Journal of Food Protection, Vol. 72, No. 6, 2009, Pages 1272–1278

Research Note

Prevalence and Enumeration of *Escherichia coli* O157:H7 and *Salmonella* in U.S. Abattoirs that Process Fewer than 1,000 Head of Cattle per Day†

JOSEPH M. BOSILEVAC,* TERRANCE M. ARTHUR, JAMES L. BONO, DAYNA M. BRICHTA-HARHAY, NORASAK KALCHAYANAND, DAVID A. KING, STEVE D. SHACKELFORD, TOMMY L. WHEELER, AND MOHAMMAD KOOHMARAIE‡

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA

MS 08-551: Received 4 November 2008/Accepted 18 January 2009

ABSTRACT

A significant portion (15 to 20%) of beef in the United States is produced in small beef processing plants that harvest fewer than 1,000 cattle per day. However, there are little data on the prevalence and levels of *Escherichia coli* O157:H7 and *Salmonella* in these processing plants. To address this lack of data, hides (n = 1,995) and carcasses (n = 1,995) of cattle at seven small processing plants located across the United States were analyzed for *E. coli* O157:H7 and *Salmonella.* Across all plants, hide prevalence of *E. coli* O157:H7 and *Salmonella* was 71 and 91%, respectively. Twelve percent of hides had *E. coli* O157:H7 at enumerable levels (≥40 CFU/100 cm^2), while 36% of hides had *Salmonella* at enumerable levels. Across all plants, the prevalence of *E. coli* O157:H7 on preevisceration carcasses was 33%, with 2% at an enumerable level (≥0.8 CFU/100 cm^2). Across all plants, *Salmonella* prevalence on preevisceration carcasses was 58%, with 8% at an enumerable level. Significant plant-to-plant variations in levels and prevalence of pathogens on carcasses were detected. Reduced levels of pathogens on carcasses were noted among small processors that had incorporated a hide-directed intervention. The results obtained are comparable to those observed previously for larger processors, showing that smaller beef processors face and address the same challenges as do larger beef processors.

The U.S. Department of Agriculture, Food Safety Inspection Service (FSIS) divides beef processing plants into three groups: (i) large plants, those that have 500 or more employees; (ii) small plants, those that have between 10 and 500 employees and generate more than $2.5 million in annual sales; and (iii) very small plants, those that employ fewer than 10 people or generate less than $2.5 million in annual sales. According to the FSIS, nearly 90% of meat and poultry processors are considered small or very small *(19).* Further, based on information from the U.S. Department of Agriculture, Economic Research Service, and the U.S. Department of Agriculture, National Agricultural Statistics Service, a significant portion (15 to 20%) of the U.S. beef supply is harvested in small and very small processing plants *(17, 20).*

Smaller beef processors have sometimes been overlooked in the dissemination of information from the FSIS, so in October 2007, the FSIS launched its Strategic Implementation Plan for Strengthening Small and Very Small Plant Outreach *(18).* This additional attention on small processors was intended to provide them consistent information and meet their key needs to help improve the quality of their food safety procedures *(18).* It is recognized that optimal hide removal techniques are essential to carcass cleanliness and reduced contamination of final beef products *(13–15).* Therefore, small beef processors need to know the status of *Escherichia coli* O157:H7 and *Salmonella* that enter their facilities and the rates at which the pathogens are transferred to carcasses.

The contribution of large and small beef processing plants to recent reports of contaminated beef products has been debated in public and industry media outlets. Some arguments favor large processors, due to their rigorous systems and superior financial resources to control pathogens, while others favor small processors that have more time for careful hide removal and increased inspector scrutiny because of slower line speeds. While there is ample data on the prevalence and levels of *E. coli* O157:H7 and *Salmonella* found during the different steps of harvest at large U.S. beef processing plants *(3–5, 7, 10),* there are limited or no data on the levels of these pathogens encountered and handled in small processing plants to support these arguments.

* Author for correspondence. Tel: 402-762-4225; Fax: 402-762-4149; E-mail: mick.bosilevac@ars.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

‡ Present address: IEH Laboratories and Consulting Group, 15300 Bothell Way N.E., Lake Forest Park, WA 98155, USA.

Our group has worked in the past to establish sampling protocols and benchmarking data for large beef processors. The benchmarking data allowed the large processors to monitor their individual pathogen reduction efforts and provided a common sampling scheme for self-evaluation *(3).* Therefore, the objective of this study was to directly address the lack of data for small processors and benchmark the prevalence and levels *E. coli* O157:H7 and *Salmonella* on hides and preevisceration carcasses of cattle harvested at small processing plants. For the purpose of this study, small processing plants were defined as processors that slaughter fewer than 1,000 cattle a day, thus representing processors classified as ''small'' by the FSIS definition.

The data presented herein describe the results of an intensive sampling of seven different small beef processors located across the United States. The data analysis establishes benchmarks, and it will aid small processors and policy makers to determine the best processes to put into place in small processing plant environments.

MATERIALS AND METHODS

Design. Seven small-scale processors from across the United States participated in this study. Ninety-five hide samples and 95 corresponding preevisceration carcass samples were collected each day for three consecutive days at each processing plant. The sample collections were completed in a 9-week span of time from October to December in 2007. The prevalence and levels of *E. coli* O157:H7 and *Salmonella* were determined for each sample. The processing steps and procedures used at each small plant were observed and documented for descriptive purposes.

Samples. Hide samples were collected as described previously *(3)* from the brisket-plate area of stunned animals prior to hide removal. Due to issues of accessibility and safety of personnel, the samples at some processors were collected preexsanguination, rather than postexsanguination. Each sample was obtained by swabbing an area of 1,000 cm^2 with a sterile sponge (Whirl-Pak, Nasco Ft. Atkinson, WI) prewetted with 20 ml of sterile buffered peptone water (Difco, Becton Dickinson, Sparks, MD). Preevisceration carcass samples were obtained by swabbing areas of 4,000 cm^2 on the inside and outside round area and the navel-plate-brisket-foreshank area to provide a combined carcass sample of 8,000 cm^2 *(1).* Each of the two sterile sponges used to collect carcass samples was prewetted with 10 ml of buffered peptone water. Carcass samples were collected immediately after hide removal, before any additional carcass directed interventions were applied. However, up to this point in processing, a variety of interventions to control contamination had been used. These interventions varied by plant and consisted of various combinations of using paper or plastic sheets, steam boots, hock blow offs, steam vacuuming, and/or knife trimming. After collection, the samples were shipped on frozen gel packs (Freez Pak, Lifoam Industries, Hunt Valley, MD) by overnight courier to the U.S. Meat Animal Research Center for analysis.

Culture isolation and confirmation of *E. coli* O157:H7. The prevalence *E. coli* O157:H7 was determined by established methods *(6)* that use a nonspecific enrichment in tryptic soy broth (TSB), which is followed by immunomagnetic separation of *E. coli* O157 and plating to CHROMagar O157 (DRG International, Mountainside, NJ) containing 5 mg/liter novobiocin and 2.5 mg/liter potassium tellurite. Suspect colonies were screened by using latex agglutination tests for the O157 antigen (Remel, Lenexa, KS), then confirmed to be *E. coli* O157:H7 and to contain at least one virulence factor (stx_1, stx_2, or *eae*) by multiplex PCR *(12).* Each daily analysis of samples included two positive control samples, one for hides and one for carcasses. The positive controls were additional samples collected at the plant and inoculated in the laboratory with approximately 50 CFU of *E. coli* O157:H7.

Culture isolation and confirmation of *Salmonella.* The *Salmonella* isolation was run concurrently to *E. coli* O157:H7 detection and used the same nonspecific TSB enrichment as *E. coli* O157:H7 isolation *(6).* The *Salmonella* was concentrated by immunomagnetic separation, and the immunomagnetic-separation beads were then selectively enriched by incubation in Rappaport-Vassiliadis–soy broth (Oxoid, Ltd., Basingstoke, UK) before plating to Hektoen enteric medium (Difco, Becton Dickinson) with 5 mg/liter novobiocin) and brilliant green agar with 80 mg/liter sulfadiazine (Difco, Becton Dickinson) *(10).* Suspect colonies were isolated and confirmed to be *Salmonella* by PCR for the *invA* gene *(16). Salmonella* isolation also included positive controls collected and set up as described above.

Enumeration of *E. coli* O157:H7 and *Salmonella.* The levels of *E. coli* O157:H7 and *Salmonella* were determined as previously described *(9).* Briefly, for pathogen enumeration from hide samples, a 50-μl aliquot of each 20-ml sponge sample was spiral plated onto ntCHROMAgar O157 to enumerate *E. coli* O157:H7 and onto xylose-lysine-desoxycholate medium (Remel) with 4.6 ml/liter tergitol (Sigma, St. Louis, MO), 15 mg/liter novobiocin, and 5 mg/liter cefsulodin to enumerate *Salmonella*. For pathogen enumeration from carcass samples, 500 μl (for *Salmonella*) and 300 μl (for *E. coli* O157:H7) of carcass sponge sample was diluted in 7 ml of phosphate-buffered saline with 1% (vol/vol) Tween 80 (Sigma). The dilutions were analyzed by hydrophobic grid–membrane filtration by using ISO-GRID membranes (Neogen, Lexington, KY) and a spread filter apparatus (FiltaFlex, Ltd., Almonte, Ontario, Canada). The membranes were then placed on the appropriate selective medium, incubated, and inspected for suspect colonies. Up to 10 presumptive isolates per plate were tested by PCR as described above to confirm their identity. The CFU counts for confirmed *E. coli* O157:H7 and *Salmonella* were adjusted for the percent of verified isolates per positive sample, and then reported as CFU/100 cm^2. Enumeration assays also included additional samples that had been collected at the plants and then inoculated with *E. coli* O157 and *Salmonella* to provide controls to verify performance of the assays.

Data analysis. The combined prevalence and numbers of enumerable *E. coli* O157:H7 and *Salmonella* on hides and carcasses were analyzed using InStat 3.0b software (GraphPad Software, La Jolla, CA). *E. coli* O157:H7 and *Salmonella* percent prevalence on hide and carcass samples was determined for each sample day and reported as the mean and standard deviation (±SD) of each plant. The percentage of hide and carcass samples found to be enumeration positive was also analyzed by plant, and reported as the mean percent enumerable (±SD). The enumeration data was log transformed for determination of geometric mean (CFU/100 cm^2) and the corresponding 95% confidence interval (CI). Comparisons of prevalence values, percent enumerable, and mean load were examined by a one-way analysis of variance and the Tukey-Kramer posttest ($P < 0.05$) comparing multiple means by using Prism 5.0a software (GraphPad).

RESULTS AND DISCUSSION

Over the course of this project, 1,995 hide and 1,995 preevisceration carcass samples were collected during 21

TABLE 1. *Overall prevalences and levels observed of* Escherichia coli *O157:H7 and* Salmonella *in hide and preevisceration carcass samples collected at seven small processing plants*[a]

Sample	*Escherichia coli* O157:H7			*Salmonella*		
	n	Avg[b]	95% CI	*n*	Avg	95% CI
Hide[c]						
Prevalence	1,995	70.9 ± 26.1	59.1–82.8	1,987[d]	90.5 ± 12.8	84.7–96.3
Enumeration[e]	246 (12.3%)	84.2	74.7–94.9	728 (36.6%)	533	455–625
Range		40–4,000			40–399,731	
Carcass[f]						
Prevalence	1,995	33.2 ± 27.5	20.7–45.7	1,995	57.8 ± 30.4	44.0–71.7
Enumeration	41 (2.1%)	1.9	1.2–2.8	159 (8.0%)	1.2	1.0–1.5
Range		0.8–189			0.5–720	

[a] Prevalence values represent the percentage of samples found positive by culture isolation. The level of pathogens found by direct plating enumeration are presented as CFU/100 cm^2.

[b] The averages of prevalence values are given as means ± SD, while the geometric means are given for enumeration values, due to their lognormal distribution.

[c] Hide samples were 1,000 cm^2, collected from the brisket-plate region of cattle after stunning.

[d] Eight samples for *Salmonella* isolation were dropped from analysis, due to technical procedural errors.

[e] Enumeration values are for samples that were enumerable (above the limit of detection) at ≥40 CFU/100 cm^2 on hides and ≥0.5 CFU/100 cm^2 on carcasses. The number over the percentage of all samples is presented.

[f] Preevisceration carcass samples were 8,000 cm^2, collected from top-hock-round and bottom-shank-brisket areas.

days (3 days each at seven different small processing plants). The prevalence and level of *E. coli* O157:H7 and *Salmonella* was determined (Table 1). Due to a technical error, eight hide samples were not processed for *Salmonella* prevalence, resulting in 1,987 tested samples. The overall hide prevalence of *E. coli* O157:H7 and *Salmonella* was 70.9 and 90.5%, respectively. The number of hides that had enumerable levels (i.e., >40 CFU/100 cm^2) of *E. coli* O157:H7 and *Salmonella* was 12.3 and 36.6%, respectively. These values are typical for cattle presented for slaughter at large processing plants in the United States *(3, 5, 10).*

The rates of carcass contamination after hide removal were directly related to the levels of hide contamination entering the plant. Overall, the prevalence of *E. coli* O157:H7 and *Salmonella* was 33.2 and 57.8%, respectively, and the number of carcasses that had enumerable levels (>0.5 CFU/100 cm^2) of *E. coli* O157:H7 and *Salmonella* was 2.1 and 8.0%, respectively. In a recent evaluation of 581 samples collected from preevisceration beef carcasses at three different large processors *(2),* overall *E. coli* O157:H7 prevalence was determined to be 17%, and ranged from 1.7 to 38.3% between the three plants, while *Salmonella* prevalence was 2.9% and ranged from 0 to 7.7%. Another recent report that used the same sampling and detection protocol used here found *E. coli* O157:H7 prevalence ranged from 6.9 to 41.5% on preevisceration carcasses at four large cull cow and bull processing plants *(10).* The prevalence of *Salmonella* on preevisceration carcasses in this same study ranged from 26.9 to 67.2% *(10).* These results show that the overall hide-to-carcass transfer rates of pathogens are not markedly different between the small plants studied and the large plants recently evaluated.

Considerable variation in hide prevalence values was observed between plants and between days at the same plant (Table 2). On a day-to-day basis, hide prevalence of *E. coli* O157:H7 ranged from a low of 18.9 to a high of 100%, while daily *Salmonella* prevalence on hides ranged from 56.5 to 100%. The day-to-day variations within a plant of *E. coli* O157:H7 and *Salmonella* on hides varied by up to 40 and 75%, respectively. Despite the variation observed, the prevalence values of pathogens on hides were not different ($P > 0.05$) between the small plants, with the exception of *E. coli* O157:H7 on cattle entering plants 1 and 6.

As mentioned, *E. coli* O157:H7 and *Salmonella* prevalence on preevisceration carcasses was directly related to the hide prevalence observed each day. Within a plant, the day of lowest preevisceration carcass prevalence correlated to the day or days of lowest hide prevalence. This was observed across all plants studied, regardless of differences in hide removal techniques and practices in place, demonstrating that preevisceration carcass contamination is primarily a function of the incoming load on hides. Preevisceration carcass prevalence of the two pathogens varied greatly, ranging from 0 to 93% for *E. coli* O157:H7 and from 16 to 99% for *Salmonella* on different days at different plants. The lowest *E. coli* O157:H7 prevalence on preevisceration carcasses was observed at plant 6, 8.4%, ranging from 0 to 23.2% daily at that plant. The highest *E. coli* O157:H7 prevalence on preevisceration carcasses was observed at plant 1, 58.6%, ranging from 12.6 to 89.5% daily. The magnitude of these daily variations in preevisceration carcass prevalence prevented the statistical measurement of any significant differences in hide-to-carcass transfer of pathogens between the plants.

Enumeration of the numbers of organisms present has recently been shown to be a valuable measurement in analysis of hide to carcass transfer of pathogens *(2, 10).* The enumeration of *E. coli* O157:H7 from hides and preevisceration carcasses at the small plants studied are shown in

TABLE 2. *Prevalence[a] of* Escherichia coli *O157:H7 and* Salmonella *on hides and carcasses observed by day at each small processing plant[b]*

Sample	Plant: 1	2	3	4	5	6	7
Hides[c]							
E. coli O157:H7							
Day 1	86.3	54.7	33.7	97.9	97.9	61.1	49.5
Day 2	95.8	94.7	56.8	93.7	50.5	21.1	76.8
Day 3	98.9	100.0	76.8	66.3	91.6	18.9	66.3
Mean ± SD	93.7 ± 6.6 A[d]	83.2 ± 24.8 AB	55.8 ± 21.6 AB	86.0 ± 17.2 AB	80.0 ± 25.7 AB	33.7 ± 23.8 B	64.2 ± 13.8 AB
95% CI	77 to 110	22 to 145	2 to 109	43 to 129	16 to 144	−25 to 93	30 to 98
Salmonella							
Day 1	98.9	97.9	69.5	100.0	74.7	88.4	81.1
Day 2	100.0	100.0	95.8	100.0	56.8	71.6	100.0
Day 3	97.9	100.0	98.9	100.0	84.2	88.4	95.8
Mean ± SD	98.9 ± 1.1 A	99.3 ± 1.2 B	88.1 ± 16.2 B	100.0 ± 0.0 B	71.9 ± 13.9 B	82.8 ± 9.7 B	92.3 ± 9.9 B
95% CI	96 to 102	96 to 102	48 to 128	100 to 100	37 to 106	59 to 107	68 to 117
Carcasses[e]							
E. coli O157:H7							
Day 1	12.6	24.2	29.5	38.9	92.6	23.2	4.2
Day 2	73.7	58.9	25.3	10.5	29.5	2.1	15.8
Day 3	89.5	48.4	49.5	6.3	45.3	0.0	16.8
Mean ± SD	58.6 ± 40.6 A	43.8 ± 17.8 A	34.7 ± 12.9 A	18.6 ± 17.7 A	55.8 ± 32.8 A	8.4 ± 12.8 A	12.3 ± 7.0 A
95% CI	−42 to 160	0 to 88	3 to 67	−25 to 63	−26 to 137	−23 to 40	−5 to 30
Salmonella							
Day 1	97.9	82.1	17.9	96.8	16.8	43.2	15.8
Day 2	93.7	84.2	83.2	72.6	22.1	20.0	31.6
Day 3	69.5	76.8	98.9	48.4	34.7	69.5	38.9
Mean ± SD	87.0 ± 15.3 A	81 ± 3.8 A	66.7 ± 43.0 A	72.6 ± 24.2 A	24.5 ± 9.2 A	44.2 ± 24.8 A	28.8 ± 11.8 A
95% CI	49 to 125	72 to 91	−40 to 173	12 to 133	2 to 47	−17 to 106	−1 to 58

[a] Values represent the percentage of samples positive each day for *E. coli* O157:H7 and *Salmonella* by culture isolation.
[b] Small processing plants are defined as those that process fewer than 1,000 cattle per day.
[c] Hide samples were 1,000 cm^2, collected from the brisket-plate region of cattle after stunning.
[d] Common letters within a row are not significantly ($P > 0.05$) different.
[e] Preevisceration carcass samples were 8,000 cm^2 collected from top-hock-round and bottom-shank-brisket areas.

Table 3. Generally, more than half of the samples that were enumeration positive, and were just above the detection limit of the enumeration methods (Table 3). It was noted that higher enumerable levels of pathogens on hides positively correlated to pathogens on corresponding preevisceration carcasses. Therefore, reducing hide levels of pathogens should be a priority for small processors. Plant 5 had the fewest hides with enumerable *E. coli* O157:H7, but these were the most heavily contaminated hides encountered in this study (up to 4,000 CFU/100 cm^2). Plant 3 had only four preevisceration carcasses that possessed enumerable levels of *E. coli* O157:H7, but these too were the highest levels of this pathogen observed on preevisceration carcasses. The levels of *E. coli* O157:H7 measured on hides was similar to that recently measured by Arthur et al. *(2)* at three large beef processors and by Brichta-Harhay et al. *(10)* at four large cull cow processing plants.

Plant 1 had one of the highest numbers of enumerable and highest levels of *E. coli* O157:H7 and *Salmonella* on hides, yet some of the lowest levels on carcasses. This seems to contradict rule of hide-to-carcass transfer, but examination of enumeration results by day (data not shown) provides an explanation. At this plant, the number of enumerable hide samples with *E. coli* O157:H7 increased from 6 to 71, while the number of enumerable samples with *Salmonella* decreased from 91 to 55, from day 1 to day 3. This directly correlates to the carcass prevalence observed each day (Table 2). This plant still appears to have been outperforming the others in hide removal technique, but careful review of their hide removal processes and visual observations at this plant showed no markedly different or unique practices to account for this result. This plant did have one of the slowest line speeds of the plants studied, and this may have been a contributing factor. Other plants had line speeds nearly as slow but similar effects on carcass contamination were not measured. Since visual cleanliness and observations do not always correlate to pathogen transfer, the hide removal practices used at this plant may have prevented contamination of carcass hot spots with pathogens, while visual cues of carcass cleanliness were absent.

Based on average CFU per 100 cm^2, *E. coli* O157:H7 was present at lower levels on hides than was *Salmonella,*

TABLE 3. *Enumeration*[a] *of* Escherichia coli *and* Salmonella *on hides and carcasses at small processing plants*[b]

Sample	*Escherichia coli* O157:H7				*Salmonella*			
	%[c]	Range	Avg[d]	95% CI	%	Range	Avg	95% CI
Hide[e]								
Plant 1	35	40–1,120	89 A[f]	75–105	83	400–399,731	6,458 A	5,494–7,593
Plant 2	20	40–1,760	79 A	61–103	36	40–4,200	202 B	156–262
Plant 3	4	40–1,320	65 A	30–143	28	40–7,880	167 B	126–222
Plant 4	13	40–1,960	76 A	53–109	61	40–27,560	160 B	130–196
Plant 5	3	40–4,000	142 A	44–462	0.7	40–80	57 B	1–4,624
Plant 6	4	40–280	63 A	41–95	27	40–14,640	163 B	116–231
Plant 7	8	40–1,120	98 A	65–149	20	40–6,400	106 B	73–156
Carcass[g]								
Plant 1	2	0.8–2.5	1.0 A	0.7–1.4	16	0.5–24	0.9 A	0.7–1.1
Plant 2	5	0.8–11.7	1.6 A	0.9–2.6	12	0.5–31	1.3 AB	0.9–1.9
Plant 3	1	0.8–189	7.4 A	0.1–519	16	0.5–720	2.2 B	1.3–3.6
Plant 4	0.4	0.8	0.8[h]		8	0.5–256	0.9 AB	0.5–1.6
Plant 5	5	0.8–50.8	2.4 A	1.1–5.1	0.7	0.5	0.5 AB	0.5–0.5
Plant 6	0				0.7	0.5	0.5 AB	0.5–0.5
Plant 7	0.7	0.8–1.7	1.2 A	0.0–96.3	1	0.5–1.5	0.9 AB	0.4–1.9

[a] Enumeration values are for samples that were enumerable (above the limit of detection) at ≥40 CFU/100 cm^2 on hides and ≥0.5 CFU/100 cm^2 on carcasses.
[b] Small processing plants are defined as those that process fewer than 1,000 cattle per day.
[c] Values represent the percentage of total samples ($n = 285$) that had enumerable levels of the pathogens present.
[d] The averages of enumeration data values are given as the geometric means, due to lognormal distribution.
[e] Hide samples were 1,000 cm^2, collected from the brisket-plate region of cattle after stunning.
[f] Common letters within a column and sample type are not significantly ($P > 0.05$) different.
[g] Preevisceration carcass samples were 8,000 cm^2, collected from top-hock-round and bottom-shank-brisket areas.
[h] No statistical value is given in this case of one observation ($n = 1$).

but found at higher levels, on carcasses. This can be explained partially by the impact of diluting the samples for the different enumeration methods, and partially by differences in the growth of each organism on selective media compared with the growth of background organisms. Each colony on a spiral plate represents 40 CFU, whereas each colony on an *E. coli* O157:H7 hydrophobic grid–membrane filtration membrane represents 0.8 CFU, and each colony on a *Salmonella* hydrophobic grid–membrane filtration membrane represents 0.5 CFU. The background organisms on hides are different from those on preevisceration carcasses *(4)* and are impacted by the dilutions used for enumeration. Therefore, when the targeted pathogen is placed on selective media with different levels and types of background organisms, the selective power of the media may be overwhelmed by the background organisms, or the specific indicators for the phenotype of the pathogens on the selective media may be diluted to the point that they were not properly detected.

Since hides are the source of pathogens entering processing plants, hide-directed interventions have been embraced by the beef processing industry *(1, 8, 11).* Hide interventions range from sophisticated wash cabinets *(8)* to minimal spray washes *(1).* Some of the small processors in this study had some form of hide-directed intervention in place. The hide interventions at the small plants studied would be considered in the minimal hide wash category *(1).* At one plant, the intervention was situated in such a way that hides were safely accessible for sampling before and after the intervention. We collected samples from before and after the hide intervention in this situation to evaluate the efficacy of the hide intervention in place and determine if pathogen levels were being reduced before hide removal. This hide wash consisted of a low-pressure, chlorinated water wash. Results of the hide intervention (Table 4) showed that it slightly lowered *E. coli* O157:H7 and *Salmonella* prevalence from 45.6 and 95.1%, to 33.7 and 82.8%, respectively. However, the greatest effect of this hide intervention was reducing by half the number of enumerable *E. coli* O157:H7 and *Salmonella* and lowering the upper range of the pathogens fivefold. The effect of this small-plant hide wash was evident on carcasses sampled at this plant. The carcasses had some of the lowest or the lowest numbers of *E. coli* O157:H7 and *Salmonella* observed in this study. Another of the small plants utilized a hide-directed intervention consisting of a water wash, but its effects could not be directly evaluated by sampling. This plant also had lower prevalences and levels of pathogens on preevisceration carcasses, likely due to the dilution and lowering of the load of pathogen on the hide. The effects of the hide washes on the preevisceration carcass cleanliness show that regardless of the size of the processor, hide intervention effectively reduces pathogens on hides, and subsequently, the associated carcasses. In our opinion, a hide-directed intervention should be a priority of all processors, large and small.

TABLE 4. *Effects of a hide[a] wash intervention in small plant processing plant[b] environment*

Sample	*Escherichia coli* O157:H7			*Salmonella*		
	n	Avg[c]	95% CI	*n*	Avg	95% CI
Preintervention						
Prevalence	285	45.6 ± 16.0	5.9–85.3	285	95.1 ± 3.4	86.7–103.5
Enumeration[d]	12 (4.2%)	62.8	41.4–95.2	76 (26.7%)	164	116–231
Range		40–280			40–14,640	
Postintervention						
Prevalence	285	33.7 ± 23.7	−25.3–92.6	285	82.8 ± 9.7	58.7–107.0
Enumeration	4 (1.4%)	40	40.0–40.0	37 (13.0%)	161	106–246
Range		40			40–3,000	

[a] Hide samples were 1,000 cm^2, collected from the brisket-plate region of cattle after stunning at a location before the hide at preintervention and again postintervention. The hide intervention consisted of a low-pressure, chlorinated water wash. Prevalence values represent the percentage of samples found positive by culture isolation over 3 days. The levels of pathogens found by direct plating enumeration are given as CFU/100 cm^2.

[b] Small processing plants are defined as those that process fewer than 1,000 cattle per day.

[c] The averages of prevalence values are given as means ± SD, while the geometric mean is given for enumeration values, due to their lognormal distribution.

[d] Enumeration values are for samples that were enumerable (above the limit of detection) at ≥40 CFU/100 cm^2 on hides and ≥0.5 CFU/100 cm^2 on carcasses. The number over the percentage of all samples is shown.

Whereas large processing plants slaughter 1,000 cattle in 2 to 3 h, smaller processors slaughter 1,000 cattle in 1 to 2 days. The small processing plants are considerably different in physical size and layout compared with large plants, but both small and large processors rely on multiple interventions to reduce contamination. The small processors in this study used different interventions on preevisceration carcasses. Some had a preevisceration wash cabinets, while others replied on extensive knife trimming to remove visible contamination. The final carcasses at all the small processors encountered an intervention of either hot water wash cabinets, steam pasteurization, and/or organic acid sprays. We did not assess the final carcasses at the small plants in this study because the primary goal of this work was to benchmark the introduction of pathogens to the carcass during hide removal. Additionally, collecting final carcass samples was logistically problematic and would have subjected the small processors to an undue burden of retaining over 10% of their product until test results were known.

Our results have established benchmark data for small processors, and show that small processors face and must address the same challenges as the larger processors. The results of this work directly helped the small processors involved evaluate their practices and implement rapid changes to improve carcass cleanliness, and will supply plant managers and policy makers data that had been missing in the literature.

ACKNOWLEDGMENTS

We thank the participating beef processors. Scientific analysis and support was supplied by Dr. Michael Guerini. Technical support was supplied by Greg Smith, Bruce Jasch, Frank Reno, Julie Dyer, and Casey Trambly. Secretarial support was supplied by Debbie Kummer. This project was funded, in part, by the Beef Checkoff.

REFERENCES

1. Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *J. Food Prot.* 70:1076–1079.
2. Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. Source tracking of *Escherichia coli* O157:H7 and *Salmonella* contamination in the lairage environment at commercial U.S. beef processing plants and identification of an effective intervention. *J Food Prot.* 71:1752–1760.
3. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae,* and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:658–665.
4. Bacon, R. T., K. E. Belk, J. N. Sofos, R. P. Clayton, J. O. Reagan, and G. C. Smith. 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. *J. Food Prot.* 63:1080–1086.
5. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
6. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
7. Bell, R. G. 1997. Distribution and sources of microbial contamination on beef carcasses. *J. Appl. Microbiol.* 82:292–300.
8. Bosilevac, J. M., X. Nou, M. S. Osborne, D. M. Allen, and M. Koohmaraie. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *J. Food Prot.* 68:265–272.
9. Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Sal-*

monella and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and faecal samples using direct plating methods. *J. Appl. Microbiol.* 103:1657–1668.

10. Brichta-Harhay, D. M., M. N. Guerini, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. *Salmonella* and *E. coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States—an evaluation of prevalence and load using immunomagnetic separation and direct plating methods. *Appl. Environ. Microbiol.* 74:6289–6297.
11. Byrne, C. M., D. J. Bolton, J. J. Sheridan, D. A. McDowell, and I. S. Blair. 2000. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. *Lett. Appl. Microbiol.* 30:142–145.
12. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. *J. Appl. Microbiol.* 87:867–76.
13. Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, and T. L. Wheeler. 2007. Interventions to reduce/eliminate *Escherichia coli* O157:H7 in ground beef. *Meat Sci.* 77:90–96.
14. Koohmaraie, M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, S. D. Shackelford, and T. L. Wheeler. 2005. Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Sci.* 71:79–91.
15. Pohlman, F. W., M. R. Stivarius, K. S. McElyea, Z. B. Johnson, and M. G. Johnson. 2002. Reduction of microorganisms in ground beef using multiple intervention technology. *Meat Sci.* 61:315–322.
16. Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galan, C. Ginocchio, R. Curtiss III, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Mol. Cell. Probes* 6:271–279.
17. U.S. Department of Agriculture, Economic Research Station. 2006. Briefing room: food marketing systems in the U.S. Available at: http://www.ers.usda.gov/Briefing/FoodMarketingSystem/processing.htm. Accessed 10 January 2009.
18. U.S. Department of Agriculture, Food Safety and Inspection Service. 2006. Food Safety Inspection Service Strategic Implementation Plan for Strengthening Small and Very Small Plant Outreach 2006 to 2007. Available at: www.fsis.usda.gov/PDF/Strategic_Plan_SVS_Plant_Outreach.pdf. Accessed 10 October 2008.
19. U.S. Department of Agriculture, Food Safety and Inspection Service. 2007. Available at: http://www.fsis.usda.gov/PDF/Small_Plant_News_Oct2007.pdf. Accessed 10 October 2008.
20. U.S. Department of Agriculture, National Agricultural Statistics Service. 2006. Economics, Statistics and Market Information System–Research Service. Briefing room: food marketing systems in the U.S. Available at: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1096. Accessed 10 January 2009.