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Research Note

Detection of *Escherichia coli* O157:H7 and *Salmonella enterica* in Air and Droplets at Three U.S. Commercial Beef Processing Plants†

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ABSTRACT

Bacteria are known to be present in the air at beef processing plants, but published data regarding the prevalences of airborne *Escherichia coli* O157:H7 and *Salmonella enterica* are very limited. To determine if airborne pathogens were present in beef processing facilities, we placed sedimentation sponges at various locations in three commercial beef plants that processed cattle from slaughter through fabrication. For the 291 slaughter area air samples, *E. coli* O157:H7 was isolated from 15.8% and *S. enterica* from 16.5%. Of the 113 evisceration area air samples, *E. coli* O157:H7 was isolated from only one sample and *S. enterica* was not isolated from any sample. Pathogens were not isolated from any of the 87 air samples from fabrication areas. Pathogen prevalences, aerobic plate counts, and *Enterobacteriaceae* counts were highest for air samples obtained from locations near hide removal operations. The process of hide removal disperses liquid droplets, which may contact neighboring carcasses. Samples were obtained both from hide removal locations that were close enough to hide pullers to be contacted by droplets and from locations that were not contacted by droplets. Higher pathogen prevalences, aerobic plate counts, and *Enterobacteriaceae* counts were observed at locations with samples contacted by the hide removal droplets. We conclude that the hide removal processes likely introduce pathogens into the air via a dispersion of liquid droplets and that these droplets may be an underappreciated source of hide-to-carcass contamination.

Airborne bacteria in beef processing plants have been demonstrated to contribute to microbial contamination of carcasses *(11, 19)*. Previous published studies on the microbial quality of air at beef processing plants have examined counts of total viable bacteria, aerobic bacteria, or total coliforms *(11, 14, 18, 19, 21)*. To our knowledge, no studies that examined the airborne prevalence of *Escherichia coli* O157:H7 or *Salmonella enterica* in beef processing plants have been published. Hides are recognized as the principle source of *E. coli* O157:H7 and *S. enterica* carcass contamination during processing *(3, 5, 7, 10, 16)*. Burfoot et al. *(11)* demonstrated that the largest amount of airborne aerobic bacteria present in beef processing plants occurred near hide pullers, mechanical devices that remove portions of hide. Additionally, the process of hide removal disperses liquid droplets, but the microbial properties of these droplets, including prevalence of *E. coli* O157:H7 or *S. enterica,* have not been examined.

The potential for contamination by airborne *E. coli* O157:H7 and *S. enterica* is not limited to the process of hide removal. Prendergast et al. *(18)* demonstrated that airborne bacterial counts increased in the area of carcass splitting saws. Additionally, plant design and airflow have been demonstrated to influence contamination *(22)*. In response to these concerns, recently constructed or renovated beef processing establishments have been designed so that the ''clean'' hide-off area and the ''less clean'' hide-on areas are separated by physical barriers. Furthermore, air handling systems are designed such that air pressure is highest in the ''cleanest'' areas and lowest in the ''dirtiest'' areas, so that air flows from clean to dirty areas *(2)*. However, there are a number of anecdotal accounts of sporadic disturbances (i.e., construction, maintenance, or door propping) disrupting the designed airflow and potentially allowing airborne pathogens to contaminate ''clean'' areas. The objective of this study was to determine the prevalences of airborne *E. coli* O157:H7 and *S. enterica,* and the sedimentation rates of airborne indicator organisms, in the slaughter, evisceration, and fabrication areas at three beef processing establishments.

MATERIALS AND METHODS

Sampling protocol. Samples were obtained with sedimentation sponges since they allow collection of all airborne bacteria contacting the sponge, including aerosols and droplets, over

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defined periods of time. Sampling with active air monitoring systems was attempted during preliminary experiments, but sampling devices frequently malfunctioned, possibly due to the high humidity and droplets present at the sampled locations. Each sedimentation sponge consisted of a sterile sponge (8.5 by 4.5 by 1.0 cm; Whirl-Pak, Nasco, Fort Atkinson, WI) prewetted with 20 ml of buffered peptone water (BPW; Difco, BD, Sparks, MD) and placed into a sterile plastic petri dish. Three cattle harvesting establishments, which processed carcasses through fabrication, were each sampled three times. Respectively, plants A, B, and C process approximately 5,440, 6,480, and 1,850 head per day at approximate rates of 340, 405, and 185 head per hour. Sampling visits occurred during the months of July, August, and September. Designs of the three plants differed, and selection of sample locations was limited by the requirement to not interfere with the activities of plant personnel; therefore, the number of samples and their specific locations varied by plant. Locations from leg transfer to preevisceration wash were classified as ''slaughter area.'' Locations from preevisceration wash to cooler entry were classified as ''evisceration area.'' Locations in chilling and grading coolers and fabrication rooms were classified as ''fabrication area.'' During each plant visit, six sedimentation sponges were placed at each sample location in slaughter and evisceration areas. Three of the sedimentation sponges at each sample location were exposed for 30 min, and the other three were exposed for 120 min. Sedimentation sponge exposure at each plant began within 2 h of the start of slaughter operations. Sedimentation sponges were only exposed while cattle were present and being processed. At the end of the exposure period, sponges were removed from petri dishes by gloved hands (gloves were changed after handling each sample) and were transferred to sterile bags (Whirl-Pak). Samples were placed in a cooler with ice packs and transported back to the laboratory, where they were processed the same day. In fabrication areas, air sampling was limited to one sedimentation sponge exposed for 120 min per location sampled, due to space limitations. The number of samples analyzed from each location sampled varied since sponges that had been disturbed (i.e., moved or overturned) were excluded.

APC and EBC. Aerobic bacteria plate counts (APC) and *Enterobacteriaceae* counts (EBC) were determined by impedance measurements obtained with a bioMérieux Bactometer (Hazelwood, MO). Each sponge was hand massaged for 30 s, then two 100-μl aliquots were removed from each sample and serial diluted. To obtain an APC value, 100 μl of an appropriate dilution was placed into 900 μl of bioMérieux general purpose medium supplemented with 18 g/liter dextrose. To obtain an EBC value, 100 μl of an appropriate dilution was placed into 900 μl of bioMérieux enteromedium. The values for APC and EBC were based on values generated from a standard curve using PetriFilm AC and EB plates (3M Microbiology, St. Paul, MN) as described previously *(9)*. CFU per sedimentation sponge values were converted to CFU per hour values (CFU/h) by multiplying the CFU per sedimentation sponge values of sponges exposed for 30 min by two while dividing the CFU per sedimentation sponge values of sponges exposed for 120 min by two. CFU/h values were then log transformed, and the geometric means for each sample point were determined. The lower limit of detection of APC and EBC was 2.0 log CFU/h.

***E. coli* O157:H7 and *S. enterica* prevalence.** The prevalences of *E. coli* O157:H7 and *S. enterica* were determined using previously described methods *(6, 8, 15)*. Briefly, 80 ml of tryptic soy broth (TSB; Difco, BD) was added to each bag containing a sedimentation sponge, following the removal of aliquots for enumeration. Samples were enriched at 25°C for 2 h, at 42°C for 6 h, then at 4°C overnight. *E. coli* O157:H7 was concentrated from enrichments by immunomagnetic separation (IMS), and the IMS beads were plated onto Chromagar O157 plates (DRG International, Mountainside, NJ) supplemented with 5 mg/liter novobiocin and 2.5 mg/liter potassium tellurite (Sigma-Aldrich Co., St. Louis, MO). Suspected *E. coli* O157:H7 colonies were screened using Oxoid DrySpot latex agglutination tests for the O157 antigen (Remel, Lenexa, KS) and were confirmed by multiplex PCR *(13)*. *S. enterica* was concentrated from enrichments by IMS, and the IMS beads were then subjected to a secondary enrichment by incubation in Rappaport-Vassiliadis soy broth (Oxoid) at 42°C for 18 h. Rappaport-Vassiliadis soy broth cultures were then swabbed onto Hektoen enteric medium (Difco, BD) supplemented with 5 mg/liter novobiocin and brilliant green agar supplemented with 80 mg/liter sulfadiazine (Difco, BD). Suspected colonies were isolated and confirmed to be *S. enterica* by PCR *(17, 20)*.

Statistical analysis. APC/h and EBC/h geometric means were compared with one-way analysis of variance with Bonferroni's correction for multiple comparisons performed with the Prism 5.0 program (GraphPad Software, La Jolla, CA); comparisons with P values <0.05 were considered significant. Differences in the proportions of prevalence positive samples were examined by Pearson's χ^2 with Bonferroni's correction for multiple comparisons performed with the Compare2 program of the WinPepi (ver. 11.7) package *(1)*; P values <0.05 were considered significant.

RESULTS AND DISCUSSION

APC were below the limit of detection for the majority of air samples obtained from fabrication areas at all three plants (Table 1). At two plants (A and C), slaughter area APC were higher than evisceration area APC, but at plant B there was no difference between slaughter and evisceration area air samples (Table 1). Indeed, the mean APC for plant B slaughter areas was more than 1.5 log lower than means observed for plant A or plant C. However, many slaughter area sample locations at plant B were more distant from carcasses than the sampled locations in plants A and C (Table 2), possibly contributing to the lower mean APC observed at this plant. The designs of the three plants examined were different, and spaces suitable for air sampling differed by plant. Thus, differences in APC, EBC, or pathogen prevalence between plants were not used to make conclusions pertaining to plant sanitation practices. However, comparisons within each plant allowed us to identify processes potentially contributing to airborne transmission of bacterial pathogens.

APC at plant A were highest at hide puller 2 (A-HP2) and hide puller 3 (A-HP3) locations (Table 2). The next highest APC at plant A were observed at the leg transfer (A-LT), hide opening (A-HO), and hide puller 1 (A-HP1) locations. The A-LT location was closest to the stunning chute and the doorway to lairage pens, and dust from these locations may have contributed to the high APC *(14, 18, 21)*. Interestingly, mean APC at A-HP1 and A-HO locations were at least 2.0 log lower than at A-HP2 and A-HP3 locations, which were further from the stunning chute and lairage pen doorway (Table 2). We theorized that the

TABLE 1. *Sedimentation rates of airborne aerobic bacteria and* Enterobacteriaceae *and prevalences of airborne* E. coli *O157:H7 and* Salmonella enterica[a]

Plant, area in plant	No. of samples	Aerobic bacteria sedimentation rate		*Enterobacteriaceae* sedimentation rate		No. (%) *E. coli* O157:H7 positive	No. (%) *S. enterica* positive
		No. < LOD	Geometric mean (log APC/h)	No. < LOD	Geometric mean (log EBC/h)		
Plant A							
Slaughter	100	0	5.8 (5.5–6.0) A	38	3.3 (3.0–3.5)	23 (23) A	15 (15) A
Evisceration	41	10	3.5 (3.1–3.9) B	34	<2.0	0 (0) B	0 (0) B
Fabrication	21	18	<2.0	18	<2.0	0 (0) B	0 (0) B
Plant B							
Slaughter	101	4	3.8 (3.7–4.0) C	75	<2.0	4 (4) C	0 (0) C
Evisceration	36	5	3.5 (3.2–3.9) C	34	<2.0	1 (3) C	0 (0) C
Fabrication	42	40	<2.0	42	<2.0	0 (0) C	0 (0) C
Plant C							
Slaughter	90	0	5.4 (5.2–5.6)	33	2.8 (2.7–3.1)	19 (21) D	33 (37) D
Evisceration	36	20	<2.0	36	<2.0	0 (0) E	0 (0) E
Fabrication	24	22	<2.0	24	<2.0	0 (0) E	0 (0) E

[a] LOD, limit of detection. Geometric means are followed by 95% confidence intervals in parentheses. Within the same column, geometric means or prevalences from each plant that do not have a common letter are significantly different ($P < 0.05$).

droplets generated by hide removal processes were responsible for the increased APC at A-HP2 and A-HP3 locations since droplets were observed impacting these sponges but were not observed impacting air sampling sponges at A-HO and A-HP1 locations. The design of plant A allowed sampling of air from two locations, hide puller 2–distant (A-HP2D) and hide puller 3–distant (A-HP3D), about 8 ft (ca. 2.4 m) farther from carcasses than the HP2 and HP3 locations, respectively. APC at each of these ''distant'' locations were at least 2.2 log lower than recorded at the corresponding locations closer to hide pullers, suggesting that a significant portion of airborne bacterial contamination generated by hide pullers does not travel beyond their immediate vicinity and further indicating that the airborne contamination risk in this area is primarily from droplets (Table 2). At plant C, APC were higher at slaughter locations where droplets were observed (hide opening [C-HO], hide puller 1 [C-HP1], and behind hide puller 1 [C-HP1B]) than at slaughter locations where droplets were not observed (hide puller 2 [C-HP2] or at the center of the slaughter room [C-CTR]). None of the plant B air sampling locations were contacted by droplets, and it is likely that this also contributed to the lower APC at this plant (Table 2).

EBC were below the limit of detection for the majority of air samples obtained from evisceration and fabrication areas at all three plants (Table 1). EBC were also below the limit of detection for the majority of slaughter area air samples at plant B (Table 1). Detectable EBC were obtained for ≥50% of air samples only at the three plant B sample locations near hide pullers: hide puller 1 (B-HP1), tail catch stand (B-TC), hide puller 2 (B-HP2; Table 2). At plants A and C, the highest EBC were observed at locations where droplets were observed (Table 2). These results demonstrate that the air most contaminated by *Enterobacteriaceae* occurs near hide removal operations and that droplets generated by hide removal contain *Enterobacteriaceae*.

At plant A, *E. coli* O157:H7 prevalence was 23% for slaughter area air samples, 0% for evisceration area air samples, and 0% for fabrication area air samples (Table 1). Plant B *E. coli* O157:H7 air sample prevalences were 4, 3, and 0% for slaughter, evisceration, and fabrication areas, respectively (Table 1). At plant C, the slaughter area air sample *E. coli* O157 prevalence was 21%, but it was 0% for both evisceration and fabrication area air samples. At plant A, *E. coli* O157:H7 was only isolated from samples obtained from hide puller locations (A-HP1, A-HP2, A-HP3) within 2 ft (ca. 0.6 m) of carcasses (Table 2). Of the 23 plant A air samples from which *E. coli* O157:H7 was isolated, 22 samples were at locations (A-HP2, A-HP3) contacted by droplets generated during hide removal (Table 2). Four of the five *E. coli* O157:H7–positive air samples at plant B were from sample locations in the vicinity of hide pullers (B-TC and B-HP2), but none of the locations sampled at plant B were contacted by droplets generated by hide removal. *E. coli* O157:H7 also was detected from a single air sample from the plant B evisceration area, specifically at the splitting saw bench approximately 1 ft (ca. 0.3 m) from carcasses. At plant C, *E. coli* O157:H7 only was recovered from slaughter area air samples located (C-HO, C-HP1, C-HP1B, C-HP2) within 4 ft (ca. 1.2 m) of carcasses (Table 2). Of the 19 plant C air samples from which *E. coli* O157:H7 was isolated, 18 samples were at locations (C-HO, C-HP1, C-HP1B) contacted by droplets generated during hide removal. These results demonstrate that *E. coli* O157:H7 was present in the air near hide removal operations and that droplets generated during removal likely harbored *E. coli* O157:H7.

S. enterica was detected from 15% of plant A slaughter area air samples, but from 0% of plant A evisceration area

TABLE 2. *Slaughter location sedimentation rates of airborne aerobic bacteria and* Enterobacteriaceae *and prevalences of airborne* E. coli *O157:H7 and* Salmonella enterica[a]

Plant, sample location	Sample location abbrev	Approx distance from carcasses (ft)	Droplets present?	No. of samples	Aerobic bacteria sedimentation rate		*Enterobacteriaceae* sedimentation rate		No. (%) *E. coli* O157:H7 positive	No. (%) *S. enterica* positive
					No. < LOD	Geometric mean (log APC/h)	No. < LOD	Geometric mean (log EBC/h)		
Plant A										
Leg transfer	A-LT	2	No	16	0	6.3 (6.0–6.6) BC	0	3.8 (3.6–4.1) B	0 (0)	1 (6)
Hide opening	A-HO	5	No	16	0	5.4 (5.0–5.8) CD	7	2.5 (2.1–3.0) C	0 (0)	0 (0)
Hide puller 1	A-HP1	2	No	12	0	4.9 (4.6–5.2) DE	12	<2.0	1 (8)	1 (8)
Hide puller 2	A-HP2	2	Yes	16	0	7.4 (6.9–7.8) A	0	5.1 (4.7–5.6) A	14 (88)	10 (63)
Hide puller 3	A-HP3	2	Yes	16	0	7.0 (6.7–7.4) AB	0	4.4 (4.0–4.8) AB	8 (50)	3 (19)
Hide puller 2–distant	A-HP2D	10	No	12	0	3.7 (3.2–4.3) E	10	<2.0	0 (0)	0 (0)
Hide puller 3–distant	A-HP3D	10	No	12	0	4.7 (4.2–5.1) DE	9	<2.0	0 (0)	0 (0)
Plant B										
Leg transfer	B-LT	6	No	17	0	3.9 (3.7–4.1) FGH	17	<2.0	0 (0)	0 (0)
Hide opening	B-HO	7	No	18	0	3.5 (3.2–3.7) GH	18	<2.0	0 (0)	0 (0)
Hide puller 1	B-HP1	6	No	12	0	4.2 (3.7–4.7) FG	6	2.5 (2.1–3.0) D	0 (0)	0 (0)
Tail catch stand (3 ft from hide puller 1)	B-TC	1	No	18	0	4.5 (4.2–4.7) F	8	2.5 (2.1–2.9) D	2 (11)	0 (0)
Hide puller 2	B-HP2	4	No	18	3	3.7 (3.1–4.4) FGH	8	2.3 (2.1–2.6) D	2 (11)	0 (0)
Catwalk above hide puller 2	B-CAT	8	No	18	1	3.3 (2.9–3.7) H	18	<2.0	0 (0)	0 (0)
Plant C										
Hide opening	C-HO	1	Yes	18	0	6.3 (6.1–6.6) I	0	3.8 (3.6–4.1) E	13 (72)	13 (72)
Hide puller 1	C-HP1	2	Yes	18	0	5.7 (5.5–6.0) J	3	3.3 (2.7–3.8) EF	3 (17)	10 (56)
Behind hide puller 1	C-HP1B	4	Yes	18	0	5.3 (5.1–5.5) JK	5	2.8 (2.6–3.1) FG	2 (11)	5 (28)
Hide puller 2	C-HP2	2	No	18	0	5.0 (4.7–5.3) KL	8	2.6 (2.1–3.0) G	1 (5)	5 (28)
Center of slaughter room	C-CTR	7	No	18	0	4.6 (4.4–4.9) L	17	<2.0	0 (0)	0 (0)

[a] LOD, limit of detection. Geometric means are followed by 95% confidence intervals in parentheses. Within the same column, geometric means from each plant that do not have a common letter are significantly different ($P < 0.05$). For distance from carcasses values, 1 ft = 0.3048 m.

and fabrication area air samples (Table 1). *S. enterica* was not detected from any sample at plant B. At plant C, *S. enterica* was detected from 37% of slaughter area air samples but was not detected from any evisceration area or fabrication area air samples (Table 1). At plant A, *S. enterica* was only isolated from sample locations (A-LT, A-HP1, A-HP2, A-HP3) within 2 ft of carcasses (Table 2). Of the 15 plant A air samples from which *S. enterica* was isolated, 13 samples were at locations (A-HP2, A-HP3) contacted by droplets generated during hide removal. At plant C, *S. enterica* was only recovered from slaughter area air samples located (C-HO, C-HP1, C-HP1B, C-HP2) within 4 ft of carcasses (Table 2). Of the 33 air samples from which *S. enterica* was isolated in Plant C, 28 were from locations (C-HO, C-HP1, C-HP1B) contacted by droplets generated during hide removal. These results demonstrate that, if hides are contaminated with *S. enterica,* then droplets generated by hide removal likely contain *S. enterica*. However, the five *S. enterica*–positive air samples from the C-HP2 sample location, a location not contacted by droplets, demonstrate that airborne pathogens are in forms other than visible droplets (aerosols).

Air handling systems in place at the studied plants are designed to ensure airflow from ''clean'' to ''dirty'' areas. Our results indicate that either these systems were working as designed or airborne contamination was limited to small localized areas since we did not detect pathogens from any of the 87 air samples from fabrication areas. Additionally, APC were below the limit of detection for 92% of fabrication area air samples, while EBC were below the limit of detection for 98% of fabrication area air samples (Table 1). Because our sampling was limited to 6 h over 3 days at each plant, we were unlikely to detect a sporadic event that would alter the designed air flow and result in higher airborne bacterial levels in fabrication areas. A more effective strategy to address the concerns relating to sporadic alterations of airflow would be for plant management to monitor and record air flow in fabrication areas and compare this data to results of routine microbial testing of products. This practice may identify activities causing the disruption of the designed airflow that result in increased microbial levels during product testing, which would allow plant managers to alter practices to prevent these activities from occurring during production.

E. coli O157:H7 was identified in 47 air samples, and 46 of these positive samples were from hide opening or removal locations. Similarly, *S. enterica* was identified in 48 air samples, and 47 of these positive samples were from hide opening or removal locations. These results lead us to conclude that contamination of air inside beef processing plants is greatest in hide removal operation areas. We further suspect that droplets generated during hide removal harbor these pathogens since 39 of the 47 *E. coli* O157:H7–positive samples and 41 of the 48 *S. enterica*–positive samples were from locations contacted by these droplets (Table 2). The highest APC and EBC were observed for air samples from locations where these droplets were present, increasing our confidence that droplets generated during hide removal are a more likely risk for contamination of carcasses than circulating air per se.

Cattle hides are frequently contaminated by both *E. coli* O157:H7 and *S. enterica (3, 10, 12)*. Levels of pathogen contamination on hides are positively correlated with subsequent carcass contamination *(3, 10, 16)*. It is hypothesized that, if pathogen concentrations on carcasses exceed an undefined critical threshold, carcass interventions will be overwhelmed, resulting in contamination of final products *(4)*. Possible mechanisms of hide-to-carcass transfer of pathogens include contact of carcasses with knives contaminated during hide opening, contact of carcasses by contaminated hides during hide opening and removal, and deposition on carcasses of airborne bacteria generated by hide removal pullers. We observed droplets generated during hide removal at all three plants examined, and air samples obtained in this study strongly suggest that carcasses could be contaminated by airborne bacteria generated during hide removal. Quantification of the contribution of airborne bacteria generated during hide removal to carcass contamination was beyond the scope of this study. Regardless of the exact mechanisms of hide-to-carcass transfer, we believe that these results demonstrate the need for studies focused on improvement of sanitary hide removal that consider the role of airborne pathogens generated by hide removal.

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REFERENCES

1. Abramson, J. H. 2011. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. *Epidemiol. Perspect. Innov.* 8:1.
2. Anonymous. 1997. USDA/NCDA&CS facility guidelines for meat processing plants. *Fed. Regist.* 62:45027–45044.
3. Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. *J. Food Prot.* 70: 280–286.
4. Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2010. Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination. *Meat Sci.* 86:32–37.
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., K. K. Edwards, X. Nou, J. M. Bosilevac, T. M. Arthur, S. D. Shackelford, and M. Koohmaraie. 2005. Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide, and carcass samples: sensitivity and improvements. *J. Food Prot.* 68: 2264–2268.
7. Bosilevac, J. M., T. M. Arthur, J. L. Bono, D. M. Brichta-Harhay, N. Kalchayanand, D. A. King, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2009. Prevalence and enumeration of *Escherichia coli* O157:H7 and *Salmonella* in U.S. abattoirs that process fewer than 1000 head of cattle per day. *J. Food Prot.* 72:1272–1278.
8. Bosilevac, J. M., M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2009. Prevalence and characterization of salmonellae

in commercial ground beef in the United States. *Appl. Environ. Microbiol.* 75:1892–1900.
9. Bosilevac, J. M., S. D. Shackelford, R. Fahle, T. Biela, and M. Koohmaraie. 2004. Decreased dosage of acidified sodium chlorite reduces microbial contamination and maintains organoleptic qualities of ground beef products. *J. Food Prot.* 67:2248–2254.
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 *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Appl. Environ. Microbiol.* 74:6289–6297.
11. Burfoot, D., R. Whyte, D. Tinker, M. Howell, K. Hall, J. Holah, D. Smith, R. White, D. Baker, and J. McIntosh. 2006. Importance of airborne contamination during dressing of beef and lamb carcasses. *J. Food Prot.* 69:2828–2836.
12. Fluckey, W. M., W. G. Loneragan, R. Warner, and M. M. Brashears. 2007. Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle feces, hides, and carcasses. *J. Food Prot.* 70:551–556.
13. 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.* 87:867–876.
14. Jericho, K. W., J. Ho, and G. C. Kozub. 2000. Aerobiology of a high-line speed cattle abattoir. *J. Food Prot.* 63:1523–1528.
15. Nou, X., T. M. Arthur, J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2006. Improvement of immunomagnetic separation for *Escherichia coli* O157:H7 detection by the PickPen magnetic particle separation device. *J. Food Prot.* 69:2870–2874.
16. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and enterobacteriaceae on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
17. Nucera, D. M., C. W. Maddox, P. Hoien-Dalen, and R. M. Weigel. 2006. Comparison of API 20E and *invA* PCR for identification of *Salmonella enterica* isolates from swine production units. *J. Clin. Microbiol.* 44:3388–3390.
18. Prendergast, D. M., D. J. Daly, J. J. Sheridan, D. A. McDowell, and I. S. Blair. 2004. The effect of abattoir design on aerial contamination levels and the relationship between aerial and carcass contamination levels in two Irish beef abattoirs. *Food Microbiol.* 21: 589–596.
19. Rahkio, T. M., and H. J. Korkeala. 1997. Airborne bacteria and carcass contamination in slaughterhouses. *J. Food Prot.* 60:38–42.
20. 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.
21. 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.
22. Worfel, R. C., J. N. Sofos, G. C. Smith, and G. R. Schmidt. 1996. Airborne contamination in beef slaughter-dressing plants with diferent layouts. *Dairy Food Environ. Sanit.* 16:440–443.