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Control of *Salmonella* on Beef Tissue Surfaces in a Model System by Pre- and Post-Evisceration Washing and Sanitizing, With and Without Spray Chilling

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ABSTRACT

Beef tissue was inoculated with a nalidixic acid resistant strain of *Salmonella californica* and processed under conditions simulating pre- and post-evisceration carcass washing and sanitizing. These treatments, using distilled water to wash and 2% acetic acid to sanitize, reduced the population of salmonellae by as much as 2-log₁₀ cycles when compared to samples which were washed only in distilled water. Increasing the acid temperature to 55°C reduced the bacterial populations further. Spray chilling, when used in series with the pre- and post-evisceration treatments, apparently resulted in recovery of some injured *Salmonella*.

Microbiological contamination of animal carcasses during slaughtering procedures is an undesirable but unavoidable problem in the conversion of live animals to meat for consumption. Proper dressing procedures at the abattoir can prevent a significant amount of this contamination, although contamination will still occur even in the best managed slaughter facilities. Much of the initial contamination of red meat carcasses comes from the hide during removal, especially when the hide is removed by mechanical means. The exposed surface of the hide and the hair accumulates dust, dirt, and fecal material during the animal's life; and this material is the primary source of bacterial contamination during slaughter (6). Much of the microflora transferred to the tissue surfaces, while aesthetically undesirable, is nonpathogenic. However, pathogens such as salmonellae (13), *Campylobacter jejuni* (7), and *Listeria monocytogenes* (11) can be present.

Because of the potential public health significance of these bacterial pathogens, research has been conducted to minimize contamination. Much of this research has focused on improved methods of washing carcasses (12,18), since

most carcasses are washed immediately prior to chilling to remove physical contaminants. Sanitizers, including chlorine (15,19), organic acids (1,4), quaternary ammonium compounds (2), and alkalis (14), have been effectively used to reduce the bacterial populations remaining on meat surfaces after washing. In addition to carcass washing systems, spray chilling, a process that sprays cold water on the surface of the carcasses to increase the cooling rate by evaporative cooling, has also been used to control bacterial populations. The process was originally introduced as the "chlor-chill" process (16,23).

The previous control measures have been employed as the final steps in the slaughter process. Recently, patents were issued for processes of washing and sanitizing intact carcasses immediately after removal of the hide, prior to evisceration (9,10). These processes consist of removing the hide from the carcass, water rinsing followed immediately by spraying with acetic acid, evisceration, rinsing with water again followed immediately by spraying with acetic acid (Fig. 1). The intent of these processes is to begin cleaning the carcass immediately after initial contamination, specifically after removal of the hide.

Figure 1. Diagram of beef slaughter operations. Solid lines indicate standard procedures. Hollow lines indicate additional procedures to control bacteria.

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²Mention of trade names, proprietary products, or specific equipment does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

The objectives of the present study were to determine the effectiveness of pre- and post-evisceration washing and sanitizing in reducing the populations of *Salmonella californica* on beef tissue. These processes were used independently and also in combination with spray chilling in a model system. This was a feasibility study to establish processing guidelines for future research with equipment which is currently under construction at the Roman L. Hruska U.S. Meat Animal Research Center (RLHUSMARC) abattoir.

MATERIALS AND METHODS

Bacterial culture

Salmonella californica (Dr. Norman J. Stern, USDA-ARS, Richard B. Russell Research Center, Athens, GA.), an isolate naturally resistant to nalidixic acid, was grown and maintained in tryptic soy broth (TSB, Difco, Detroit, MI) at 37°C. The culture was transferred to individual tubes of TSB and incubated aerobically in a gyratory incubator at 37°C for 18 h prior to use. Manure was collected from cattle fed on a corn silage diet and used within 2 h after collection. A suspension of *S. californica* was prepared with 35 ml of the 18 h culture diluted to 99 ml in Butterfield's phosphate buffer (20). This suspension was added to 100 g of manure and mixed in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH) for 2 min.

Tissue preparation

Postrigor beef tissue was obtained as boneless trim from the abattoir at the RLHUSMARC. The tissue was separated into lean and fat tissue, sliced into 0.5 cm thick slices, frozen in sterile sealed bags, sterilized with gamma radiation at a minimum dose of 42 kGy, and stored at -20°C until use. The tissue slices were partially thawed and aseptically cut into 3.0 x 1.0 x 0.5 cm pieces (10 cm² surface area) as needed. Tissue samples were inoculated by immersion in inoculated manure for 5 min at 23°C and were then removed to a sterile petri dish for 5 min, allowing for additional bacterial attachment. Treatment protocols began immediately after inoculation. Tissue samples were transferred to sterile square jawed alligator clips (Radio Shack, Fort Worth, TX) to facilitate handling during the simulated spray chilling cycles.

Experimental design

Carcass washing and sanitizing were simulated by vortexing the tissue samples in 20 ml of sterile distilled water or sterile 2% (v/v) acetic acid volumes on a Vortex Genie 2 (Scientific Industries, Bohemia, NY) set at 75% maximum speed for 10 s. Spray chilling was simulated by briefly dipping the tissue samples in 5°C sterile distilled water or sterile 2% acetic acid at 30 min intervals for 4 h. Between each time interval, the tissue samples were suspended by the clips in separate sterile beakers at 5°C, such that the samples did not contact the sides of the beakers. The beakers were loosely covered with foil which minimized additional contamination but still allowed for the normal dehydration of tissue surfaces. Protocols for experiments 1 through 4 are given in Fig. 2 and 3. Samples were processed in the "pre-" (wash and sanitize) treatments as indicated, and then held for 10 min in a sterile tube prior to the final wash and sanitizing treatments. This holding period simulated the delay in processing which would normally occur between the removal of the hide and washing of beef carcasses in processing situations.

Experiment 1. This initial experiment was intended to quantify reductions in bacterial populations attributable to a second washing and sanitizing procedure (Fig. 2). Both water and acid solutions were maintained at 23°C.

EXPERIMENT 1

TREATMENT	TREATMENT NUMBER	WATER PRE-WASH	ACID PRE-SANIT.	WATER WASH	ACID SANITIZE
WASH	1-1	Y	-	-	-
ACID	1-2	-	Y	-	-
WSH/ACD	1-3	Y	Y	-	-
WSH/WSH	1-4	Y	-	Y	-
WSH/ACD/WSH	1-5	Y	Y	Y	-
WSH/ACD/WSH/ACD	1-6	Y	Y	Y	Y

EXPERIMENT 2

TREATMENT	TREATMENT NUMBER	WATER PRE-WASH TEMP.	ACID PRE-SANIT. TEMP.	WATER WASH TEMP.	ACID SANITIZE TEMP.
WASH	2-1	23	NA	NA	NA
WSH/ACD/WSH/ACD	2-2	23	23	23	23
WSH/ACD/WSH/ACD	2-3	23	40	23	40
WSH/ACD/WSH/ACD	2-4	23	55	23	55

EXPERIMENT 3

TREATMENT	TREATMENT NUMBER	WATER PRE-WASH TEMP.	ACID PRE-SANIT. TEMP.	WATER WASH TEMP.	ACID SANITIZE TEMP.
WASH	3-1	23	NA	NA	NA
WSH/WSH	3-2	23	NA	23	NA
WSH/ACD/WSH/ACD	3-3	23	23	23	23
WSH/WSH	3-4	55	NA	55	NA
WSH/ACD/WSH/ACD	3-5	23	55	23	55

Figure 2. Experimental protocols for experiments 1, 2, and 3. Temperatures expressed as °C; Acid = 2% acetic acid; Y = simulated treatment applied; - or NA = not applicable.

EXPERIMENT 4

TREATMENT	TREATMENT NUMBER	WATER PRE-WASH TEMP.	ACID PRE-SANIT. TEMP.	WATER WASH TEMP.	ACID SANITIZE TEMP.	SPRAY CYCLE
WASH	4-1	23	NA	NA	NA	NA
WSH/WSH	4-2	23	NA	23	NA	A
WSH/ACD/WSH/ACD	4-3	23	55	23	55	A
WSH/WSH	4-4	23	NA	23	NA	B
WSH/ACD/WSH/ACD	4-5	23	55	23	55	B
WSH/WSH	4-6	23	NA	23	NA	C
WSH/ACD/WSH/ACD	4-7	23	55	23	55	C

SPRAY CYCLES

TREATMENT	0	30	60	90	120	150	180	210	240
SPRAY A	W	W	W	W	W	W	W	W	W
SPRAY B	W	W	W	W	A	W	W	W	A
SPRAY C	A	A	A	W	W	W	W	W	W

Figure 3. Experimental protocol for experiment 4, including spray cycles. Temperature expressed as °C; Acid = 2% acetic acid; NA = not applicable. Spray cycles: W = distilled water; A = 2% acetic acid; all solutions maintained at 5°C.

Experiments 2 and 3. Experiment 2 (Fig. 2) evaluated the effect of elevated acid temperature and elevated water temperature, respectively, on numbers of residual bacteria.

Experiment 4. This experiment included each previous treatment in combination with simulated spray chilling cycles.

Bacterial enumeration

Tissue samples were homogenized immediately after the final procedure in 99 ml Butterfield's phosphate buffer for 2 min in a Stomacher 400. Samples were serially diluted in phosphate

buffer and bacterial populations enumerated on tryptic soy agar (TSA, Difco) with added nalidixic acid and novobiocin, MacConkey agar base (Difco) with 10% (wt/vol) lactose) and added nalidixic acid, and EF-18 media (QA Laboratories, Toronto, Canada) with nalidixic acid using the surface plate technique (8). Nalidixic acid sodium salt and Novobiocin (Sigma, St. Louis, MO) were prepared as aqueous solutions, filter sterilized through 0.45 μ m filters, and added to tempered media to a final concentration of 200 mg/L (nalidixic acid) and 15 mg/L (novobiocin). The plates were incubated at 37°C for 24-48 h, and colonies typical of *Salmonella* sp. on the selective media were counted.

Statistical analysis

The numbers of salmonellae attached to the tissue surfaces were converted to $\log_{10}$ colony forming units/cm². Statistical analysis was conducted using the General Linear Models procedure of the Statistical Analysis System (21) using models appropriate to the completely randomized design of the experiments. Numbers of salmonellae on the washed samples (1-1, 2-1, 3-1, and 4-1) for each replication were used as a covariate in the analysis. Three independent replications were performed for each experiment. Unless otherwise noted, significance is expressed at the 5% level.

RESULTS AND DISCUSSION

Salmonellae on the inoculated tissue samples, prior to any treatment, typically averaged 1- to 1.5- $\log_{10}$ cycles higher than populations on washed (treatments 1-1, 2-1, 3-1, and 4-1) samples. However, because of the extreme variation in amounts of manure adhering on individual tissue surfaces after inoculation, these counts were not included in the data analysis. Counts of washed samples were used as control values since washing removed the adhering manure and provided a stable reference point for all other treatments. Although the following discussion will focus on reductions of bacterial populations in reference to the washed samples, the actual reductions would be at least 1 $\log_{10}$ greater than those reported here if the original unwashed inoculated samples were used as a reference point.

Initial experiments were conducted to determine the reductions attributable to a second washing and sanitizing treatment (Table 1). A single washing and sanitizing treatment (treatment 1-3) reduced the population of salmonellae by an additional 1.2- (lean) to 1.5- (fat) $\log_{10}$ cycles as determined with MacConkey and EF-18 agars when compared to washing alone (treatment 1-1). This is in agreement with previously published research, which indicates that carcass washing will reduce bacterial populations by approximately 1 $\log_{10}$, and that washing followed by acid sanitizing will reduce bacterial populations by an additional $\log_{10}$ (3). When two washing and sanitizing cycles separated by a 10-min interval were used in combination (treatment 1-6), there was a reduction in the populations when compared to the single wash and sanitizing treatment, although this reduction was generally not statistically significant. The average population reduction was approximately 2- $\log_{10}$ cycles when compared to the washed samples. Treatments including sanitizing procedures (1-2, 1-3, 1-5, and 1-6) were generally producing significantly lower counts than treatments which only included washing procedures.

TABLE 1. Reduction in numbers of *Salmonella californica* on beef tissue surfaces by treatments simulating pre- and post-evisceration washing and sanitizing. See Fig. 2, experiment 1, for protocols of each treatment number.

Tissue	Treatment number	Bacterial population ¹		
		TSA	Mac	EF
Lean	1-1	4.09 ^{2a}	4.02 ^a	4.00 ^a
	1-2	3.50 ^b	3.16 ^b	3.19 ^b
	1-3	2.97 ^{c,d}	2.68 ^{b,c}	2.85 ^b
	1-4	3.23 ^{b,d}	2.91 ^b	2.94 ^b
	1-5	2.91 ^c	2.80 ^{b,c}	2.57 ^c
	1-6	2.72 ^c	1.98 ^c	2.12 ^c
Fat	1-1	4.20 ^a	4.04 ^a	3.99 ^a
	1-2	3.73 ^a	3.37 ^b	3.42 ^{a,b}
	1-3	2.72 ^b	2.44 ^c	2.53 ^c
	1-4	3.07 ^b	3.17 ^b	3.21 ^b
	1-5	3.00 ^b	2.79 ^{b,c}	2.77 ^{b,c}
	1-6	2.37 ^b	2.28 ^c	2.32 ^c

¹Mean $\log_{10}$ colony forming units/cm²; average of three independent replications; TSA = tryptic soy agar + novobiocin + nalidixic acid, Mac = MacConkey agar + nalidixic acid, EF = EF-18 agar + nalidixic acid.

²Means with different superscripts are significantly different ($P < 0.05$). Bacterial populations from each medium analyzed separately.

As temperature of the acetic acid was raised in 15°C increments, there was a reduction in populations as determined by all three media, although this decrease was generally not statistically significant (Table 2). This trend is similar to previously published reports on the interactions of temperature and acid concentration, when samples were dipped once (4,5). The populations of bacteria enumerated on TSA averaged approximately 1.7 $\log_{10}$ cycles higher than those on MacConkey agar and approximately 1.2 cycles higher than those on EF-18. Part of this difference is attributable to the probable failure of injured salmonellae cells to grow on the more selective media. Also, TSA enumerated all novobiocin and nalidixic acid resistant aerobic bacteria. The actual salmonellae population, both noninjured and injured cells, is most likely an intermediate figure between the populations estimated by TSA and the more selective media.

Hot water has been used effectively as a decontaminant with mutton carcasses (22). However, when water was applied as in a simulated pre- and post-evisceration washing process (treatments 3-2 and 3-4), there was no significant difference in bacterial populations between the 23 and 55°C water temperatures (Table 3). Significant population reductions were seen with pre- and post-evisceration washing and sanitizing with 55°C acetic acid (treatment 3-5) when compared to washing alone with either 23 or 55°C water. As previously noted, the estimated populations were also higher on TSA than on the selective media.

Since spray chilling is one of the dominant cooling methods used in commercial meat operations (17), the pre- and post-evisceration washing and sanitizing treatments were used in series with simulated spray chilling cycles (Fig. 3). The spray cycles included a standard treatment

TABLE 2. Reduction in numbers of *Salmonella californica* on beef tissue surfaces by treatments simulating pre- and post-evisceration washing and sanitizing with elevated 2% acetic acid temperatures. See Fig. 2, experiment 2, for protocols of each treatment number.

Tissue	Treatment number	Acid temp.	Bacterial population ¹		
			TSA	Mac	EF
Lean	2-1	NA ²	5.36 ^{3a}	5.16 ^a	5.33 ^a
	2-2	23	3.83 ^b	2.85 ^b	3.28 ^b
	2-3	40	3.50 ^b	1.94 ^{b,c}	2.07 ^b
	2-4	55	3.68 ^b	1.73 ^c	2.39 ^b
Fat	2-1	NA	4.84 ^a	4.59 ^a	4.72 ^a
	2-2	23	3.04 ^a	1.56 ^b	2.10 ^b
	2-3	40	2.76 ^b	1.24 ^b	1.37 ^b
	2-4	55	2.41 ^b	0.99 ^b	1.19 ^b

¹Mean log₁₀ colony forming units/cm²; average of three independent replications; TSA = tryptic soy agar + novobiocin + nalidixic acid, Mac = MacConkey agar + nalidixic acid, EF = EF-18 agar + nalidixic acid.

²NA = not applicable; other temperatures given as °C.

³Means with different superscripts are significantly different (P<0.05). Bacterial populations from each medium analyzed separately.

TABLE 3. Reduction in numbers of *Salmonella californica* on beef tissue surfaces by treatments simulating pre- and post-evisceration washing with 55°C water or washing and sanitizing with 55°C 2% acetic acid. See Fig. 2, experiment 3, for protocols of each treatment number.

Tissue	Treatment number	Bacterial population ¹		
		TSA	MAC	EF
Lean	3-1	4.86 ^{2a}	4.76 ^a	4.82 ^a
	3-2	4.13 ^{ab}	4.07 ^a	4.11 ^{ab}
	3-3	3.58 ^{bc}	2.24 ^b	2.62 ^c
	3-4	4.14 ^{ab}	3.92 ^a	3.87 ^b
	3-5	3.45 ^c	1.86 ^b	2.15 ^c
Fat	3-1	4.63 ^a	4.37 ^a	4.54 ^a
	3-2	3.90 ^a	3.82 ^{ab}	3.79 ^{ab}
	3-3	2.98 ^b	1.87 ^c	1.86 ^c
	3-4	3.74 ^{ab}	3.28 ^b	3.45 ^b
	3-5	2.04 ^c	1.01 ^c	1.16 ^c

¹Mean log₁₀ colony forming units/cm²; average of three independent replications; TSA = tryptic soy agar + novobiocin + nalidixic acid, Mac = MacConkey agar + nalidixic acid, EF = EF-18 agar + nalidixic acid.

²Means with different superscripts are significantly different (P<0.05). Bacterial populations from each medium analyzed separately.

using 5°C water (cycle A), as well as two modified cycles which incorporated 5°C, 2% acetic acid at certain points in the cycle (cycles B and C). There was a reduction in bacterial populations on lean tissue of less than 1-log₁₀ cycle on TSA and EF-18 when pre- and post-evisceration washing and sanitizing with 55°C acetic acid treatments were used in series with spray chilling with water only (Table 4, treatment 4-3). Spray chilling apparently permit-

TABLE 4. Reduction in numbers of *Salmonella californica* on beef tissue surfaces by treatments simulating pre- and post-evisceration washing and sanitizing in series with spray chilling cycles. See Fig. 3 for protocols of each treatment number.

Tissue	Treatment number	Bacterial population ¹		
		TSA	Mac	EF
Lean	4-1	5.80 ^{2a}	5.61 ^a	5.62 ^a
	4-2	5.13 ^b	4.93 ^b	4.98 ^b
	4-3	5.13 ^b	4.29 ^c	4.73 ^b
	4-4	5.01 ^b	3.79 ^d	4.47 ^{b,c}
	4-5	4.90 ^b	3.48 ^d	4.02 ^c
	4-6	5.23 ^b	4.79 ^b	4.66 ^b
	4-7	5.07 ^b	3.64 ^d	4.04 ^c
Fat	4-1	5.66 ^a	5.54 ^a	5.51 ^a
	4-2	4.89 ^b	4.73 ^b	4.71 ^b
	4-3	3.11 ^c	2.83 ^c	2.75 ^c
	4-4	4.43 ^d	3.40 ^d	3.94 ^d
	4-5	2.86 ^c	2.66 ^c	2.69 ^c
	4-6	4.45 ^d	3.40 ^d	3.80 ^d
	4-7	3.54 ^c	2.42 ^c	2.83 ^c

¹Mean log₁₀ colony forming units/cm²; average of three independent replications; TSA = tryptic soy agar + novobiocin + nalidixic acid, Mac = MacConkey agar + nalidixic acid, EF = EF-18 agar + nalidixic acid.

²Means with different superscripts are significantly different (P<0.05). Bacterial populations from each medium analyzed separately.

ted recovery of injured cells, since these reductions were less than those seen in identical treatments without spray chilling (compare treatment 4-3 with 2-4 and 3-5). This apparent recovery was limited to lean tissue, since comparable reductions were seen on fat tissue with or without spray chilling.

The inclusion of acetic acid in the spray chilling cycle resulted in additional reductions in the bacterial populations on lean tissue as determined by the selective agars, although there was no difference in the TSA counts. There was no difference in bacterial populations on fat tissue with any of the treatments using pre- and post-evisceration 55°C acetic acid (treatments 4-3, 4-5, and 4-7), irrespective of the type of spray cycle. When used in series with pre- and post-washing without sanitizing, acetic acid in the spray cycle reduced the bacterial populations on fat tissue, although there were no significant differences on lean tissue.

This study indicates that modifying existing slaughter procedures to include pre- and post-evisceration washing and sanitizing procedures can reduce the numbers of salmonellae on beef tissue surfaces. Greatest reductions in bacterial populations were seen on fat tissue, which is significant in that much of the surface of a beef carcass is covered with a layer of fat. Further investigations will be conducted to determine the fate of the population of salmonellae, both viable and injured, remaining on the tissue after washing and sanitizing.

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