration on bacteria inoculated beef

Application of LC for 5 sec significantly reduce aerobic bacteria by 4.47, 4.22 and 3.75 log CFU/mL for treatment

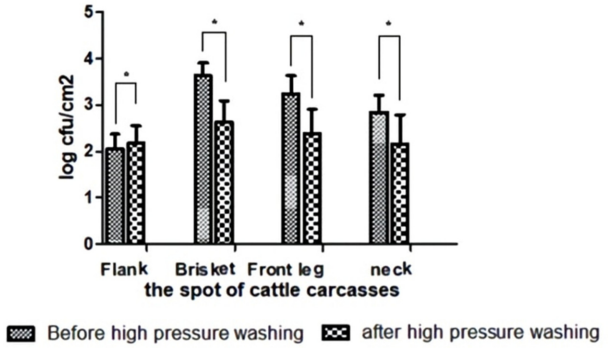

Fig. 1. The contamination level of aerobic bacteria before/after high-pressure water based washing. Four spots (flank, brisket, front leg, neck) of cattle carcasses are collected (n = 25). All data are expressed as mean ± SD.
*$p < 0.05$.

concentration of 1%, 2% and 3%, respectively. Application of LC for 10 sec significantly reduce aerobic bacteria by 4.53, 4.12 and 3.84 log CFU/mL for treatment concentration of 1%, 2% and 3%, respectively. ClO_2 at the concentration of 10, 20 and 40 ppm is significantly reduced aerobic bacteria by 4.24, 4.21 and 3.53 log CFU/mL after treatment for 5 sec, respectively, and by 4.25, 4.15 and 3.54 log CFU/mL after treatment for 10 sec, correspondingly. In addition, SAEW at the concentration of 10, 20 and 40 ppm was significantly reduced aerobic bacteria by 4.66, 4.52 and 3.88 log CFU/mL after treatment for 5 sec, respectively, and by 4.64, 4.49 and 3.86 log CFU/mL after treatment for 10 sec, correspondingly. There are no significant difference between treatment time ($p > 0.05$). However, when it comes to concentration there are significant reduction. ($p < 0.05$).

Discussion

The reduction of aerobic bacteria on chest, front leg and neck after high-pressure washing was 1.0, 0.86 and 0.68 log CFU/mL, respectively, compared with those before high-pressure washing (Fig. 1). According to the previous study, it is not very effective on a small number of common bacteria, coliform, and *E. coli* cells [6]. Also coincide with other studies reporting that high-pressure washing with only water offers an extremely low bacteria reduction rate and even causes contamination on the surface of other pieces of meat [2-4,7]. Moreover, the log reduction value was negative for flanks, which demonstrates that microorganism contamination increased after high-pressure washing than before. In the study that was conducted on a large-scale abattoir, the level of microorganismal contamination increased from 0.78 log CFU/cm^2 to 1.01 log CFU/cm^2, revealing that the method acts to redistribute the bacteria than to remove them [8]. These results show the methods used in abattoir are not quite effective for meat hygiene. Therefore, the alternative methods using disinfectants should be considered. LC has 1.24–

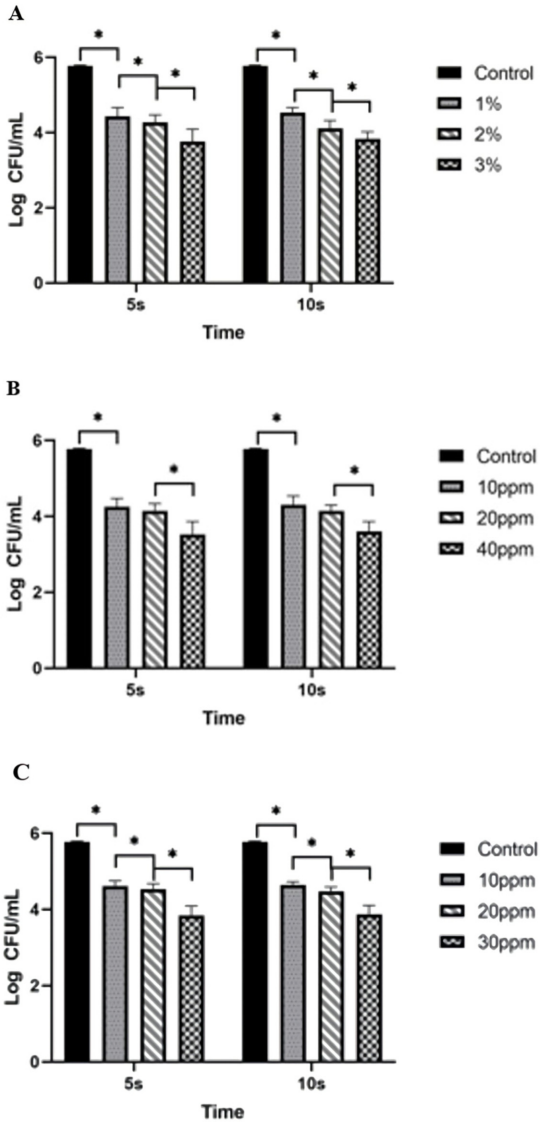

Fig. 2. The decontamination effects of lactic acid (A), chlorine dioxide (B), slightly acidic electrolyzed water (C) regarding aerobic bacteria on beef samples (n = 15). All data are expressed as mean ± SD.
*$p < 0.05$.

2.02 log reduction for aerobic bacteria (Fig. 2). This coincides with the results of previous studies reporting that 2% LC sprayed onto beef head for 26 sec led to 1.52 log reduction in microorganismal count, with 2% LC sprayed on beef resulting in 1.6 log reduction in microorganismal count [9,10]. Moreover, 2% LC applied for 15 sec on meat inoculated with *E. coli* was found to result in 1.5–2.2 log reduction in *E. coli* count [11]. Similarly, when LC was sprayed in the abattoir, there was a 0.9–2.3 log reduction in aerobic bacteria [12]. Another study also reported an improvement in the storage quality of meat to which LC was applied [13]. ClO_2 has 1.44–1.96 log reduction for aerobic bacteria. This is similar to a previous study reporting that when 10 ppm of ClO_2

was used, it resulted in ≥ 1.53 log reduction [14], as well as another study reporting that when ClO_2 was used on chicken carcasses, significant reduction was found even at concentrations below 10 ppm [15,16]. However, when 800 ppm of ClO_2 was used on beef inoculated with *E. coli*, the result did not exceed 1.3 log reduction in *E. coli* count [14]. The differences in reduction of this study compared to the previously mentioned may be due to different inoculation methods, as well as initial microbial concentration, contact time, type of fruit, disinfection system, disinfectant forms, and enumeration method. According to a previous study, application of ClO_2 for 1 min or less did not lead to significant reduction and this shows the importance of long contact time [12]. SAEW has 1.1–1.91 log reduction for aerobic bacteria. According to previous studies, 20 ppm of SAEW resulted in 1.5 log reduction of aerobic bacteria, and 40 ppm of SAEW showed a significant reduction in mesophilic bacteria in pork meat [17,18]. However, when meat was treated with SAEW for 5 min using the dipping method, aerobic bacteria reduced from 3.06 log CFU to 2.28 log CFU [19]. Free chlorine concentration is the most important factor that determines the sterilization effect of SAEW, it is most effective at pH between 6.0 to 7.5 [20]. Therefore, the results may differ due to differences in PH caused by technicalities.

These results indicate that application of chemical disinfectants can be alternative method to get over high-pressure and time which is disadvantages of high-pressure water based washing. Results indicate chemical disinfectants show constant effects not greatly affected by low pressure and application time, and show higher decontamination efficacy than high-pressure washing method. The results could be serve as a basis for other decontamination methods on abattoir model.

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References

1. Koohmaraie M, Arthur TM, Bosilevac JM, Guerini M, Shackelford SD, Wheeler TL. Post-harvest interventions to reduce/eliminate pathogens in beef. Meat Sci 2005;71:79-91.
2. Bell RG. Distribution and sources of microbial contamination on beef carcasses. J Appl Microbiol 1997;82:292-300.
3. Jericho KW, Bradley JA, Kozub GC. Microbiologic evaluation of carcasses before and after washing in a beef slaughter plant. J Am Vet Med Assoc 1995;206:452-455.
4. Reagan JO, Acuff GR, Buege DR, Buyck MJ, Dickson JS, Kastner CL, Marsden JL, Morgan JB, Nickelson R 2nd, Smith GC, Sofos JN. Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat. J Food Prot 1996;59:751-756.
5. Ji IB, He D, Kim HJ, Seo GC, Ju JS. Mid to Long-Term Development Strategy for Slaughtering Industry. pp. 33-35, Korea Rural Economic Institute, Naju, 2015.
6. Gill CO, Landers C. Microbiological effects of carcass decontaminating treatments at four beef packing plants. Meat Sci 2003;65:1005-1011.
7. Yalçin S, Nizamlioğlu M, Gürbüz Ü. Fecal coliform contamination of beef carcasses during the slaughtering process. J Food Saf 2001;21:225-231.
8. Yang X, Badoni M, Youssef MK, Gill CO. Enhanced control of microbiological contamination of product at a large beef packing plant. J Food Prot 2012;75:144-149.
9. Bosilevac JM, Nou X, Barkocy-Gallagher GA, Arthur TM, Koohmaraie M. Treatments using hot water instead of lactic acid reduce levels of aerobic bacteria and *Enterobacteriaceae* and reduce the prevalence of *Escherichia coil* O157:H7 on preevisceration beef carcasses. J Food Prot 2006;69:1808-1813.
10. Kalchayanand N, Arthur TM, Bosilevac JM, Brichta-Harhay DM, Guerini MN, Wheeler TL, Koohmaraie M. Evaluation of various antimicrobial interventions for the reduction of *Escherichia coli* O157:H7 on bovine heads during processing. J Food Prot 2008;71:621-624.
11. Dorsa WJ, Cutter CN, Siragusa GR. Effects of acetic acid, lactic acid and trisodium phosphate on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157: H7, *Listeria innocua*, and *Clostridium sporogenes*. J Food Prot 1997;60:619-624.
12. Prasai RK, Acuff GR, Lucia LM, Hale DS, Savell JW, Morgan JB. Microbiological effects of acid decontamination of beef carcasses at various locations in processing. J Food Prot 1991;54:868-872.
13. Echeverry A, Brooks JC, Miller MF, Collins JA, Loneragan GH, Brashears MM. Validation of intervention strategies to control *Escherichia coli* O157:H7 and *Salmonella* typhimurium DT 104 in mechanically tenderized and brine-enhanced beef. J Food Prot 2009;72:1616-1623.
14. Cutter CN, Siragusa GR. Application of chlorine to reduce populations of *Escherichia coli* on beef. J Food Saf 1995; 15:67-75.
15. Lillard HS. Levels of chlorine and chlorine dioxide of equivalent bactericidal effect in poultry processing water. J Food Sci 1979;44:1594-1597.
16. Lillard HS. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. Poult Sci 1980;59:1761-1766.
17. Aday MS. Application of electrolyzed water for improving postharvest quality of mushroom. Lebenson Wiss Technol 2016;68:44-51.
18. Koide S, Takeda JI, Shi J, Shono H, Atungulu GG. Disinfection efficacy of slightly acidic electrolyzed water on fresh cut cabbage. Food Control 2009;20:294-297.
19. Sheng X, Shu D, Tang X, Zang Y. Effects of slightly acidic electrolyzed water on the microbial quality and shelf life extension of beef during refrigeration. Food Sci Nutr 2018; 6:1975-1981.
20. Nan S, Yongyu LI, Baoming LI, Wang C, Cui X, Cao W. Effect of slightly acidic electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Staphylococcus aureus* analyzed by transmission electron microscopy. J Food Prot 2010;73: 2211-2216.