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Evaluation of decontamination efficacy of commonly used antimicrobial interventions for beef carcasses against Shiga toxin-producing *Escherichia coli*

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ABSTRACT

In Argentina, Shiga toxin producing *Escherichia coli* (STEC) serogroups O157, O26, O103, O111, O145 and O121 are adulterant in ground beef. In other countries, the zero-tolerance approach to all STEC is implemented for chilled beef. Argentinean abattoirs are interested in implementing effective interventions against STEC on carcasses. Pre-rigor beef carcasses were used to determine whether nine antimicrobial strategies effectively reduced aerobic plate, coliform and *E. coli* counts and *stx* and *eae* gene prevalence. These strategies were: citric acid (2%; automated), acetic acid (2%; manual and automated), lactic acid (LA 2%; manual and automated), LA (3%; automated), electrolytically-generated hypochlorous acid (400 ppm; manual), hot water (82 °C; automated) and INSPEXX (0.2%; automated). Automated application of 2% LA after 30–60-min aeration and 3% LA at 55 °C were the most effective interventions. Automated application was more effective than manual application. Decontamination of beef carcasses through automated application of lactic acid and hot water would reduce public health risks associated with STEC contamination.

1. Introduction

Zoonotic pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) carried by cattle are unavoidably spread to carcasses during slaughter (Duarte, Nauta, & Aabo, 2016). STEC (especially STEC O157:H7) is recognized as an etiological agent of hemolytic uremic syndrome (HUS) through foodborne infections since 1982. In Argentina, > 400 HUS cases per year are declared. Additionally, HUS is recognized as the second major cause of chronic kidney failure (Spizzirri, Rahman, Bibiloni, Ruscasso, & Amoreo, 1997). Although STEC foodborne outbreaks were historically linked to meat products, this pattern has changed since a larger number of food products (*e.g.* fresh produce and unpasteurized juices) are now associated with serious outbreaks (Erickson & Doyle, 2007; Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005).

In Argentina, previous studies showed that the prevalence of STEC O157 and STEC in beef cattle carcasses was 2.6 (Masana et al., 2010) and 12.3% (Etcheverria et al., 2010), respectively. A recent study reported that 5.8% of carcasses were positive for non-O157 STEC and that its prevalence in anatomical cuts and trimmings was 5.8 and 7.0%, respectively (Brusa et al., 2017).

In the United States (US), the absence of detectable O157, O26, O45, O103, O111, O121 and O145 serogroups in ground beef and beef trimmings is mandatory (FSIS, 2012). Likewise, the absence of the six major STEC serogroups (O157, O26, O103, O111, O145 and O104:H4) in sprouts from the European Union or ground beef and beef trim from the US is also mandatory (European Commission, 2013b). While in Argentina the search of O157:H7/NM, O26, O103, O111, O145 and O121 STEC serogroups in ground beef, ready-to-eat food, sausages and vegetables is compulsory (Ministerio de Justicia, 2017), other countries

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have zero-tolerance for all STEC in chilled beef (RASFF from https://webgate.ec.europa.eu/rasff-window/portal). Consequently, the Argentinean beef industry is focused on the improvement of intervention methods to reduce the prevalence of STEC on beef.

Different physical and chemical carcass decontamination procedures have been evaluated to reduce the prevalence of STEC. For instance, hot water (Algino, Ingham, & Zhu, 2007; Bosilevac, Nou, Barkocy-Gallagher, Arthur, & Koohmaraie, 2006; Castillo, Lucia, Goodson, Savell, & Acuff, 1998; Kalchayanand et al., 2012), organic acids (Algino et al., 2007; Castillo et al., 2001; Geornaras et al., 2012; Kalchayanand et al., 2012, 2015) and electrolytically-generated hypochlorous acid (EGHA) (Bosilevac, Shackelford, Brichta, & Koohmaraie, 2005; Jadeja & Hung, 2014; Kalchayanand et al., 2008) proved effective to reduce *E. coli* O157:H7 and *Salmonella* spp. contamination on bovine heads and beef hides. In 2011, the European Food Safety Authority (EFSA) evaluated the safety and efficacy of lactic acid to reduce microbial contamination in carcasses, cuts and trimmings (EFSA, 2011), recommending its use in the framework of a hazard analysis and critical control point (HACCP) plan. Consequently, certain parameters of the process had to be verified (lactic acid concentration, application temperature, any other factor that could affect the effectiveness of microbiological decontamination) and validated (lactic acid efficacy considering the specific abattoir processing conditions). In 2013, the use of lactic acid was authorized by the European Commission (European Commission, 2013a). Recently, the US Department of Agriculture and the Food Safety and Inspection Service (USDA-FSIS) approved several substances for use in meat production (FSIS, 2017). In Argentina, the National Service of Agrifood Health and Quality (SENASA, for its Spanish acronym) approved different treatments to decontaminate bovine carcasses (SENASA, 2014). Besides good manufacturing practices (GMP) and HACCP, the application of intervention measures such as washing carcasses and cuts with acid is necessary to meet the zero-tolerance criteria for non-O157 STEC from beef (Brusa et al., 2017).

In general, studies about the efficacy of different beef decontamination interventions were primarily concerned with applications under controlled conditions in research laboratories (Dorsa, Cutter, & Siragusa, 1997; Huffman, 2002). Further research could help determine the effectiveness of decontamination treatments to control microbiological contamination on beef carcasses (especially STEC) at commercial abattoirs.

The aim of this study was to evaluate the antimicrobial effect of nine different interventions, including citric, acetic and lactic acid, the combination of peroxyacetic, octanoic, acetic, hydrogen peroxide, peroxyoctanoic and 1-hydroxyethylidene-1, 1-diphosponic acid (INSPEXX 200), hot water and electrolytically-generated hypochlorous acid (EGHA), against STEC on beef carcasses at commercial abattoirs.

2. Material and methods

2.1. Abattoir selection

The study was carried out in cattle abattoirs producing beef for export and for local markets of Argentina between August 2015 and April 2016. Eight abattoirs designated A to H were invited and accepted to participate voluntarily in this study. They were selected considering the number of cattle slaughtered over a five-year period (> 800,000), their geographic location (Buenos Aires, 33°46′S 60°05′W; 34°18′S 60°15′W; 34°25′S 58°35′W; 34°53′S 58°02′W; Santa Fe, 32°57′S 60°39′W; 29°14′S 59°56′W; 33°48′S 61°20′W; San Luis, 33°40′S 65°28′W) and their capacity to apply all the intervention treatments. Sampling was approved by SENASA. All sampled carcasses presented the organoleptic and commercial characteristics established in National Decree No 4238/68 for meats (SENASA, 1968).

2.2. Intervention treatments

Interventions were evaluated during slaughter after water washing. Decontamination procedures were both manual and automated. Manual procedures included portable spray equipment with a high-resistance rotomolded tank (100 l capacity), suction filter, pressure regulating valve, glycerin bath pressure gauge, electric pump (12 V) and one adjustable flow spray nozzle with 5-m hose. One or two operators sprayed both surfaces of each carcass from top to bottom during 8 working h, depending on each day. Automated procedures were performed using a double stainless steel cabinet with an electric pump (220 V), 12–30 nozzles homogeneously distributed in two or three lines to cover all of the two sides of each carcass at the pressure of 1.5–3 bar, and a sensor to detect the presence of carcasses. In the case of water, high-pressure hot water spray was applied in a stainless steel cabinet with an EZ Heater® (Hydro Thermal, Waukesha, WI, USA) and 40 nozzles in two lines to cover all of the two sides of each carcass. It also included a sensor to detect the presence of carcasses.

The decontamination treatments evaluated were:

Abattoir A (automated). Citric acid (2%) spray at 45–50 °C using 0.5–1 l per carcass (RZBC, Shandong, China).
Abattoir B (manual). Acetic acid (2%) fine droplets at 20–25 °C using 0.1–0.3 l per carcass (Samsung Bp Chemicals Co., Ltd., Ulsan, Korea).
Abattoir C (automated). Acetic acid (2%) spray at 20–25 °C using 0.2–0.3 l per carcass (Samsung).
Abattoir D (manual). Lactic acid (2%) fine droplets at 20–25 °C using 0.8–1 l per carcass (Purac, Netherlands).
Abattoir D (automated). INSPEXX 200 (0.2%) spray at 20–25 °C using 2–3 l per carcass (Ecolab, Minnesota, USA).
Abattoir E (automated). Lactic acid (2%) spray at 20–25 °C using 2–3 l per carcass (Purac) after a 30–60-min aeration period.
Abattoir F (automated). Lactic acid (3%) spray at 55 °C using 0.8–1 l per carcass (Purac).
Abattoir G (manual). EGHA (400 ppm) fine droplets using 0.1–0.2 l per carcass (Envirolife, Buenos Aires, Argentina).
Abattoir H (automated). Hot water spray at 82–87 °C (1–1.5 bar).

Tap water to mix the antimicrobial compounds fulfilled the manufacturer's requirements. Additional characteristics of the interventions are depicted in Table 1.

The standardized conditions of each abattoir were analyzed before the validation process. Each abattoir was included in a training program to ensure the systematic collection and processing of samples. This program included the person responsible for quality control, all samplers and a SENASA official veterinarian in each abattoir. Specifications about mode and time of application, temperature of the solution and any other relevant information are depicted in Table 1.

2.3. Sample collection

Samples were taken by the personnel in charge of quality control of the abattoirs, supervised by the SENASA veterinarian. Sampling was carried out for 10 consecutive weeks in each abattoir considering the natural variability about presence and concentration of spoilage and pathogenic microorganisms in the cattle slaughtered.

Samples (n = 5 each sampling day) were taken from the same carcass three times per week before (three right and two left half-carcasses; n = 150) and after (two right and three left half-carcasses; n = 150) applying the intervention. A total of 2700 samples (N = 300 for each intervention; 1350 before and 1350 after the interventions) were collected. The five samples collected each sampling day were from at least two different herds.

Sample size was enough to detect differences of at least 0.25 log cfu/400 cm^2 with a standard deviation of 1.2 log cfu/400 cm^2 (95.0%

Table 1
Descriptive characteristics of interventions on beef carcasses in the slaughter line.

Intervention	Concentration (%)	Mode of application	Time of application (s)	Volume applied (liters/carcass)	Distance between carcass washing and application point (m)	Temperature (°C)	Organoleptic changes in carcasses
Citric acid (RZBC, 100%)	2	Cabinet with 12 nozzles in two lines	10	0.250	5	45–50	NO
Acetic acid (Samsung, 99.8%)	2	Manual	7	0.130	50	20–25	NO
Acetic acid (Antarctica)	2	Cabinet with 12 nozzles in two lines	11	0.200	1–2	20–25	NO
Lactic acid (Purac, 85%)	2	Manual	10–15	1 ± 0.100	50	20–25	NO
Lactic acid (Purac, 85%)	2	Cabinet with 30 nozzles in three lines	10	2–3	100[a]	20–25	NO
Lactic acid (Purac, 85%)	3	Cabinet with 12 nozzles in two lines	11	1	30	45–50	NO
EGHA (Envirolife)	400 ppm	Manual	12–15	0.100	15	20–25	NO
Hot water	–	Cabinet with 40 nozzles in two lines	3–4	≥10	5	82–87	NO
Peroxyacetic acid (INSPEXX 200)	0.2	Cabinet with 30 nozzles in two lines	8 ± 2	2–3	5	20–25	NO

EGHA: electrolytically-generated hypochlorous acid.
[a] After a 30–60-min aeration period.

confidence level) among microorganism counts.

Pre-intervention samples were collected after carcass washing with water and before applying the intervention. Post-intervention samples were collected approximately 30–45 min after treatment, prior to entering the chilling rooms (Table 1). Two samples were taken from each carcass, one for the count of indicator microorganisms and the other for the detection of *stx* and *eae* genes. For mesophilic aerobic, coliform and *E. coli* counts, a combination of four carcass areas of 100 cm^2 each (chest, neck, buttock and posterior lateral hock) was swabbed with a sterile sponge (Whirl-Pak speci-sponge, Nasco, USA) previously soaked in 10 ml buffered peptone water (Biokar, Zac de Ther, France). First, the chest and neck area was swabbed with one side of the sponge (ten strokes in two directions, from left to right and from top to bottom). The same sponge was then flipped to the other side to swab the buttock and posterior lateral hock in the same as aforementioned.

For *stx* and *eae* gene detection, the carcass surface, covering a total half carcass including the anterior and the posterior regions, was swabbed with another sterile sponge (Whirl-Pak) previously soaked in 10 ml buffered peptone water (Biokar). The posterior area was first swabbed with ten strokes of the sponge in two directions. The same sponge was then flipped and the anterior area was covered by another ten strokes in both directions as mentioned previously. After swabbing, sponges were placed into sterile stomacher bags, stored at 4 °C and immediately sent to the laboratory for analysis.

2.4. Microbiological analyses

2.4.1. Count of total mesophilic aerobic microorganisms, coliforms and E. coli

Each sample sponge was hand squeezed three times and 1-ml aliquot was used for enumerations. Mesophilic aerobic organism enumerations were performed with Petrifilm method AOAC 990.12 (3 M Minnesota, USA). Coliform and *E. coli* enumerations were performed using Petrifilm method AOAC 991.14 (3 M). All Petrifilm plates were incubated following the manufacturer's recommendations. Results were expressed as log cfu/400 cm^2.

2.4.2. Prevalence of stx and eae genes

All samples were analyzed for non-O157 STEC according to ISO/CEN 13136:2012 (ISO, 2012), with some modifications. Briefly, each sampling day the sponge used for swabbing was put into a stomacher bag with 500 ml of modified trypticase soy broth containing 8 mg/l novobiocin plus casamino acids (mTSB-8, Acumedia Manufacturers, Minnesota, USA). Then, the sponge was mixed in the stomacher bag for 2 min and incubated for 20 h at 41.5 °C. After the enrichment incubation step, samples were screened for stx_1, stx_2 and *eae* genes by RT-PCR BAX (Dupont, Minnesota, USA). It should be noted that *stx* and *eae* gene detection using PCR methods does not necessarily mean that only O157:H7 and non-O157 STEC carry these two genes. All data were collected in an Excel spreadsheet.

2.5. Statistical analyses

Bacterial counts were log transformed before statistical analyses. Differences in microorganism counts and presence of *stx* and *eae* genes between the right and left half-carcasses before the interventions were evaluated with Student's *t*-test and Pearson's Chi-squared test. Comparisons of microorganism counts and presence of *stx* and *eae* genes in carcasses among the abattoirs before the interventions were performed using ANOVA and the generalized linear model (GLM). Presence/absence of *stx* and *eae* genes was the outcome variable in the GLM, using a binary logistic distribution as linked function. The effectiveness of each intervention on the count of aerobic microorganisms, coliforms and *E. coli* was evaluated using paired Student's *t*-test. The effect of each intervention on the prevalence of *stx* and *eae* genes was determined using MacNemar test. The comparison of effectiveness

Table 2
Effect of interventions on aerobic plate counts.

Intervention	Before the intervention (log cfu/400 cm^2)	After the intervention (log cfu/400 cm^2)	Log reduction	$P=$[1]
	Mean (SD)[2]	Mean (SD)	Mean (SD)[2]	
2% Citric acid automated	4.71 (0.88) [cb]	3.92 (1.27)	0.79 (1.23) [b]	< .001
2% Acetic acid manual	4.22 (0.69) [e]	3.81 (0.91)	0.41 (0.66) [cd]	< .001
2% Acetic acid automated	4.94 (0.69) [b]	4.30 (0.70)	0.63 (0.85) [bc]	< .001
2% Lactic acid manual	4.61 (1.68) [cd]	4.61 (1.84)	0.02 (2.38) [e]	.993
2% Lactic acid automated	5.51 (1.23) [a]	4.37 (1.92)	1.18 (1.14) [a]	< .001
3% Lactic acid automated	5.69 (0.62) [a]	4.74 (0.91)	0.95 (0.96) [ab]	< .001
EGHA manual	4.90 (0.92) [b]	4.88 (0.97)	0.02 (0.93) [e]	.760
Hot water automated	4.43 (0.80) [de]	3.65 (1.17)	0.77 (1.41) [b]	< .001
INSPEXX 200 automated	5.00 (1.63) [b]	4.81 (1.65)	0.18 (1.82) [de]	.249

EGHA: electrolytically-generated hypochlorous acid.
[a–e] Interventions with no common letter differed significantly ($P < .05$).
[1] Paired Student's *t*-test.
[2] Differences among abattoirs using ANOVA.

among intervention on the reduction of aerobic microorganisms, coliforms and *E. coli* was evaluated using ANOVA. All statistical analyses were performed using InfoStat software (Universidad Nacional de Córdoba).

3. Results

3.1. Microbial population counts

Differences in microbial counts (total aerobic microorganisms, *E. coli* and coliforms) between the right and left half-carcasses before each intervention were not significant ($P > .05$). However, we identified differences among abattoirs ($P < .001$). Thus, interventions with 2 or 3% lactic acid and hot water reduced the highest population of indicator microorganisms, while manual spray of 2% lactic acid, EGHA, and peroxyacetic acid reduced the lowest microbial population (Tables 2–4).

Whereas manual spray of 2% lactic acid, EGHA and INSPEXX 200 did not reduce aerobic plate counts significantly, the rest of the interventions significantly reduced them, in the range of 0.41 and 1.14 log cfu/400 cm^2. Based on the findings, automated application of 2% or 3% lactic acid was the most effective in reducing aerobic bacteria on surfaces of beef carcasses (Table 2).

Acetic acid efficacy to reduce aerobic counts was not apparently influenced by the mode of application, either manual or automated. Conversely, lactic acid spray cabinet greatly reduced aerobic counts compared with manual application.

Except for INSPEXX 200 and EGHA, differences in coliform counts were significant for all antimicrobial solutions. Intervention effectiveness ranged from 0.34 to 1.21 log cfu/400 cm^2. Therefore, considering the control of the effect of the initial coliform counts, 2% and 3% lactic acid automated spray treatments were the most effective antimicrobial intervention (Table 3).

Similarly to that observed in aerobic plate counts, lactic acid application elicited different reductions in coliform populations. Although differences in counts after manual and automated lactic acid spray application were significant, reductions were more evident with the latter mode of application.

Reductions of *E. coli* population were significant with all intervention treatments except manual application of 2% lactic acid, EGHA, and automated spray treatment with INSPEXX 200. Antimicrobial intervention significantly reduced *E. coli* on surface of beef carcasses ranging from 0.42 to 1.03 log cfu/400 cm^2, while the treatment with 2 or 3% lactic acid were the most effective (Table 4).

3.2. Prevalence of stx and eae genes

Differences in the prevalence of these genes on carcasses before the interventions among the abattoirs were significant ($P < .001$). The highest *stx* and *eae* prevalence was detected in abattoirs where 3% lactic acid (automated) and hot water were implemented. In contrast, the lowest prevalence of both genes was found in abattoir D, where INSPEXX 200 and 2% lactic acid (manual) were applied (Tables 5 and 6).

Interventions with 2 and 3% lactic acid (automated) and hot water showed the most important reductions in the prevalence of both *stx* (Table 5) and *eae* (Table 6) genes related with STEC. Manual or automated application of 2% acetic acid only reduced *stx* prevalence.

Table 3
Effect of interventions on coliform counts.

Intervention	Before the intervention (log cfu/400 cm^2)	After the intervention (log cfu/400 cm^2)	Log reduction	$P=$[1]
	Mean (SD)[2]	Mean (SD)	Mean (SD)[2]	
2% Citric acid automated	2.09 (0.99) [c]	1.68 (1.11)	0.40 (1.33) [d]	< .001
2% Acetic acid manual	2.21 (0.87) [c]	1.71 (1.19)	0.49 (0.99) [cd]	< .001
2% Acetic acid automated	2.92 (1.13) [b]	2.15 (1.34)	0.78 (1.48) [bc]	< .001
2% Lactic acid manual	0.77 (1.09) [e]	0.43 (1.05)	0.34 (1.43) [d]	.004
2% Lactic acid automated	1.42 (1.30) [d]	0.21 (1.05)	1.21 (1.23) [a]	< .001
3% Lactic acid automated	3.39 (0.71) [a]	2.33 (1.05)	1.06 (1.07) [ab]	< .001
EGHA manual	1.48 (1.36) [d]	1.24 (1.28)	0.24 (1.58) [d]	.059
Hot water automated	1.56 (1.39) [d]	0.81 (1.18)	0.75 (1.81) [bc]	< .001
INSPEXX 200 automated	0.83 (1.14) [e]	0.67 (1.17)	0.15 (1.47) [d]	.208

EGHA: electrolytically-generated hypochlorous acid.
[a–e] Interventions with no common letter differed significantly ($P < .05$).
[1] Paired Student's *t*-test.
[2] Differences among abattoirs using ANOVA.

Table 4
Effect of interventions on *E. coli* counts.

Intervention	Before the intervention (log cfu/400 cm^2)	After the intervention (log cfu/400 cm^2)	Log reduction	$P=$[1]
	Mean (SD)[2]	Mean (SD)	Mean (SD)[2]	
2% Citric acid automated	1.92 (0.95) [b]	1.50 (1.00)	0.41 (1.23) [bc]	< .001
2% Acetic acid manual	1.40 (0.96) [c]	0.89 (0.91)	0.50 (1.04) [b]	< .001
2% Acetic acid automated	1.79 (1.35) [b]	1.10 (1.17)	0.70 (1.50) [ab]	< .001
2% Lactic acid manual	0.49 (0.63) [e]	0.42 (0.60)	0.08 (0.85) [d]	.218
2% Lactic acid automated	1.13 (1.02) [d]	0.31 (0.47)	0.83 (0.97) [a]	< .001
3% Lactic acid automated	2.83 (0.93) [a]	1.80 (1.06)	1.03 (1.07) [a]	< .001
EGHA manual	1.17 (0.92) [d]	1.05 (0.98)	0.12 (1.18) [cd]	.171
Hot water automated	1.52 (1.15) [c]	0.92 (0.89)	0.59 (1.41) [b]	< .001
INSPEXX 200 automated	0.60 (0.75) [e]	0.47 (0.68)	0.13 (0.89) [cd]	.066

EGHA: electrolytically-generated hypochlorous acid.
[a–e]Interventions with no common letter differed significantly ($P < .05$).
[1] Paired Student's *t*-test.
[2] Differences among abattoirs using ANOVA.

Table 5
Effect of interventions on *stx* gene prevalence.

Intervention	Before the intervention (%) [2]	After the intervention (%)	$P=$[1]
2% Citric acid automated	16.0 [d]	12.0	.307
2% Acetic acid manual	32.0 [c]	21.3	.029
2% Acetic acid automated	46.6 [b]	32.0	.003
2% Lactic acid manual	5.3 [e]	7.3	.997
2% Lactic acid automated	20.7 [d]	6.1	< .001
3% Lactic acid automated	72.6 [a]	43.3	< .001
EGHA manual	70.8 [a]	57.3	< .001
Hot water automated	65.3 [a]	38.0	< .001
INSPEXX 200 automated	8.0 [e]	4.0	.180

EGHA: electrolytically-generated hypochlorous acid.
[a–e]Interventions with no common letter are significantly different ($P < .05$).
[1] McNemar test.
[2] Generalized linear model.

Table 6
Effect of interventions on *eae* gene prevalence.

Intervention	Before the intervention (%)[2]	After the intervention (%)	$P=$[1]
2% Citric acid automated	23.3 [c]	30.6	.099
2% Acetic acid manual	35.3 [b]	30.6	.392
2% Acetic acid automated	40.0 [b]	36.0	.504
2% Lactic acid manual	4.0 [d]	4.0	1.000
2% Lactic acid automated	16.4 [c]	3.6	< .001
3% Lactic acid automated	58.0 [a]	26.0	< .001
EGHA manual	42.6 [b]	43.3	.998
Hot water automated	35.3 [b]	18.6	< .001
INSPEXX 200 automated	4.6 [d]	2.6	.508

EGHA: electrolytically-generated hypochlorous acid.
[abcde]Interventions with no common letter are significantly different ($P < .05$).
[1] McNemar test.
[2] Generalized linear model.

Analysis of the intervention with 2% lactic acid (automated) showed that from 20.7% of *stx*-positive carcasses before treatment, 85% were negative after the intervention, whereas from 16.4% of carcasses positive for the *eae* gene before, > 80% were negative after the intervention.

Almost half of carcasses positive for the *stx* gene before applying 3% lactic acid (automated) were negative after the intervention. The prevalence of the *eae* gene was lower than that of the *stx* gene (58.0%), and approximately 65% of carcasses were negative after application of 3% lactic acid (26.0% reduction).

Hot water was the other successful intervention. Half of *stx*-positive carcasses before the intervention were negative after treatment. Although *eae* prevalence (35.3%) was lower than that of the *stx* gene, > 70% of *eae*-positive carcasses before the intervention were negative after its application. However, it is important to mention that some day's water temperature decreased to 80 °C in the last two working hours.

Manual spray of 2% lactic acid and INSPEXX 200 did not reduce the prevalence of both genes. Probably, the initial low prevalence in carcasses before the intervention may have influenced the low efficacy observed.

Sensory characteristics of carcasses were not modified after hot water, lactic acid (2% and 3%), EGHA, citric acid and acetic acid application. However, the strong odor produced by acetic acid application in the production environment could be considered a negative effect on the part of operators.

4. Discussion

Pathogenic *E. coli* strains are part of the natural gut microbiota in cattle. Carcass contamination may occur because of transfer of fecal material from the hide and ruptured gut or by cross-contamination at different stages of processing (Greig et al., 2012; Kanankege et al., 2017; Reyes Carranza et al., 2013). Despite many laboratory studies have shown the effectiveness of different interventions in reducing the microbial load of beef carcasses, such interventions may not be suitable for all abattoirs due to cost or space restrictions (Koohmaraie et al., 2007). Additionally, interventions should be safe, economic and feasible along the production process, widely accepted by consumers, and should not change the organoleptic properties of beef carcasses (Loretz, Stephan, & Zweifel, 2011). Our main objective was to evaluate and recommend an effective and commercially available intervention to reduce STEC contamination of beef carcasses.

Cattle from different production lots were included in each treatment group, resulting in 300 samples for each intervention. The same carcass was sampled before and after treatment to accurately evaluate the effects of each intervention and properly compare the resulting

microbial reductions. In general, the conditions created by decontamination methods to reduce overall bacterial levels, as measured by total aerobic plate counts or total coliforms, reflect the potential effects on pathogens of concern. However, this does not hold true for all cases (Huffman, 2002). In this study, the efficacy of interventions against STEC was determined under commercial abattoir conditions, assuming that natural carcass contamination would be low to provide significant results and conclusions (Greig et al., 2012; Signorini, Ponce-Alquicira, & Guerrero-Legarreta, 2006). Considering the particular problems of abattoirs, results of laboratory-tested interventions cannot be extrapolated to the actual conditions of production.

The analysis of the results obtained after application of the different interventions and of the experiences gathered in each abattoir allowed us to identify a large number of variables potentially associated with the effectiveness of the interventions. Consequently, each intervention should be evaluated independently considering the variables inherent to each abattoir and specific to each intervention.

In some abattoirs, the low prevalence of *stx* genes did not allow to obtain definitive conclusions about the effectiveness of all the evaluated interventions, which could be influenced by the initial levels of bacterial contamination. High levels of contamination, normally associated with dirt particles, are more easily removed by the interventions, whereas low levels of microbial contamination are associated with intimate adhesion to meat tissues and therefore significant reductions are not easily observed (Greig et al., 2012).

Chemical compounds, such as organic acids, have been extensively used for beef carcass decontamination. Their bactericidal activity is mainly based on the disruption of cellular membranes, other cellular constituents and physiological cellular processes (Loretz et al., 2011; Wheeler, Kalchayanand, & Bosilevac, 2014; Zweifel & Stephan, 2014). An important aspect considered in the automated application of 2% lactic acid was the high volume of the solution (2–3 l for each medium). Undoubtedly, the higher the volume of organic acid applied, the greater the effectiveness of the intervention. When organic acids are applied using automated cabinets, the volume applied will depend on factors such as the line speed, which determines the dwell time for the carcasses in the cabinet. Therefore, the optimal line speed for effective organic acid applications should also be considered.

After carcass washing with water, the drying process is different in each abattoir. We found that drying periods longer than 30 min resulted in better intervention efficacy.

Castillo et al. (2001) considered that lactic acid was more effective when applied at 55 °C and sprayed for 30 s to deliver a total volume of 0.5 l per carcass. According to DeGeer et al. (2016), lactic acid is most effective when applied at 50–55 °C. In the present study, no differences in microbial reduction were found when lactic acid was applied at 20–25 °C and sprayed for 10 s to deliver a total minimum volume of 1 l per carcass, or at > 45 °C and sprayed for 10 s using the same volume. It is likely that the temperature reached by the solution was not high enough to generate an additional antibacterial effect. However, the volume of lactic acid used per carcass must be considered, since in our study the highest efficacy was obtained when 2% lactic acid in a volume of 2 and 3 l per carcass was used. Consequently, the effect of lactic acid might have been greater if it had been applied at higher temperatures (> 55 °C). Furthermore, the corrosive effect of lactic acid on the equipment should be noted, since it would increase as temperature rises (Kalchayanand et al., 2012).

Hot water was another effective intervention to reduce microbial load on carcasses, as shown by different studies (Algino et al., 2007; Bosilevac et al., 2006; Castillo et al., 1998; Greig et al., 2012; Kanankege et al., 2017). The main factor to reduce carcass microbial load is the temperature achieved on the carcass surface rather than water temperature. In this study, hot water was applied at 85 °C during 4 s. However, Castillo et al. (1998) used hot water at 96 °C during 5 s and Bosilevac et al. (2006) applied hot water at 74 °C during 5.5 s, obtaining effective results. In the present study under real conditions, it was not possible to maintain water at least 80 °C throughout the slaughter process. Thus, strict control of water temperature would be the most important factor to consider when adopting this intervention.

Concerning the manual application of organic acids, numerous variables should be properly controlled to achieve the desired results. Some of the sensitive points identified were: a) a dependent operator, so the volume applied and its homogeneity will vary depending on the fatigue of the operator and the speed of the line, b) loss of pressure by the manual equipment, generating continuous oscillations that impact on the volume of acid applied, c) the surface of all the half-carcass is not homogeneously covered, d) the use of a single nozzle, and e) failure to spray the product.

INSPEXX 200 was approved for use on meat carcasses by FSIS (21 CFR 173.370). In our study, no significant differences in coliform and *E. coli* counts were found after treatment with peroxyacetic and octanoic acid solution *versus* control water-sprayed samples, probably due to the low coliform and *E. coli* counts observed in all sample sets. Regarding the effect of INSPEXX 200 and lactic acid manually applied, no conclusive data could be obtained due to the low carcass bacterial load of this specific abattoir before the interventions. Since intervention efficacy depends on the initial bacterial load, we could not infer that these interventions were ineffective in view of the good microbiological conditions of this abattoir. To prevent this effect, all the study should be performed in the same abattoir. However, using several abattoirs allowed us to observe and analyze different working practices and intervention applications.

Citric acid at 1 to 3% concentrations has been used to reduce *E. coli* O157: H7 and serotypes of *Salmonella* and *Listeria monocytogenes* when sprayed on beef, causing intracellular acidification (loss of homeostasis) (Laury et al., 2009). In a recent study, chilled meat samples from a bovine slaughterhouse experimentally contaminated with several bacterial pathogens, including *E. coli*, were decontaminated with organic acids (Dan, Mihaiu, Reget, Oltean, & Tăbăran, 2017). Lactic acid was the most effective, followed by acetic acid and citric acid, which was the least effective. Hussain, Rahman, Hussain, Uddin, and Ali (2015) reported that citric acid effect was significantly higher than that of 1, 3 and 5% lactic acid. Moreover, the authors recommended the use of 5% citric acid in Pakistani slaughtering environments. Itelima and Agina (2014) concluded that citric and lactic acids could not completely eliminate *E. coli* O157:H7 from food samples; however, citric acid may have more value than lactic acid as surface decontaminant of food. On the other hand, potential carcass discoloration has been reported after organic acid interventions (Loretz et al., 2011). In our study, 4% citric acid caused changes in meat color and an adequate dilution of the product was difficult to achieve. However, 2% citric acid was not enough to obtain a significant reduction in bacterial load or *stx* and/or *eae* presence.

Algino et al. (2007) reported that spraying with 2.5% acetic acid at the end of slaughter reduced *E. coli* and total coliform counts on carcasses by 0.34 and 0.22 log cfu/cm^2, respectively. The same authors also reported a reduction of aerobic bacteria > 0.5 log cfu/cm^2 in 50.8% of carcasses. In another study, (Reyes Carranza et al., 2013) reported an improved efficacy of the combination of water washing plus 2% acetic acid spray. In the present study, manual and automated application of acetic acid showed good efficacy in the reduction of indicator microorganisms, although its effect on the presence of *stx* and/or *eae* could not be definitively verified. Interestingly, the strong odor produced by acetic acid application in the production environment could be considered a negative effect on the part of operators. Thus, a better aeration system should be considered when applying acetic acid.

Electrochemically-activated solutions (ECAS) have broad-spectrum antimicrobial activity. Particularly, EGHA has been tested on different surfaces and it use authorized by FSIS (FSIS, 2017). Bosilevac et al. (2005) reported that the prevalence of *E. coli* O157 on hides was reduced from 82 to 35% following EGHA treatment, and reductions of aerobic bacteria and *Enterobacteriaceae* counts were 3.5 and 4.3 log cfu/

100 cm^2, respectively. The bactericidal activity of EGHA at free available chlorine concentration of 38 ± 2 mg/l was examined against *E. coli* O157:H7 in fish, chicken and beef, obtaining about 1.0 and 1.5 log reductions after 5 and 10 min treatment, respectively (Al-Holy & Rasco, 2015). Conversely, Kalchayanand et al. (2008) determined the effectiveness of electrolyzed oxidizing water using a model carcass spray-washing cabinet for beef head, but the decontamination strategy for *E. coli* O157:H7 reduction was not effective. In the present study, we could not obtain significant differences after manual EGHA application. In addition, some drawbacks associated with this intervention include the need to prepare the solution daily and avoid storing it. However, we did not identify sensory modifications in the product and it was widely accepted by operators.

Some variables can affect the success of bovine carcass decontamination: pressure, temperature, chemical type and concentration, exposure time, application method, cabinet design and steps in the slaughter process (Edwards & Fung, 2006). In agreement with the above mentioned, we found that the time elapsed between carcass washing and application of the interventions, the volume of the product used to wash the carcass and automated application would play a fundamental role in intervention efficacy. Carcass washing previous to decontamination would dilute the chemical product when sprayed as a mist onto the wet surfaces of carcasses (Gill, 2009). However, in the present study product application was more effective as spray than in fine droplets. Therefore, intervention effectiveness will increase if interventions are performed after sufficient time has elapsed to ensure the correct drainage of the carcass and their application is carried out under standardized conditions.

5. Conclusions

In this study, automated application of 2% lactic acid in a volume of 2 to 3 l per carcass after 30–60-min aeration, 3% lactic acid at 55 °C and hot water were the most effective interventions to reduce the counts of aerobic microorganisms, coliforms and *E. coli* and the prevalence of *stx* and *eae* genes related with STEC presence. The effectiveness of interventions improved using the automated procedure in the production line as compared with the manual procedure, since it guaranteed the homogeneous application of the product in suitable volumes. The implementation of any of the interventions evaluated here will require a precise control of variables such as application pressure, temperature, homogeneity, product concentration and time elapsed between carcass washing and application of the intervention to ensure the desired effect. Abattoir decontamination of beef carcasses through lactic acid automated application and hot water would effectively reduce public health risks associated with STEC contamination. However, these methods together with good practices during the production process should be considered as complementary measures to increase the safety of meat.

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