Extent of Beef Carcass Contamination with *Escherichia coli* and Probabilities of Passing U.S. Regulatory Criteria

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ABSTRACT

In the 1996 U.S. Meat and Poultry Inspection Regulations, *Escherichia coli* biotype I counts were included as "performance criteria" of the slaughtering process. The criteria were based on a three-class attributes sampling plan applied in a moving window. The values for m and M and c and n were set at 5 and 100 CFU/cm^2, and 3 and 13 samples, respectively, for beef carcasses after overnight chilling following slaughter. In this study, beef carcasses were analyzed for counts of *E. coli,* and the results were expressed according to the above criteria. Furthermore, probabilities of passing *E. coli* performance criteria were determined. Carcasses were sampled in seven slaughtering plants (four steer and heifer; three cow and bull), during two seasons, and at three plant locations (pre-evisceration, after final carcass washing, and after 24 h of carcass chilling). Each entire carcass sample (100 cm^2 from the brisket, flank, and rump) was analyzed individually for *E. coli* counts. Compared with the regulation, which set the value of m and the acceptable range based on the 80th percentile of *E. coli* contamination data from U. S. Food Safety and Inspection Service nationwide baseline studies, our results showed that, on the average and depending on plant and season, 84.2 to 100% of the chilled carcass samples were in the acceptable range. The average percentages of chilled samples in the unacceptable range, set at the 98th percentile, were 0 to 6.7%. Depending on plant and season, the overall probabilities of chilled carcasses passing the regulatory requirement were 0.597 to 1.0 (brisket), 0.471 to 1.0 (flank), and 0.485 to 1.0 (rump). The results indicated substantial variation among plants and between seasons in ability to meet the *E. coli* performance criteria.

The 1996 U. S. Meat and Poultry Inspection Regulations *(4)* established the requirement for *Escherichia coli* enumeration as a means of "verifying that the slaughter process is under control." This decision was based on the premise *(6)* that *E. coli* testing is an indicator of fecal contamination. Establishment of "*E. coli* performance criteria to verify process control" used data from previously published U.S. Food Safety and Inspection Service (FSIS) beef carcass contamination baseline studies *(2, 3)*. It is stated in the regulation *(4)* that the established "criteria are not enforceable regulatory standards" but "are intended to assist slaughter establishments and FSIS in ensuring that establishments are meeting their current statutory obligation to prevent and reduce contamination of carcasses by fecal material, ingesta, and associated bacteria" *(4)*.

The *E. coli* testing performance criteria established by FSIS were based on the principles of a three-class attributes sampling plan *(6, 9)* applied in a moving window *(4)*. The three-class plan concept specifies contamination level cutoff points denoted by m and M, with m > M, which define an "acceptable" (≤m), a "marginal" (>m and ≤M), and an "unacceptable" (>M) result. The levels of contamination selected by FSIS to establish the m and M cutoff points were based on baseline contamination data collected by FSIS during nationwide surveys of carcasses, sampled by excision *(2, 3)*. For steer and heifer and for cow and bull

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carcasses, the m point was set at 5 CFU/cm^2, which was the limit of detection in the FSIS baseline studies, corresponded with the 80th percentile of those baselines, and was considered as the current industry-wide performance in terms of *E. coli* contamination levels *(4)*. The point M was set at 100 CFU/cm^2, which was the 98th percentile of the FSIS baseline studies. Furthermore, FSIS limited the number of samples in the marginal range to no more than 3 (value of c) results above m and up to M within any consecutive 13 (value of n) samples tested in a moving window. These numbers were also selected on the basis of an 80% probability level *(4)*. Based on this, if the results of one test are above M, or if more than 3 of 13 test results are between m and M, FSIS indicated that this would raise a question as to whether the establishment was maintaining adequate process control and would trigger a review of those process controls. However, FSIS indicated that these *E. coli* criteria were guidelines and not regulatory standards and that, ideally, each establishment would develop its own criteria for process control based on its own data and/or industry-developed benchmarks *(4)*.

Results above the value of M are considered unacceptable and are expected to trigger immediate review of slaughter process controls to determine the reason for their occurrence and prevent reoccurrence *(4)*. FSIS indicated that it would use *E. coli* test results in its efforts to assess how well the establishment was controlling the slaughter and dressing process. According to the regulation, a single

failure would "trigger greater inspection activity," while repeated failures would be considered an indication of "inadequate process control." The objective was, with implementation of hazard analysis critical control point (HACCP) programs, to continue testing for *E. coli* as "a HACCP verification activity" *(4)*.

The 1996 U.S. Meat and Poultry Inspection Regulations *(4)* set sampling frequency for *E. coli* testing based on production volume, which for large-volume cattle establishments was set at 1 test for every 300 carcasses. Small-volume establishments are required to test less frequently (once per week or less for very small volume establishments). The regulation also indicated that the establishments were required to take samples from three anatomical areas (brisket, flank, and rump) on each carcass, with one sponge used to swab all three sites. It should be noted, however, that the FSIS baseline studies were done following the method of sample excising instead of swabbing with sponges. Following discussion of this controversial issue, FSIS allowed sampling either by sponging or excising. However, the above expressed criteria apply only to excised samples, while data obtained from carcasses sampled with sponges should be evaluated on the principles of statistical process control based on data obtained by each company, until baseline levels based on sampling by sponging are established.

A study was designed to determine *E. coli* contamination on carcasses in seven beef (four steer and heifer and three cow and bull) slaughtering operations. Even though sampling procedures were different than those used in the FSIS baseline studies, the results were also used to calculate probabilities of passing the "process performance criteria" established in the 1996 U. S. Meat and Poultry Inspection Regulations.

MATERIALS AND METHODS

Experimental design. Of the seven plants examined, four were classified as primarily slaughtering fed steers and heifers and three were classified as primarily slaughtering nonfed cows and bulls *(7)*. Two of the plants were in Texas, while there was one plant each in California, Washington, Nebraska, Wisconsin, and Pennsylvania. Each facility was visited twice, once during the "wet" season (November through January) and once during the "dry" season (May through June) of 1995 to 1996. Following collection, all samples were placed in coolers with ice packs for shipment by overnight air express to a laboratory (Agri-West Laboratory, San Antonio, Tex.) for analysis.

Carcass sampling. Carcass sampling was performed at three different locations in the slaughtering chain of each plant and at three different anatomical sites on the carcass. The plant locations were designated as pre-evisceration, after final carcass washing, and after 24 h of carcass chilling. The sampling sites on the carcass were (i) brisket—anterior to the navel on the ventral mid-line, (ii) flank—posterior to the navel on the ventral mid-line, and (iii) rump—the cushion of the round. These sampling sites on the carcasses are equivalent to the brisket, flank, and rump areas, respectively, as listed in the U.S. Meat and Poultry Inspection Regulations *(4)*.

Carcass samples were taken during three days (per visit and season in each plant) to obtain a sampling population from different lots of cattle. A 100 cm² portion (10 × 10 cm; 0.2-cm thick) of the adipose and muscle tissue surface was aseptically removed from each sampling area of the carcass at each location in the plant by use of a sterile rubber template, forceps, and scalpel. The sample from a single anatomical carcass area and plant location was placed in a sterile Whirl-Pak bag (Nasco, Modesto, Calif.) and analyzed separately. For each sampling site on the carcass and at each location in the plant, samples were taken from 30 carcasses, but carcasses sampled at each location in the plant were different (i.e., carcasses were not followed through the entire production chain for subsequent sampling). The overall total (all plants and both visiting periods) of samples taken for analysis was 3,780, representing 1,260 carcasses.

Microbiological analysis. In this study, the entire excised sample (100 cm²) from each anatomical carcass site and plant location was analyzed microbiologically, while in the FSIS baseline studies *(2, 3)* subsamples (20 cm²) from each of the three anatomical carcass areas were combined (60 cm² total) for analysis. Each carcass tissue sample (100 cm²) was analyzed for *E. coli* counts by adding 100 ml of sterile phosphate buffer (Difco Laboratories, Detroit, Mich.) in the Whirl-Pak bag containing the sample and agitating for 1 min using a Stomacher-3500 (Tekmar, Cincinnati, Ohio). Appropriate dilutions were plated in duplicate on Petrifilm *E. coli* Count Plates (3M Health Care Products, St. Paul, Minn.) and incubated at 35°C for 48 ± 2 h. Counts of *E. coli* were determined by counting blue colonies with adjacent gas bubbles. The calculated detection limit was 1 CFU/cm².

Statistical analysis. The data were transformed to CFU/cm² and were expressed as percentage of samples with counts below the FSIS baseline negative of <5 CFU/cm² (m), between 5 and 100 CFU/cm², and greater than 100 CFU/cm² (M). In addition, the data were used to calculate the probabilities of passing the regulatory *(4)* microbiological "performance criteria," according to an equation developed by Dr. Robert S. Elder of FSIS *(1)*. The procedure used to calculate the probability of passing is described in the Elder manuscript as follows: "One way to evaluate the performance of a particular verification procedure is in terms of the long-term probability of meeting the procedure's criteria at a given process performance level. For the procedure chosen by FSIS, this probability is the fraction of tests for which (1) the current test does not exceed M and (2) not more than 3 of the last 13 tests exceed m. The process performance level for the three-class plan has two performance-determining dimensions: the percent of marginal units and the percent of unacceptable units. Curves can be produced relating the probability of passing to the process percentages of marginal and unacceptable samples. This probability is found using the trinomial distribution, assuming that the moving count of results above m starts at zero and runs without resetting thereafter.

The formula for the probability of passing is:

$$Pa = B(p_1 + p_2; n; c) - p_2 \times B(p_1 + p_2; n - 1; c - 1)$$

where n = number of tests in the window; c = number of marginal or unacceptable results allowed in the windows; p_o = process proportion acceptable units; p_1 = process proportion marginal units; and p_2 = process proportion unacceptable units, with $p_o + p_1 + p_2 = 1$ and B(p;n;c) = binomial probability of c or fewer successes in n trials, with probability p of success on each trial."

RESULTS AND DISCUSSION

As indicated, the 1996 U.S. Meat and Poultry Inspection Regulations *(4)* established that beef carcasses should be sampled to determine whether they meet certain process

TABLE 1. *Percentages (means ± SD) of brisket, flank, and rump samples taken at different plant locations from four steer and heifer plants and three cow and bull plants with* E. coli *biotype I counts (CFU/cm^2) in the three ranges (acceptable, marginal, and unacceptable) of the 1996 U.S. Meat and Poultry Inspection Regulations, during the "wet" (November to January) and "dry" (May to June) seasons*

			Season					
			Wet			Dry		
Plant type	Plant location	Carcass site	Acceptable	Marginal	Unacceptable	Acceptable	Marginal	Unacceptable
Steer and heifer	Preevisceration	Brisket	76.7 ± 26.0	22.5 ± 26.0	0.8 ± 1.7	75.8 ± 9.9	20.0 ± 7.2	4.2 ± 6.3
		Flank	63.4 ± 28.9	32.5 ± 27.0	4.2 ± 6.3	83.3 ± 15.6	13.4 ± 9.0	3.3 ± 6.7
		Rump	66.7 ± 39.7	27.5 ± 32.4	5.8 ± 7.4	66.6 ± 21.4	31.8 ± 20.5	1.7 ± 1.9
	Final carcass washing	Brisket	99.2 ± 1.7	0.8 ± 1.7	0	93.4 ± 7.7	5.8 ± 6.9	0.8 ± 1.7
		Flank	97.4 ± 5.2	2.6 ± 5.2	0	93.4 ± 6.1	5.0 ± 5.8	1.7 ± 3.4
		Rump	93.3 ± 9.4	6.7 ± 9.4	0	90.9 ± 5.0	7.5 ± 3.2	1.7 ± 1.9
	24-h carcass chilling	Brisket	100	0	0	85.0 ± 9.9	7.5 ± 6.9	6.7 ± 6.1
		Flank	99.2 ± 1.7	0.8 ± 1.7	0	84.2 ± 6.9	9.2 ± 9.6	6.7 ± 6.1
		Rump	98.4 ± 1.9	1.7 ± 1.9	0	92.5 ± 8.4	5.8 ± 5.0	1.7 ± 3.4
Cow and bull	Preevisceration	Brisket	51.1 ± 30.2	27.8 ± 13.9	21.1 ± 28.3	62.2 ± 34.7	30.0 ± 24.1	7.8 ± 10.7
		Flank	81.1 ± 10.7	16.7 ± 8.8	2.2 ± 3.8	91.1 ± 12.6	6.7 ± 8.8	2.2 ± 3.8
		Rump	70.0 ± 34.8	6.7 ± 3.4	23.4 ± 31.8	80.0 ± 10.0	17.8 ± 6.9	2.2 ± 3.9
	Final carcass washing	Brisket	94.5 ± 9.6	4.4 ± 7.7	1.1 ± 1.9	93.3 ± 11.5	6.7 ± 11.5	0
		Flank	97.8 ± 1.9	2.2 ± 1.9	0	90.0 ± 8.8	3.3 ± 0	6.7 ± 8.8
		Rump	94.4 ± 5.1	4.5 ± 3.9	1.1 ± 1.9	92.2 ± 10.7	4.4 ± 5.1	3.3 ± 5.8
	24-h carcass chilling	Brisket	95.6 ± 5.1	4.4 ± 5.1	0	98.9 ± 1.9	1.1 ± 1.9	0
		Flank	90.0 ± 14.6	4.4 ± 5.1	5.6 ± 9.6	97.8 ± 3.8	1.1 ± 1.9	1.1 ± 1.9
		Rump	91.1 ± 15.4	4.4 ± 7.7	4.4 ± 7.7	100	0	0

"performance criteria" based on analyses for counts of *E. coli*. The results presented herein were derived from analysis of 3,780 samples from seven plants, during two seasons, at three sampling locations within each plant, and by excision sampling (100 cm^2) of each of three anatomical carcass sites (brisket, flank, and rump) analyzed separately. The data were expressed as percentages of samples falling within each of the three ranges (acceptable, marginal, or unacceptable) of counts established in the three-class attributes sampling plan of the regulation. Percentages of samples with *E. coli* contamination levels in the various regulatory ranges varied with season, individual plants, sampling plant location, and carcass site sampled (Table 1). The impact of plant-to-plant variation was major, while carcass decontamination processes before chilling increased the overall percentages of samples with contamination levels <5 CFU/cm^2 (the detection limit of the FSIS baseline studies) *(2, 3)*. The average percentages of samples at preevisceration with counts >100 CFU/cm^2, depending on carcass site and season, were 0.8 to 6.7% and 2.2 to 23.4% for steer and heifer plants and for cow and bull plants, respectively; carcass decontamination changed these average percentages to 0 to 1.7% and 0 to 6.7%, respectively (Table 1). The average percentages of preevisceration samples with *E. coli* counts in the acceptable range for the 1996 U.S. Meat and Poultry Inspection Regulations *(4)*, depending on carcass site and season, were 63.4 to 83.3% and 51.1 to 91.1% for steer and heifer and for cow and bull plants, respectively; final carcass washing changed these average percentages to 90.9 to 99.2% and 90.0 to 97.8%, respectively (Table 1).

The results indicated that decontamination interventions applied in the plants had a major impact in reducing carcass contamination compared with counts from samples taken immediately following hide removal (preevisceration). This is indicated by the increase in percentages of samples passing the regulatory "performance criteria" following the stage of final carcass washing, compared with those for carcasses sampled after hide removal but before evisceration (Table 1). The characteristics of the plants in terms of carcass decontamination interventions and parameters were described by Sofos et al. *(7)*. Briefly, the slaughtering speed of the plants, which operated in one shift, was 90 to 216 animals per hour, and one of the seven plants applied preevisceration carcass washing, while four plants used steam vacuuming for spot cleaning of carcasses *(5)*. Final carcass washing was applied by all plants with no chemicals added and at pressures and temperatures of 6.9 to 24.8 bar and 21 to 38°C, respectively. Four of the seven plants applied final carcass rinsing at 3 to 51°C with plain water (one plant), acetic acid (two plants), or lactic acid (one plant). Four of the seven plants sprayed carcasses during chilling. Application of these carcass decontamination interventions not only had a major impact in reducing *E. coli* contamination, but also reduced variability in contamination compared with samples taken immediately after hide removal and before application of any decontamination interventions, as evidenced by the lower standard deviations in percentages of samples with different contamination levels (Table 1). This indicates that application of decontamination technologies can help plants improve the microbiological status of carcasses in their attempts to meet

TABLE 2. *Ranges of probabilities of passing the* E. coli *testing performance criteria for four steer and heifer plants and three cow and bull plants during two seasons ("wet": November to January; "dry": May to June) for each of three individual carcass sites at preevisceration, after final carcass washing, and after 24 h of carcass chilling*

			Season	
Plant type	Plant location	Carcass site	Wet	Dry
Steer and heifer	Preevisceration	Brisket	0.008–0.999	0.299–0.966
		Flank	0.004–0.937	0.158–0.991
		Rump	0.000–1.00	0.004–0.907
	Final carcass washing	Brisket	0.999–1.00	0.897–1.00
		Flank	0.962–1.00	0.906–1.00
		Rump	0.747–1.00	0.891–0.999
	24-h carcass chilling	Brisket	1.00	0.597–1.00
		Flank	0.999–1.00	0.641–0.960
		Rump	0.999–1.00	0.710–0.999
Cow and bull	Preevisceration	Brisket	0.000–0.841	0.000–0.966
		Flank	0.505–0.991	0.608–1.00
		Rump	0.001–0.960	0.404–0.966
	Final carcass washing	Brisket	0.821–1.00	0.747–1.00
		Flank	0.999–1.00	0.654–0.999
		Rump	0.937–1.00	0.692–1.00
	24-h carcass chilling	Brisket	0.966–1.00	0.999–1.00
		Flank	0.471–1.00	0.960–1.00
		Rump	0.485–1.00	1.00

the regulatory requirements *(8)*. However, since plants varied in facility design, decontamination interventions applied, geographic location, and types of animals slaughtered, our statistician advised us not to analyze data for influence of individual plants or processing parameters. The effect of season on *E. coli* contamination depended on type of plant and carcass area and may have been influenced by geographic location of each plant, as well as individual plant design and air flow patterns within plants.

After 24 h of carcass chilling (the point at which the regulation requires carcass sampling), average percentages of steer and heifer carcasses with *E. coli* counts in the acceptable and unacceptable ranges were 84.2 to 100% and 0 to 6.7%, respectively, depending on season and carcass site (Table 1); for cow and bull plants, the corresponding average percentages were 90.0 to 100.0% and 0 to 5.6%. More chilled steer and heifer carcass samples exceeded the limit of 100 CFU/cm^2 during the dry season, while the opposite was found for chilled carcass samples from cow and bull plants. At 24 h of carcass chilling, overall percentages of brisket, flank, and rump samples, respectively, exceeding 100 CFU/cm^2 were 0 to 6.7%, 0 to 6.7%, and 0 to 1.7% for steer and heifer plants and 0%, 1.1 to 5.6%, and 0 to 4.4% for cow and bull plants (Table 1). Plant variation was a major factor in variability of samples with higher or lower contamination loads and was a greater source of variability than was carcass site sampled. These observations indicate that individual plants should examine facility design and operation and make appropriate modifications to reduce contamination, taking into consideration the climatic conditions as they operate under the system of HACCP.

Applying the formula of Elder (described in the "Sta-

tistical analysis" section above) for calculating probabilities of passing the *E. coli* testing "performance criteria" of the U.S. Meat and Poultry Inspection Regulations *(4)*, and recognizing differences in sampling between this study and the regulation *(4)*, the results confirmed that variation in counts was also reflected in probabilities of passing the criteria among plants, between wet and dry seasons, and among carcass anatomical sampling sites. For comparative purposes, probabilities of passing the criteria at preevisceration, which is not a regulatory requirement, ranged from very low (0.0) to very high (1.0) during both seasons, in both steer and heifer plants and cow and bull plants (Table 2). When samples were obtained immediately after final carcass washing, the probabilities of passing ranged from 0.654 to 1.0, depending on season, type of plant, and carcass sampling site. For 24-h carcass-chilling samples, the probabilities of passing ranged from 0.471 to 1.0, based on type of plant, season, and each carcass site tested individually. In general, the probabilities of passing the *E. coli* testing "performance criteria" for steer and heifer plants were greater during the wet season and of passing for the cow and bull plants were greater during the dry season. However, this study was not designed to determine whether this difference was due to type of animals slaughtered or geographic location, plant design, and operation. For samples obtained after 24 h carcass chilling, the overall probabilities of passing for the brisket, flank, and rump samples were 0.597 to 1.00, 0.471 to 1.00, and 0.485 to 1.00, respectively (Table 2).

It should be noted that the results of this study, including the calculated probabilities of passing the "process control performance criteria," cannot be compared directly with data collected during the FSIS baseline studies *(2, 3)*

or with data obtained by sampling with sponges *(4)* because of differences in sampling. In this study, we analyzed entire (100 cm^2) excised samples from individual carcass anatomical areas, while the FSIS studies analyzed composite subsamples from all three anatomical areas combined *(2, 3)*. Counts of *E. coli* and probabilities of passing reported herein may have been different if the three samples excised from each carcass were analyzed as a composite.

In this study, for the plants examined, under their current practices, and considering the sampling procedures followed, the probabilities of passing the ''process control performance criteria'' set in the 1996 U.S. Meat and Poultry Regulations for *E. coli* testing after 24 h of carcass chilling *(4)* were as low as 0.471 to 0.597, depending on carcass area sampled; for other plants or season, the probability was 1.0. It is unknown how much meat is produced by plants with probabilities of meeting the criteria of approximately 0.50, but these plants will need to modify their practices for the criteria to be met. Also, it should be noted that the regulation *(4)* requires analysis of all three carcass sites combined, while in this study each site was analyzed separately. Furthermore, sampling in these plants was performed in 3 days with no adherence to the regulatory requirement for testing 1 of 300 carcasses processed. It is unknown how this may have affected the probabilities of passing the regulatory requirements. The regulation *(4)* allows sampling by sponging, in addition to excising used in this study and in the FSIS baseline studies *(2, 3)*. However, it will be interesting to compare these results with those of future analyses to determine the impact of the new regulations on the *E. coli* contamination of beef carcasses in the United States.

REFERENCES

1. Elder, R. S. 1996. Unpublished data.
2. Food Safety and Inspection Service. 1994. Nationwide Beef Microbiological Baseline Data Collection Program: steers and heifers, October 1992–September 1993. U.S. Department of Agriculture, FSIS, Science and Technology, Microbiology Division, Washington, D.C.
3. Food Safety and Inspection Service. 1996. Nationwide Beef Microbiological Baseline Data Collection Program: cows and bulls, December 1993–November 1994. U.S. Department of Agriculture, FSIS, Science and Technology, Microbiology Division, Washington, D.C.
4. Food Safety and Inspection Service. 1996. Pathogen reduction; Hazard Analysis and Critical Control Point (HACCP) systems; Final Rule. Fed. Regist. 61:38806–38989.
5. Kochevar, S. L., J. N. Sofos, R. R. Bolin, J. O. Reagan, and G. C. Smith. 1997. Steam-vacuuming as a pre-evisceration intervention to decontaminate beef carcasses. J. Food Prot. 60:107–113.
6. National Academy of Sciences. 1985. An evaluation of the role of microbiological criteria for foods and food ingredients. National Research Council, Committee on Food Protection, Food and Nutrition Board, Subcommittee on Microbiological Criteria, National Academy Press, Washington, D.C.
7. Sofos, J. N., S. L. Kochevar, G. R. Bellinger, D. R. Buege, D. D. Hancock, S. C. Ingham, J. B. Morgan, J. O. Reagan, and G. C. Smith. 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. J. Food Prot. 62: 140–145.
8. Sofos, J. N., and G. C. Smith. 1998. Nonacid meat decontamination technologies: model studies and commercial applications. Int. J. Food Microbiol. 44:171–189.
9. Sperber, W. H. 1998. Three-class sampling plans aid HACCP efficiency for processors and suppliers. Food Testing Anal. 4:14–16.