

Evaluation of "Zero Tolerance" Final Rail Inspection Conducted in Commercial Beef Processing Facilities

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SUMMARY

This study evaluated "Zero Tolerance" final rail inspection, at its present location in the slaughter/dressing process, to determine its impact or contribution to the production of microbiologically acceptable beef carcasses. Carcasses (N = 300) were evaluated by sponge swabbing and subsequently were tested to determine total plate counts (TPC), total coliform counts (TCC) and *Escherichia coli* counts (ECC). Sampling occurred at three different in-plant locations in each of five commercial beef slaughter facilities. Overall, there were no differences ($P > 0.05$) in mean log CFU/100 cm² values for TPC, TCC and ECC between a point immediately before "Zero Tolerance" final rail inspection and a point immediately following. However, average post-inspection TPC, TCC and ECC were reduced ($P < 0.05$) by 1.4, 1.1 and 1.0 log CFU/100 cm², respectively, following the application of thermal pasteurization and post-evisceration carcass washing/organic acid solution rinsing.

INTRODUCTION

In 1992-1993, undercooked ground beef, contaminated with *Escherichia coli* O157:H7, was involved in an outbreak of foodborne illness that affected several hundred people in the western United States (Bell *et al.*, 1994; Sofos and Smith, 1993). Following that outbreak, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) issued a policy, known as "Zero Tolerance", that

required the removal of feces, ingesta and udder contents from beef carcasses, by trimming, before carcass washing as a means of improving the cleanliness and microbiological status of beef (FSIS-USDA, 1993).

MATERIALS AND METHODS

Microbiological samples (N = 300) were collected at five beef packing plants, of which four were steer/heifer plants and the other was a bull/cow plant. In the first three steer/heifer operations, the decontamination system evaluated consisted of: (a) steam vacuuming, (b) pre-evisceration washing plus acetic acid solution rinsing, (c) thermal pasteurizing, and (d) final washing plus acetic acid solution rinsing. In steer/heifer operation number 4, the decontamination system was identical to that used in plants 1 through 3 except that acetic acid solution rinsing was not used in association with either pre-evisceration washing or final washing. In the bull/cow plant, the decontamination system consisted of: (a) steam vacuuming, (b) thermal pasteurizing, and (c) final washing plus lactic acid solution rinsing. Sponge sampling took place at the following three locations: (1) after the activities of in-house trimmers, but immediately before the "Zero Tolerance" final rail inspection; (2) immediately following the "Zero Tolerance" final rail inspection, and; (3) after final washing plus organic acid solution rinsing but before chilling. At each in-plant sampling location, for each system evaluated, 20 carcass sides were sampled and tested for total plate counts (TPC), total coliform counts (TCC) and *Escherichia coli* counts (ECC). Sampled carcasses were tracked through the entire slaughtering/dressing process and tag transfer was documented in order to prevent the resampling of any carcass and to insure that, when needed, samples were collected from adjacent, same-lot carcasses as those sampled previously.

Sample Collection and Enumeration. Sampling by sponging followed the procedures described in the

USDA/FSIS Meat and Poultry Inspection regulations (FSIS-USDA, 1996). Immediately before sampling, sterile sponges (BioPro Enviro-Sponge Bags, International BioProducts, Redmond, WA) were hydrated with 10 ml of 0.1% sterile, buffered peptone water (BioPro, International BioProducts). Sampling of each carcass side was achieved using a 100 cm² disposable, sterile template (USDA Template, International BioProducts) at each of three anatomical locations, for a total sampling area of 300 cm². Sponging at each carcass site, within the 100 cm² template area, consisted of 10 passes vertically (up-and-down being considered as 1 pass) and 10 passes horizontally (side-to-side being considered as 1 pass) with a pressure equivalent to that which would be used to remove dried blood from the carcass.

The three anatomical carcass sites included: (a) flank, at a point where the medial border of the cutaneous flank muscle came within 7.62 cm of the midline, (b) brisket, at a point on the midline that was level with the elbow and (c) rump, at a point where a line from the posterior aspect of the aitch bone to the achilles tendon intersected the cut surface of the round (FSIS-USDA, 1996b). Samples were collected aseptically using sterile, latex gloves (International BioProducts) which, in addition to the template, were changed between sampling of each carcass side. An additional 15 ml of refrigerated 0.1% sterile, buffered peptone water (Difco Laboratories, Detroit, MI) was added to the sponge after sampling to bring the total volume of buffer to 25 ml. After excess air was expelled and the sponge bags were folded down, the samples were packed with icepacks and a cardboard pad, to prevent direct contact with the samples, into shipping coolers for overnight delivery to the laboratory (Warren Analytical Laboratory, Greeley, CO) for analysis.

Following arrival of samples at the laboratory, the sponges and associated buffer were pummeled in a stomacher (Seward Model 400, Tekmar

Company, Cincinnati, OH) for 1 min and appropriate serial dilutions were made based on past test and background sample results. Appropriate dilutions were plated on Plate Count Agar (PCA) (Difco Laboratories) using a spiral plating system (Spiral Systems Instruments, Inc., Cincinnati, OH). Plates were incubated at 35°C +/- 2°C for 48 h at which point colonies were counted using a laser bacteria colony counter (Model 500A, Spiral Systems Instruments, Inc.) and a computer assisted spiral bio-assay (CASBA) data processor with Bacterial Enumeration Program E20 (Spiral Systems, Inc.). Additionally, appropriate dilutions were placed on Petrifilm™ *Escherichia coli* count plates (3M™ Health Care, St. Paul, MN) and, following a 24 +/- 2 h incubation period at 35°C +/- 2°C, colonies associated with trapped gas and possessing a dark red or blue color were counted as coliforms, while dark blue colonies associated with trapped gas were separately counted as *E. coli* biotype I.

Statistical Analysis. The data (TPC, TCC and ECC) were transformed to log₁₀ CFU/100 cm² for statistical analyses. Minimum detection limits for TPC, TCC and ECC were 2.2, 0.9 and 0.9 log CFU/100 cm², respectively, based on the maximum sensitivity of the tests with no further dilution of the samples beyond the original buffer volume of 25 ml. TPC, TCC and ECC falling below the minimum detection limit were entered as 2.2, 0.9, and 0.9 log CFU/100 cm², respectively, so that statistical analysis could be performed. Values for the mean log (\bar{x}) and standard deviation of each set of bacterial counts were calculated on the assumption of a log-normal distribution of microorganisms (Brown and Baird-Parker, 1982; Gill *et al.*, 1996; Gill and Bryant, 1997; Kilsby and Pugh, 1981). A value for the log mean (log α) for each set of bacterial counts was calculated from the formula $\text{Log } \alpha = \mu + \ln 10\sigma^2$ in an attempt to estimate the true log average count (Gill *et al.*, 1996; Gill and Bryant, 1997; Kilsby and Pugh, 1981). Data were evaluated with

analysis of variance (AOV) using the model $y = a + x_1 + x_2 + x_1x_2$ and means were computed for TPC, TCC and ECC by plant (x_1), site (x_2), and plant x site fixed effects using the General Linear Models procedure of SAS® (SAS, 1995). Due to the significant Plant x Site interaction (P = 0.0001), only interaction subclass means are reported (Tables 1-3). When AOV detected effects (P < 0.05), means were separated using Tukey's HSD (SAS, 1995).

RESULTS AND APPLICATION

"Zero Tolerance" final rail inspection made no difference (P > 0.05) in TPC, TCC and ECC in plants 1 and 2, while TPC increased (P < 0.05) following inspection in plant 3 and TPC, TCC and ECC increased (P < 0.05) following inspection in plant 4 (Tables 1-3). This increased contamination could be a result of the additional handling of the carcass that takes place during the inspection process. Conversely, TPC and ECC were reduced (P < 0.05) following inspection in plant 5 (Tables 1 and 3). Across all plants, there were no differences (P > 0.05) in mean log CFU/100 cm² values for TPC, TCC and ECC between a point immediately before "Zero Tolerance" final rail inspection and a point immediately following "Zero Tolerance" final rail inspection (Tables 1-3). There were, however, reductions (P < 0.05) in TPC, TCC and ECC of 1.4, 1.1 and 1.0 log CFU/100 cm², respectively, as mean counts of 5.3, 2.5 and 1.9 log CFU/100 cm², respectively, following "Zero Tolerance" final rail inspection were reduced to 3.9, 1.4 and 0.9 log CFU/100 cm², respectively, at a point following thermal pasteurizing and final washing plus organic acid solution rinsing (Tables 1-3).

Lastly, results of the present study demonstrate that microbiological populations found on beef carcasses are not solely confined to, or in close proximity of, areas of visible contamination. However, the effective removal of visible contamination will continue to remain esthetically important.

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Table 1. Means (\bar{x}) and standard deviations (s) for the \log_{10} values of total plate counts (CFU/100 cm²), the number of samples (n) in which total plate counts were not detected at a detection limit of 2.2 log CFU/100 cm², and estimated logs for the arithmetic means (log α) of total plate counts on beef carcasses at each plant and in-plant location in the harvesting sequence.

Plant	In-Plant Location in the Harvesting Sequence											
	Before “Zero Tolerance” Final Rail Inspection				After “Zero Tolerance” Final Rail Inspection				After Post-Evisceration Carcass Washing and any Organic Acid Solution Rinsing			
	\bar{x}	s	n	log α	\bar{x}	s	n	log α	\bar{x}	s	n	log α
1	5.2 ^a	0.00	0	5.2	5.0 ^a	0.61	0	5.4	3.3 ^b	0.84	4	4.1
2	4.5 ^a	1.54	0	7.2	3.9 ^a	0.61	0	4.3	2.9 ^b	0.91	6	3.9
3	4.9 ^b	0.67	0	5.4	5.6 ^a	1.10	0	7.0	3.6 ^c	0.50	0	3.9
4	4.8 ^b	0.57	0	5.2	5.4 ^a	0.90	0	6.3	4.4 ^b	0.57	0	4.8
5	8.5 ^a	0.69	0	9.0	6.9 ^b	0.95	0	7.9	5.5 ^c	1.55	0	8.3
Average	5.6 ^a	1.71	0	9.0	5.3 ^a	1.27	0	7.2	3.9 ^b	1.31	10	5.9

Number of samples analyzed at each location in each plant: 20.

$$\text{Log } \alpha = \bar{x} + \ln 10 s^2 2^{-1}.$$

^{abc} Means in the same row with different superscript letters are different (P < 0.05).

Table 2. Means (\bar{x}) and standard deviations (s) for the \log_{10} values of total coliform counts (CFU/100 cm²), the number of samples (n) in which total coliform counts were not detected at a detection limit of 0.9 log CFU/100 cm², and estimated logs for the arithmetic means (log α) of total coliform counts on beef carcasses at each plant and in-plant location in the harvesting sequence.

Plant	In-Plant Location in the Harvesting Sequence											
	Before “Zero Tolerance” Final Rail Inspection				After “Zero Tolerance” Final Rail Inspection				After Post-Evisceration Carcass Washing and any Organic Acid Solution Rinsing			
	\bar{x}	s	n	log α	\bar{x}	s	n	log α	\bar{x}	s	n	log α
1	1.4 ^a	0.39	4	1.6	1.3 ^a	0.46	3	1.5	0.9 ^b	0.00	20	0.9
2	1.7 ^a	0.59	1	2.1	2.0 ^a	0.77	3	2.7	0.9 ^b	0.15	16	0.9
3	2.2 ^b	0.81	1	3.0	2.8 ^a	0.87	0	3.7	0.9 ^c	0.14	17	0.9
4	2.3 ^a	0.71	1	2.9	2.8 ^a	0.71	0	3.4	1.5 ^b	0.79	10	2.2
5	4.2 ^a	1.27	1	6.1	3.3 ^{ab}	0.84	0	4.1	2.7 ^b	1.47	3	5.2
Average	2.3 ^a	1.26	8	4.1	2.5 ^a	1.00	6	3.7	1.4 ^b	1.02	66	2.6

Number of samples analyzed at each location in each plant: 20.

$$\text{Log } \alpha = \bar{x} + \ln 10 s^2 2^{-1}.$$

^{abc} Means in the same row with different superscript letters are different (P < 0.05).

Table 3. Means (\bar{x}) and standard deviations (s) for the \log_{10} values of *Escherichia coli* counts (CFU/100 cm²), the number of samples (n) in which total coliform counts were not detected at a detection limit of 0.9 log CFU/100 cm², and estimated logs for the arithmetic means ($\log \alpha$) of *Escherichia coli* counts on beef carcasses at each plant and in-plant location in the harvesting sequence.

Plant	In-Plant Location in the Harvesting Sequence											
	Before “Zero Tolerance” Final Rail Inspection				After “Zero Tolerance” Final Rail Inspection				After Post-Evisceration Carcass Washing and any Organic Acid Solution Rinsing			
	\bar{x}	s	n	$\log \alpha$	\bar{x}	s	n	$\log \alpha$	\bar{x}	s	n	$\log \alpha$
1	1.1 ^{ab}	0.32	10	1.2	1.1 ^a	0.38	6	1.3	0.9 ^b	0.00	20	0.9
2	0.9 ^{ab}	0.01	18	0.9	1.0 ^a	0.20	15	1.0	0.9 ^b	0.00	20	0.9
3	1.6 ^b	0.68	3	2.1	2.3 ^a	0.95	2	3.3	0.9 ^c	0.01	19	0.9
4	1.8 ^a	0.76	3	2.5	2.2 ^a	0.79	2	2.9	1.0 ^b	0.41	16	1.2
5	4.1 ^a	1.28	1	6.0	3.0 ^b	0.79	0	3.7	1.0 ^c	0.25	16	1.1
Average	1.9 ^a	1.37	35	4.1	1.9 ^a	1.03	25	3.1	0.9 ^b	0.22	91	1.0

Number of samples analyzed at each location in each plant: 20.

$$\log \alpha = \bar{x} + \ln 10 s^2 2^{-1}.$$

^{abc} Means in the same row with different superscript letters are different (P < 0.05).