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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†

TERRANCE M. ARTHUR,* JOSEPH M. BOSILEVAC, DAYNA M. BRICHTA-HARHAY, NORASAK KALCHAYANAND, DAVID A. KING, STEVEN 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

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ABSTRACT

Transportation from the feedlot and lairage at the processing plant have been identified as potential sources of *Escherichia coli* O157:H7 and *Salmonella* hide contamination. The objective of this study was to perform a comprehensive tracking analysis of *E. coli* O157:H7 and *Salmonella* associated with beef cattle from the feedlot through processing. Cattle ($n = 581$) were sampled in a feedlot, then transported in multiple lots to three commercial, fed beef processing plants in the United States, where they were sampled again. Samples were collected from the tractor trailers prior to loading cattle and from the lairage environment spaces prior to entry of the study cattle. Pathogen prevalence on cattle hides increased on every lot of cattle between exiting the feedlot and beginning processing. Prior to loading cattle, *E. coli* O157:H7 was found in 9 (64%) of 14 tractor trailers. *E. coli* O157:H7 was detected in over 60% of the samples from each lairage environment area, while *Salmonella* was detected in over 70% of the samples from each lairage environment area. *E. coli* O157:H7 and *Salmonella* isolates ($n = 3,645$) were analyzed using pulsed-field gel electrophoresis. The results of the pulsed-field gel electrophoresis tracking indicate that the transfer of bacteria onto cattle hides that occurs in the lairage environments of U.S beef processing plants accounts for a larger proportion of the hide and carcass contamination than does the initial bacterial population found on the cattle exiting the feedlot. Finally, the results of this study indicate that hide wash cabinets are effective in removing contamination derived from the lairage environment.

The complete pathway for ground beef contamination by foodborne bacteria, such as *Escherichia coli* O157:H7 and *Salmonella,* continues to be elucidated. The general model begins with cattle shedding the bacteria in their feces, occasionally at levels exceeding 10^6 CFU/g *(22).* The hide of the individual animal as well as those of the other animals in the same feedlot pen or pasture become laden with the fecal-bacteria mixture including *E. coli* O157:H7 and other pathogens. At slaughter, a proportion of the bacterial inhabitants of the hide is transferred to the carcass during the hide-removal process *(7, 8, 19).* Any bacterial pathogens transferred to the carcass must be killed or removed by antimicrobial interventions, or they will potentially contaminate the ground product.

Recently, a segment of this pathway (transportation and lairage) has been identified as a major contributor of hide, and subsequently carcass, contamination, separate from the feedlot. The lairage environment, defined for this study as the areas cattle pass through from arrival at the processing plant until shackling, has been implicated for contributing to the load of bacterial pathogens on the hides of cattle at slaughter. Marked bacteria have been used to demonstrate the potential for the spread of bacterial pathogens between lots of cattle via the lairage environment *(15).* Other studies have found lairage pen floors to harbor *E. coli* O157:H7 and *Salmonella* from one day to the next, even after the routine cleaning processes have occurred *(23, 25, 26).* Childs et al. *(13)* reported that isolates with similar genotypes were obtained from the lairage environment surfaces and on cattle hides during processing, but hide samples were not collected prior to cattle shipment to determine if the organisms in question were present before transport. Previously, we have shown that 67 and 83% of the hide and carcass isolates, respectively, obtained postharvest could not be attributed to the feedlot or transport trailers *(1).* The results of that study indicated that the isolates of unknown origin may have originated in the lairage environment, but no samples of the lairage environment were collected in that study; thus, the comparison could not be made. That study was done at one processing plant, and because the results were so unexpected, the study described herein was conducted. The study described herein was a comprehensive tracking of *E. coli* O157:H7 and *Salmonella* associated with beef cattle from the feedlot through processing at multiple beef processing plants. In analyzing the

* Author for correspondence: Tel: 402-762-4227; Fax: 402-762-4149; E-mail: Terrance.Arthur@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 U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

TABLE 1. *Number of cattle transported for processing and prevalence of* E. coli *O157:H7 and* Salmonella *in truck samples*

Trip no.	Plant	Lot	Cattle	No. (%) of positive truck samples[a]	
				E. coli O157:H7	*Salmonella*
1	A	A	46 heifers	1 (16.7)	2 (33.3)
		B	48 steers	2 (33.3)	1 (16.7)
2	B	A	40 steers	1 (16.7)	0
		B	47 steers	0	0
3	C	A	44 heifers	6 (100)	1 (16.7)
		B	44 steers	5 (83.3)	3 (50.0)
4	C	A	48 heifers	2 (33.3)	3 (50.0)
		B	49 steers	0	1 (16.7)
5	A	A	32 heifers	0	0
		B	32 heifers	0	3 (50.0)
		C	32 heifers	5 (83.3)	2 (33.3)
		D	32 heifers	3 (50.0)	4 (66.7)
6	B	A	45 steers	1 (16.7)	1 (16.7)
		B	42 steers	0	0

[a] Number of truck samples that were positive for each pathogen prior to loading cattle. Six samples were collected for each truck.

results from this study, an intervention that is effective in reducing the extent of lairage contamination was identified.

MATERIALS AND METHODS

Study design. Cattle ($n = 581$) were sampled in a feedlot, then transported in multiple lots to three large, commercial, fed beef processing plants in the United States. Each plant received cattle shipments on two separate sampling days. Two lots of cattle were sent to each plant for each of the sampling days with the exception of plant A, which received four lots of cattle on the second sampling day. On a sampling day, the individual lots of cattle were housed in holding pens in separate areas of the cattle yard. One lot of cattle would enter the processing plant approximately 2 h after arrival in the lairage area, while the second lot would enter approximately 4 h after arrival. For the second shipment to plant A, two lots were processed at 2 h postarrival, with a nonstudy lot of cattle processed between them. The remaining two lots were processed at 4 h postarrival, also with a lot of nonstudy cattle processed between the two lots. Hide and carcass samples were collected along the processing line during normal operation.

Animals. Cattle were transported to the processing plants on six separate occasions. All animals were transported on commercial tractor trailers. At the processing plant, the animals were treated as any other lots of commercial cattle would be, with the exception that the time interval between arrival of the cattle at the plant and slaughter was limited to 2 or 4 h, as described above. No other special accommodations were made. Each tractor-trailer load of animals was maintained as a single lot through processing and was not intermingled with other cattle. The test cattle could have come in contact with other cattle through the bars of the lairage pens. The number of cattle in each lot is presented in Table 1.

Sample collection: feedlot. Hide and fecal samples were collected from every animal on the day prior to transport. Animals to be shipped were restrained in a squeeze chute, samples were collected, and the animals were returned to their original pens to await load out the following morning. Hide samples were collected by using a sterile sponge (Nasco, Fort Atkinson, Wis.) premoistened with buffered peptone water (Difco, Becton Dickinson, Sparks, Md.) to swab an area of approximately 1,000 cm^2 of the brisket, using five strokes (one motion back and forth constituted a stroke) on each side of the sponge. Fecal samples (10 g) were collected by rectal palpation. Hide and fecal samples were collected between 8:30 and 11:00 a.m. one day prior to transport. Cattle were loaded on tractor trailers at approximately 6:00 a.m. the day of processing.

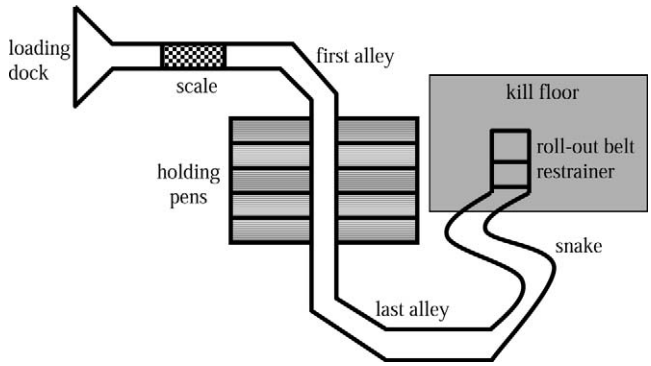

FIGURE 1. *General schematic for layout of lairage environment.*

Truck samples. Commercial tractor trailers transported the study cattle from the feedlot to the processing plant. Prior to loading the cattle, the interior of each tractor trailer was sampled. (The tractor trailer samples will be referred to as ''truck'' samples for the purposes of this report.) No truck transported more than one lot of study animals per day. Individual trucks may have transported multiple lots of study animals throughout the course of the study, but multiple transports of nonstudy cattle and cleaning cycles would have occurred between transport of each lot of study animals. Six samples were collected from the interior of each truck. The sample locations were spread among the animal holding areas. Truck samples were collected using a sterile sponge attached to a handle (SpongeSicle, Biotrace International, Inc., Bothell, Wash.) premoistened with 20 ml of buffered peptone water to swab an area of ≈1,000 cm^2 on the truck floor.

Lairage environment samples. Lairage environment samples were collected using a sterile, nonhydrated SpongeSicle and swabbing an area of ≈500 cm^2. Multiple samples were collected throughout the lairage environment immediately prior to the test cattle passing through each area. The lairage environment by definition would consist of the spaces encountered by the animals from the time of unloading from trucks to the stunning event. However, contamination of the hide could occur outside of the strict lairage environment after stunning as the animal moves from the restrainer to the rollout belt prior to the location where the hide sample was taken. Therefore, for the purposes of this report, the lairage environment definition has been broadened to include this area. The samples were collected from the following locations: scale (samples [$n = 4$] collected from the loading dock to the scale); first alley (samples [$n = 4$] collected in alleyways leading from scale to holding pens); holding pen (samples [$n = 8$] collected from the holding pen); last alley (samples [$n = 4$] collected in alleyways leading from the holding pen to the crowd pen); snake (samples [$n = 4$] collected in the single-file race that leads from the crowd pen to the restrainer); restrainer (samples [$n = 2$] collected from the restrainer); and rollout belt (samples [$n = 2$] collected from the rollout belt) (Fig. 1). Two sets of lairage

environment samples were collected per sampling trip. One set ($n = 28$) was collected for each lot of test cattle. Samples collected for the scale, first alley, holding pens, last alley, and snake area were collected by swabbing the floor of each of these locations. Due to a lack of substantial organic matter found on visual inspection, walls and holding pen bars were not sampled. Restrainer and rollout belt samples were collected by swabbing the animal contact surfaces for each piece of equipment.

In-plant sampling. Postharvest hide and carcass samples were collected in the processing plants. The processing plants differed in that two utilized hide-on carcass wash cabinets (plants B and C), while the other plant did not (plant A). Hide samples were collected immediately after stunning and bleeding in plants A and B. A second set of hide samples was collected in plant C after carcasses exited the hide cabinet. Due to space constraints, hide samples could not be collected prior to the hide wash cabinet in plant B; thus, hide samples were collected after exit of the carcasses from the cabinet. The hide prevalence and enumeration data are presented herein as ''before cabinet'' and ''after cabinet.'' The before-cabinet data includes plant A, which did not have a hide wash cabinet, and plant C. The after-cabinet data includes plants B and C. Hide samples were collected before and after the hide cabinet in plant C. Hide samples were collected using sterile sponges premoistened with 20 ml of buffered peptone water to swab an area of ≈1,000 cm^2 in approximately the same location that was sampled at the feedlot. Preevisceration carcass samples were obtained after hide opening, before application of any antimicrobial interventions. Carcass samples were collected using two sterile sponges (Nasco) premoistened with 20 ml of buffered peptone water. Each sponge was used to sample an area of approximately 4,000 cm^2, as previously described *(3).*

Enumeration. *E. coli* O157:H7 and *Salmonella* were enumerated from carcass, hide, fecal, lairage environment, and truck samples, using either hydrophobic grid membrane filtration or spiral plating (Spiral Biotech, Norwood, Mass.), following the protocol used by Brichta-Harhay et al. *(12).* For carcass, hide, and truck samples, the sponge samples were homogenized by hand massage, and 250 μl of solution was removed to a 1.5-ml microcentrifuge tube prior to the addition of enrichment media. Each tube was vortexed and then held static for 3 min to allow the debris to settle.

Following the settling period, 50 μl was used for hide and truck sample spiral plating onto ntCHROMAgar (CHROMAgar-O157 [DRG International, Mountainside, N.J.] supplemented with novobiocin [5 mg/liter; Sigma, St. Louis, Mo.] and potassium tellurite [2.5 mg/liter; Sigma]) for *E. coli* O157:H7, and XLD_{tnc} medium (XLD medium [Remel, Lenexa, Mo.] with 4.6 ml/liter Tergitol, 15 mg/liter novobiocin, and 5 mg/liter cefsulodin [Sigma]) for *Salmonella.* For pathogen enumeration from carcass samples, 300 μl (for *E. coli* O157:H7) or 500 μl (for *Salmonella*) of carcass sponge sample was added to 7 ml of phosphate-buffered saline (PBS) with 1% (vol/vol) Tween 80 (Sigma) for hydrophobic grid membrane filtration analysis. The samples were applied to ISO-Grid membranes (Neogen, Lansing, Mich.), filtered with a spread-filter apparatus (FiltaFlex, Ltd., Ontario, Canada), and the membranes were then placed on the appropriate selective medium. *E. coli* O157:H7 ISO-Grid membranes were placed on nt-CHROMAgar and incubated at 42°C for 18 to 20 h, and then inspected for the presence of *E. coli* O157:H7. These putative *E. coli* O157:H7 colonies were tested using the DrySpot agglutination test kit (Oxoid, Ltd., Basingstoke, UK), as above. Suspect colonies were confirmed by PCR *(16). Salmonella* ISO-Grid membranes were placed on XLD_{tnc} medium and incubated at 37°C for 18 to 20 h, and then for an additional 18 to 20 h at room temperature (23 to 25°C). Black colonies on the XLD_{tnc} plates were considered presumptive *Salmonella.* Suspect colonies were confirmed by PCR *(20, 27).*

When enumerating from fecal and lairage environment samples, the enrichment media (90 ml of tryptic soy broth [TSB; Difco, Becton Dickinson] with phosphate buffer [TSB+PO_4: 30 g of TSB, 2.31 g of KH_2PO_4, and 12.54 g of K_2HPO_4] per liter of solution) was added to the 10-g fecal sample or the Sponge-Sicle, and the mixture was homogenized by hand massage. One milliliter of the sample mixture was removed to a 1.5-ml microcentrifuge and vortexed. The enumeration was then carried out as described for carcass, hide, and truck samples. After incubating the *E. coli* O157:H7 and *Salmonella* plates overnight at 42 and 37°C, respectively, the plates were counted and suspect colonies confirmed by PCR *(16, 20, 27).* Limits of detection for the enumeration assay were 200 CFU/g, 40 CFU/100 cm^2, 400 CFU/100 cm^2, 40 CFU/100 cm^2, and 0.5 CFU/100 cm^2 for the fecal, hide, lairage environment, truck, and carcass samples, respectively. The means for enumeration data from lairage environment samples were calculated using the following definitions: samples that were enumeration and enrichment negative were assigned a value of 4.0 CFU/100 cm^2; those that were enumeration negative but enrichment positive were assigned a value of 40 CFU/100 cm^2; and samples that were enumeration positive were given their calculated value. All values were log converted, and the average and standard deviation were determined.

Sample processing for prevalence. Samples were processed according to methods previously described, with slight modifications *(9, 18).* Carcass, hide, and truck sponge samples were enriched with 80 ml of TSB after the 250-μl aliquot for enumeration was removed. Fecal and lairage environment samples were enriched in the 90 ml of TSB+PO_4 used for enumeration dilution. The sample bags were incubated at 25°C for 2 h, then at 42°C for 6 h prior to being held at 4°C overnight. After incubation, the samples were processed by immunomagnetic separation, during which 1 ml from each enrichment was subjected to anti-*Salmonella* immunomagnetic bead cell concentration, using 20 μl of anti-*Salmonella* beads (Invitrogen, Carlsbad, Calif.). The beads were extracted from enrichment samples and washed two times in PBS–Tween 20 (Sigma), using an automated magnetic particle processor (KingFisher 96, Thermo Fisher Scientific, Inc. Waltham, Mass.). For *Salmonella,* the immunomagnetic separation beads were transferred to Rappaport-Vassiliadis–soy (Remel) broth and incubated at 42°C overnight. *Salmonella* present in these samples was detected by swabbing the Rappaport-Vassiliadis–soy enrichment onto Hektoen enteric agar (Difco, Becton Dickinson) containing novobiocin (5 mg/liter), and brilliant green medium with sulfadiazine (Difco, Becton Dickinson). All plates were incubated at 35 to 37°C for 18 to 20 h. After incubation, up to three suspect colonies were picked for confirmation. PCR was used to confirm that each *Salmonella* isolate contained the *invA* gene *(20, 27).* Isolates were maintained as frozen stocks for use in strain typing by pulsed-field gel electrophoresis (PFGE).

After *Salmonella* immunomagnetic separation, 20 μl of anti-O157 beads (Invitrogen) was added to the same 1-ml enrichment aliquots and recovered as described for *Salmonella.* The beads were resuspended in 100 μl of PBS–Tween 20. Fifty microliters of the final bead-bacteria complexes was spread plated onto ntChromagar, and sorbitol MacConkey agar (Difco, Becton Dickinson) supplemented with cefixime (0.05 mg/liter) and potassium tellurite (2.5 mg/liter; Invitrogen). All plates were incubated at 35 to 37°C for 18 to 20 h. After the plates were incubated, up to

TABLE 2. E. coli *O157:H7 enumeration*[a] *and prevalence*[b] *from feedlot and plant samples*

Plant	No. of cattle	Enumeration				Prevalence			
		Feedlot hide	Plant hide before cabinet	Plant hide after cabinet	Plant preevisceration	Feedlot hide	Plant hide before cabinet	Plant hide after cabinet	Plant preevisceration
A	222	1.4	28.4	NC[c]	0.5	57.2	88.7	NC[c]	38.3
B	174	1.7	—[d]	4.0	0	73.3	—[d]	97.7	1.7
C	185	2.2	12.4[e]	2.2	0	69.7	62.5[e]	38.4	5.9
Overall	581	1.7	21.1	3.0	0.2	66.0	76.8	65.2	17.0

[a] Enumeration data are presented as the percent of total samples that were above the limit of detection for enumeration. Enumeration limit of detection for *E. coli* O157:H7 was ≥200 CFU/g for fecal samples, ≥40 CFU/100 cm^2 for hide samples, and ≥0.5 CFU/100 cm^2 for preevisceration samples.

[b] Prevalence data are presented as the percent of total samples in which *E. coli* O157:H7 was detected.

[c] NC, no cabinet; plant A did not have a hide wash cabinet.

[d] Cattle were not accessible for hide sampling prior to hide wash cabinet for plant B.

[e] Before-cabinet samples from the second trip to plant C had very high background microflora. Prevalence and enumeration are believed to be underestimated.

three presumptive positive colonies were picked for confirmation. PCR was used to confirm that each *E. coli* isolate harbored genes for the O157 antigen, H7 flagella, and at least one of the Shiga toxins *(16).* All isolates were maintained as frozen stocks for later use in PFGE.

PFGE. Isolates (n = 3,645) from three of the six trips (one trip for each processing plant) were analyzed using PFGE. *E. coli* O157 (n = 2,004) and *Salmonella* (n = 1,641) isolate fingerprints generated and analyzed in this study were based on PFGE separation of *Xba*I-digested genomic DNA, as described previously *(8)*; this is the method currently used by members of PulseNet (http://www.cdc.gov/pulsenet/protocols.htm). Pulsed-field gel–certified agarose (SeaKem Gold agarose) was obtained from Cambrex Bio Science Rockland, Inc. (Rockland, Maine); Tris-borate-EDTA running buffer and proteinase K were purchased from Sigma. *Xba*I was purchased from New England Biolabs (Beverly, Mass.). *Salmonella* serotype Braenderup strain H9812 was used as a control and for standardization of gels *(17).* Banding patterns were analyzed and comparisons made using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium), employing the Dice similarity coefficient in conjunction with the unweighted pair group method using arithmetic averages for clustering. Isolates were grouped into types that likely had the same origin based on fingerprint pattern similarities. Types were defined strictly as isolates that grouped together and had identical banding patterns.

Statistics. Appropriate comparisons of pathogen prevalence were made using the DIFFER procedure of PEPI (version 2, USD, Inc., Stone Mountain, Ga.), which calculates a continuity-adjusted chi square for the difference between two proportions.

RESULTS

Prevalence and enumeration analysis of *E. coli* O157:H7 and *Salmonella.* The overall prevalence of *E. coli* O157:H7 on the cattle hides at the feedlot was 66%, with 10 (1.7%) animals harboring the bacteria on their hides at enumerable levels (≥40 CFU/100 cm^2) (Table 2). Fecal prevalence at the feedlot was 24.5%, with 49 (8.4%) animals shedding *E. coli* O157 at enumerable levels (≥200 CFU/g) (data not shown). Increases ($P < 0.001$) were detected in the hide prevalence and enumeration results from all three plants, with the exception of the hide prevalence before the cabinet in plant C (Table 2). The before-cabinet samples from the second trip to plant C had high levels of background flora present, making detection of the *E. coli* O157:H7 colonies difficult. The prevalence of 62.5% on the hides before the cabinet is most likely an underestimation of the true prevalence. Transfer of *E. coli* O157 from hide to carcass resulted in an overall prevalence at preevisceration of 17%. The carcass prevalence and enumerable counts of *E. coli* O157:H7 were lower at the processing plants utilizing hide wash cabinets than at the plant without a hide wash cabinet ($P < 0.001$).

For *Salmonella* analysis, the data were much clearer due to the low prevalence of *Salmonella* in the feedlot and a secondary selective enrichment used to reduce background microflora. *Salmonella* was detected in the hide and fecal samples collected at the feedlot on the first sampling trip, but not on the other five trips. The overall hide prevalence was 0.7%, with no animals harboring enumerable levels on their hides (Table 3). The fecal prevalence was 1.9%, with no animals shedding *Salmonella* at levels ≥200 CFU/g (data not shown). Increases ($P < 0.001$) were detected in hide prevalence and levels at all three plants to the point where the majority of cattle on each trip harbored *Salmonella* on their hide during processing. The overall preevisceration prevalence of *Salmonella* was 2.9%, with carcass isolates recovered only at the plant not utilizing a hide wash cabinet (plant A) (Table 3).

Prior to loading cattle, *E. coli* O157:H7 was found in 9 (64%) of 14 trucks, with one truck having all samples (n = 6) positive for the pathogen (Table 1). Overall, 26 (31.0%) of 84 truck samples were positive for *E. coli* O157 H7. Seven (8.3%) truck samples, which came from 4 of the 14 trucks, harbored *E. coli* O157:H7 at levels ≥40 CFU/100 cm^2, with the highest level being 1,600 CFU/100 cm^2 (data not shown). *Salmonella* was detected in 10 (71.4%) of 14 trucks prior to loading cattle (Table 1), with an overall prevalence of 25% (21 of 84). No truck samples contained *Salmonella* at levels ≥40 CFU/100 cm^2.

With the exception of the restrainer on the second trip to plant C, where only *E. coli* O157:H7 was detected, every lairage environment area sampled was found to harbor *E.*

TABLE 3. Salmonella *enumeration*[a] *and prevalence*[b] *from feedlot and plant samples*

Plant	No. of cattle	Enumeration				Prevalence			
		Feedlot hide	Plant hide before cabinet	Plant hide after cabinet	Plant preevisceration	Feedlot hide	Plant hide before cabinet	Plant hide after cabinet	Plant preevisceration
A	222	0	3.6	NC[c]	0.5	1.8	62.6	NC[c]	7.7
B	174	0	—[d]	2.8	0	0	—[d]	87.4	0
C	185	0	6.5	0.5	0	0	88.1	24.3	0
Overall	581	0	4.9	1.7	0.2	0.7	74.2	51.3	2.9

[a] Enumeration data are presented as the percent of total samples that were above the limit of detection for enumeration. Enumeration limit of detection for *Salmonella* was ≥200 CFU/g for fecal samples, ≥40 CFU/100 cm^2 for hide samples, and ≥0.5 CFU/100 cm^2 for preevisceration samples.

[b] Prevalence data are presented as the percent of total samples in which *Salmonella* was detected.

[c] NC, no cabinet; plant A did not have a hide wash cabinet.

[d] Cattle were not accessible for hide sampling prior to hide wash cabinet for plant B.

coli O157:H7 and *Salmonella* on every trip (data not shown). *E. coli* O157:H7 was detected in over 60% of the samples from each lairage environment area, with the scale samples having the highest prevalence at 87.5% (42 of 48 samples) (Table 4). Several samples from the lairage environment carried *E. coli* O157:H7 at levels ≥400 CFU/100 cm^2. A last alley sample had the highest load at 23,200 CFU/100 cm^2, while a holding pen sample also was high at 22,400 CFU/100 cm^2 (data not shown). *Salmonella* was detected in over 70% of the samples from each lairage environment area, with 100% (48 of 48 samples) of the samples from the snake carrying the organism (Table 4). Several samples harbored *Salmonella* at levels ≥400 CFU/100 cm^2 (Table 4), but the maximum levels were not as high as those for *E. coli* O157:H7, with the highest *Salmonella* load coming from a scale sample at 1,600 CFU/100 cm^2 (data not shown).

PFGE analysis of *E. coli* O157:H7 and *Salmonella*. Isolates (n = 3,645) collected on three of the six trips, one trip to each plant, were analyzed by PFGE. The breakdown of isolate origins is 599 *E. coli* O157:H7 and 40 *Salmonella* from the feedlot, 990 *E. coli* O157:H7 and 1,057 *Salmonella* from hides and carcasses at the plant, and 415 *E. coli* O157:H7 and 544 *Salmonella* from the lairage environment and trucks. Of the 746 *E. coli* O157:H7 isolated from hides postharvest, 304 (40.8%) matched genotypes found in the feedlot (Tables 5 and 6). Several of these genotypes also were isolated from the lairage environment prior to entry of the test cattle, putting the true source of these bacteria in question. Only 64 (8.6%) isolates were of genotypes that were identified solely in the feedlot. The largest source for the postharvest hide isolates was the lairage environment. Postharvest hide PFGE patterns for 599 (80.3%) of the 746 isolates matched patterns found in the lairage environment. Again, some of these overlapped with feedlot patterns, but 350 (46.9%) were attributable to only the lairage environment. One percent of postharvest hide isolate genotypes matched genotypes found in the trucks, and 11% (82 of 746) were of unknown origin, most likely from the lairage environment, but were not discovered in the limited sampling.

The distribution of *E. coli* O157:H7 carcass isolate sources followed that of the postharvest hide isolates, as would be expected. The majority of the carcass isolates (184 [75.1%] of 245) matched genotypes found in the lairage environment (Table 5). While some of these genotypes were seen in the feedlot, the majority (n = 164, 66.9%) of the preevisceration carcass isolates solely matched lairage derived genotypes (Table 6). Sixty (24.5%) of the carcass isolates matched feedlot sources, but when removing the

TABLE 4. E. coli *O157:H7 and* Salmonella *enumeration*[a] *and prevalence*[b] *in lairage environment samples (%)*

Sample location	No. of samples per lot	No. of lots	*E. coli* O157:H7		*Salmonella*	
			% prevalence (SD)	Log CFU/100 cm^2 (SD)	% prevalence (SD)	Log CFU/100 cm^2 (SD)
Scale	4	12	87.5 (29.2)	1.65 (0.42)	97.9 (7.2)	1.81 (0.44)
First alley	4	12	83.3 (19.5)	1.61 (0.48)	95.8 (22.9)	1.67 (0.25)
Holding pen	8	14[c]	71.4 (31.6)	1.63 (0.68)	89.3 (22.9)	1.52 (0.25)
Last alley	4	12	66.7 (37.4)	1.77 (0.94)	97.9 (7.2)	1.65 (0.12)
Snake	4	12	62.5 (32.9)	1.45 (0.44)	100 (0.0)	1.65 (0.10)
Restrainer	2	12	75.0 (39.9)	1.52 (0.65)	79.2 (33.4)	1.60 (0.30)
Rollout belt	2	12	83.3 (32.6)	1.48 (0.38)	70.8 (39.6)	1.36 (0.48)

[a] Enumeration data are presented as the average log CFU/100 cm^2. The data are averages of the cell counts per location per lot.

[b] Prevalence data are presented as the percent of samples in which *Salmonella* was detected. The data are averages of the prevalence per location per lot. Standard deviations are given in parentheses.

[c] For the second trip to plant A, two additional holding pens were used for the two additional lots.

TABLE 5. E. coli *O157:H7 and* Salmonella *PFGE types of postharvest hide and carcass isolates after harvest of cattle*

Organism	Sample site	Total no. of isolates	No. (%) of isolates matching PFGE types from[a]:			
			Feedlot	Lairage	Truck	Unknown origin
E. coli O157:H7	Hide	746	304 (40.8)	599 (80.3)	10 (1.3)	82 (11.0)
	Preevisceration	245	60 (24.5)	184 (75.1)	0	21 (8.6)
Salmonella	Hide	1,007	0	959 (95.2)	351 (34.9)	48 (4.8)
	Preevisceration	50	0	42 (84.0)	27 (54.0)	8 (16.0)

[a] Number of isolates within a row may add up to more than the total number of isolates due to identical PFGE patterns being found in multiple locations.

genotypes that overlapped with other sources, there were a remaining 40 (16%) isolates that could be attributed only to the feedlot. Genotypes of unknown origin accounted for 8.6% (21 of 245) of the carcass isolates, while genotypes found in the truck samples were not identified on any carcasses.

Salmonella isolates were recovered from samples at the feedlot on only one of the five sampling times. None of the genotypes found in the feedlot matched any of the genotypes from the 1,007 hide or 50 carcass isolates recovered from the processing plant samples (Table 5). There was overlap of genotypes identified in the lairage environment and those from truck samples (Table 7). Most of the *Salmonella* hide isolates (959 [95.2%] of the 1,007) matched genotypes identified in the lairage environment. On removal of those that also were identified in truck samples, 656 (65.1%) *Salmonella* postharvest hide isolates were found to match genotypes solely from the lairage environment samples. The genotypes found in the truck were identical to those of 351 (34.9%) of the 1,007 postharvest hide isolates, but all of these overlapped with genotypes from the lairage samples (Table 7). The remaining 48 (4.8%) *Salmonella* postharvest hide isolate genotypes could not be attributed to a source.

The *Salmonella* carcass isolates also followed the trend of the postharvest hide isolates with respect to the genotype distribution. No genotypes matched those from the feedlot. Genotypes from the lairage made up the majority of the

TABLE 6. *Lairage environment locations where* E. coli *O157:H7 isolates were obtained that matched hide and carcass isolate genotypes*

No. of isolates of particular genotypes[a]		Locations where identical genotypes were recovered[b]									
Hide	Pre-evisceration	F	TK	SC	FA	HP	LA	SN	RN	RB	UK
157	4	■		■	■	■	■	■		■	
21	4			■	■	■	■		■	■	
40	38				■	■	■	■	■	■	
14	12	■			■	■			■	■	
9				■	■		■	■	■		
23	42				■	■		■	■	■	
31	24				■	■	■		■	■	
2				■	■	■		■			
4				■			■	■		■	
33	19				■	■		■		■	
69	4	■					■	■			
8			■					■		■	
25				■	■	■					
9				■	■		■				
2				■		■			■		
40	2			■				■		■	
3					■			■			
7						■	■		■		
19	24					■			■	■	
1			■			■					
16				■				■			
26	1				■	■					
64	40	■									
1			■								
2				■							
2					■						
10						■					
18	10						■				
1								■			
4									■		
3										■	
82	21										■

[a] Number of hide and carcass isolates with genotypes found in indicated locations. One row may consist of multiple genotypes as long as they were all found in the same lairage environment spaces.

[b] F, feedlot; TK, truck; SC, scale; FA, first alley; HP, holding pen; LA, last alley; SN, snake; RN, restrainer; RB, roll-out belt; UK, unknown origin.

TABLE 7. *Lairage environment locations where* Salmonella *isolates were obtained that matched hide and carcass isolate genotypes.*

No. of isolates of particular genotypes[a]		Locations where identical genotypes were recovered[b]									
Hide	Pre-evisceration	F	TK	SC	FA	HP	LA	SN	RN	RB	UK
252	27		■	■	■	■	■	■	■	■	
45			■	■		■	■	■	■	■	
264				■	■	■	■	■	■	■	
17				■	■		■	■	■	■	
21				■	■	■	■	■			
35				■			■	■	■	■	
153						■	■	■	■	■	
11				■	■	■	■				
7				■	■						
1				■	■			■			
2				■		■			■		
3						■	■	■			
54			■	■							
48	12			■	■						
15	3					■		■			
11				■							
12						■					
7								■			
1										■	
48	8										■

[a] Number of hide and carcass isolates with genotypes found in indicated locations. One row may consist of multiple genotypes as long as they were all found in the same lairage environment spaces.

[b] F, feedlot; TK, truck; SC, scale; FA, first alley; HP, holding pen; LA, last alley; SN, snake; RN, restrainer; RB, roll-out belt; UK, unknown origin.

carcass isolate genotypes (42 [84%] of 50), but 27 (54%) of these overlapped with genotypes from truck isolates (Tables 5 and 7). Eight (16%) isolates could not be attributed to a source by PFGE analysis.

DISCUSSION

Previous studies have reported the potential for cross-contamination of cattle during transport to the processing facility. Barham et al. isolated *E. coli* O157:H7 and *Salmonella* from 7 and 74.5%, respectively, of the samples taken from cattle trucks prior to loading cattle *(6).* In another study, *E. coli* O157:H7 was detected in 8 of 12 trucks prior to loading cattle *(15).* It was not determined in either of these studies if these isolates were found on the hides of cattle after transport, or if they were ever transferred to the carcasses. Reicks et al. reported that *Salmonella* prevalence on the hides of animals increased after transport, but could not attribute the change to the cleanliness of the trailers *(21).* In previous work, Arthur et al. found 7 of 8 tractor trailers to harbor *E. coli* O157:H7 prior to loading cattle *(1).* After transportation, it was determined that 19 (2.5%) of the 764 *E. coli* O157:H7 isolates recovered from hide and carcass samples matched the PFGE patterns of isolates found in the trailers prior to loading cattle, but did not match any genotypes of *E. coli* O157:H7 isolates collected in the feedlot *(1).*

The lairage environment, defined for this study as the areas cattle pass through from arrival at the processing plant until shackling, has been implicated for contributing to the load of bacterial pathogens on the hides of cattle at slaughter. Several studies have shown the potential for bacterial pathogens to persist in the lairage environment even through routine cleaning cycles *(23, 25, 26).* Our group presented data showing that over 65% of the hide *E. coli* O157:H7 isolates and 83% of the carcass isolates collected at a beef processing plant did not originate in the feedlot from which the animal came *(1).* A small portion of these isolates were of identical genotypes to those found on the transport trucks prior to loading cattle, but due to the fact that lairage environment samples were not collected in that study, the sources of the remaining isolates were not identified.

In the study described herein, 67 and 30% of the carcass *E. coli* O157:H7 and *Salmonella* isolates, respectively, could be attributed solely to the lairage environment. Bacteria from the lairage environment most likely account for a larger percentage of carcass contamination than this, but could not be definitively proven so, due to overlap of genotypes with those found at the feedlot and in truck samples. In addition, isolates were recovered on the hides and carcasses for which a source could not be found. These isolates, categorized as ''unknown,'' most likely originated from the lairage environment, but were not detected due to the sampling areas being of small size relative to the entire lairage environment. The fact that the lairage environment sources account for such high levels of carcass contamination reflects on the scope of the hide contamination that is occurring in the lairage environment. One can easily envision that as multiple cattle in multiple lots pass through the same spaces, bacteria could be picked up on the hide, even leading to a high prevalence of such isolates on the hide. For these isolates to be present on the carcass at the high frequencies detected in this study indicates that not only are these bacteria getting onto the cattle hides at high rates, but also that they do so in high numbers. In earlier work, our lab reported a large turnover in the *E. coli* O157:H7 population on cattle hides between transport and processing. In that study, the cattle were sampled at the feedlot and loaded onto trucks the same day. The cattle arrived at the processing plant that evening, and they were held until the next morning for processing. It was hypothesized that the long hold time in the lairage environment led to the high prevalence of nonfeedlot bacteria on the cattle hides *(1).* In the present study, the cattle were treated as typical commercial cattle and were held between 2 to 4 h in lairage. The present study also included three beef processing plants, as opposed to one, to ensure that the results were indicative of routine plant operations in the United States.

One of the most striking results from this study is that *Salmonella* was detected only on the first feedlot sampling, leading to an overall hide prevalence of 0.7% prior to transport, yet on sampling at the processing plant, *Salmonella* was found on the majority of the cattle hides on all six trips, and none of the hide or carcass isolates could be attributed to the feedlot, based on PFGE analysis. This observation indicates that the absence of pathogens on cattle hides when the animals arrive at a processing plant does not ensure that carcasses of those animals will remain free of pathogens. Further, it suggests that the fecal and hide status, with regard to pathogen prevalence, of animals sent to slaughter is of little relevance in determining the risk of carcass contamination. With large processing plants receiving over 3,000 head per day, the lairage environment will be a composite of the pathogen prevalence and levels associated with each animal that has passed through the individual spaces. Therefore, cattle that have undergone successful preharvest intervention regimes may readily pick up contamination during lairage, in effect nullifying the intervention. As was seen in this study, *E. coli* O157:H7 and *Salmonella* were detected in at least one sample from all lairage environment spaces in all three plants on each trip, with the one exception being one space, the restrainer, on one trip to one plant that only had *E. coli* O157:H7 and not *Salmonella.*

Other studies have reported similar observations regarding the potential for transfer of bacterial pathogens among cattle in lairage environments. Collis et al. *(14)* inoculated the hides of cattle and lairage environment surfaces with marked bacterial strains, and observed the spread of these bacteria to the hides of multiple animals prior to processing, It was noted in that report that besides within-lot transfer, both the hide marker and the environmental marker were found on the carcasses of animals from lots whose members or environment had not been inoculated, demonstrating the cross contamination potential in the lairage environment *(14).* Small et al. *(24)* also identified the potential for spread of pathogens in the lairage environment by finding *E. coli* O157:H7, *Salmonella,* and *Campylobac-*

ter in several lairage environment samples, but was not able to show direct transfer.

Molecular analysis by PFGE has been used to show the transfer of bacteria to cattle hides in the lairage environment. Avery et al. identified a predominant *E. coli* O157:H7 genotype on the hides of cattle at processing *(5).* The cattle sampled in that study originated from multiple locations, removing the feedlot from the list of potential sources. The lairage environment was the only common factor identified that could be a source of the bacteria *(5).* Tutenel et al. *(26)* also utilized PFGE to identify a predominant *E. coli* O157:H7 type present on the hides of cattle over multiple days at a processing plant, in spite of the fact that the animals originated from different farms. Another study concluded that *E. coli* O157:H7 contamination of cattle hides could be traced, using PFGE, to the feedlot, transport trailers, and plant holding pens, but could not give a magnitude for these sources *(13).* In looking at this question from a different point of view, *E. coli* O157:H7 isolates of the same genotype were obtained from cattle hides at multiple U.S. beef processing plants, separated by large geographical distances *(4).* One potential explanation the authors gave for this result focused on the lairage environment. With individual feedlots sending cattle to multiple plants for processing, it is logical to conclude that the lairage environments at those plants will become contaminated with bacteria of the same genotypes. Those bacteria have the potential to spread to other animals in lairage, resulting in cattle from unrelated sources carrying pathogens of the same genotype. The current study used PFGE analysis to determine the frequency with which cattle pick up new *E. coli* O157:H7 and *Salmonella* contamination during lairage, and indicates that the source is the lairage environment in general and not any particular space within that environment.

The two plants that employed hide cabinets had carcass prevalence rates for *E. coli* O157:H7 and *Salmonella* of below 6 and 0%, respectively, while the plant that did not use a hide wash cabinet had carcass prevalence rates of 38 and 8%, respectively. Previous work has shown that hide wash cabinets reduce hide and subsequently carcass prevalence rates significantly *(2, 10, 11).* However, when the genotypes of carcass isolates were analyzed, it was determined that not only did the prevalence go down, but also that in the plants with hide cabinets, 61.5% (16 of 26) *E. coli* O157:H7 preevisceration carcass isolates matched feedlot genotypes, whereas only 25.5% (40 of 157) of the carcass isolates matched feedlot genotypes in the plant without a hide wash cabinet. As for *Salmonella,* the plant without a hide wash cabinet was the only one to have carcass contamination with *Salmonella,* with none of the isolates matching feedlot genotypes. These results lead to the conclusion that hide wash cabinets are effective in removing contamination derived from the lairage environment. It stands to reason that lairage-derived contamination is of a recent nature when the animal enters that slaughter floor and could be more easily washed away, leading to a lower proportion of lairage-derived contamination on the carcass. Thus, while it was expected that the hide wash cabinets would be effective, it was not anticipated that they would preferentially remove the more recent lairage contamination.

In summary, the sources of *E. coli* O157:H7 and *Salmonella* contamination of carcasses at U.S. beef processing plants were tracked using PFGE. The results of the tracking indicate that the transfer of bacteria onto cattle that occurs in the lairage environments of U.S beef processing plants accounts for a larger proportion of the hide and carcass contamination than does the initial bacterial population found on the cattle upon exiting the feedlot. A large proportion of this lairage-derived bacterial load can be removed using hide wash cabinets.

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REFERENCES

1. 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 and levels of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. *J. Food Prot.* 70:280–286.
2. 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.
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. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Comparison of the molecular genotypes of *Escherichia coli* O157:H7 from the hides of beef cattle in geographically distant regions of North America. *J. Food Prot.* 70:1622–1626.
5. Avery, S. M., E. Liebana, M. L. Hutchison, and S. Buncic. 2004. Pulsed field gel electrophoresis of related *Escherichia coli* O157 isolates associated with beef cattle and comparison with unrelated isolates from animals, meats and humans. *Int. J. Food Microbiol.* 92: 161–169.
6. Barham, A. R., B. L. Barham, A. K. Johnson, D. M. Allen, J. R. Blanton, Jr., and M. F. Miller. 2002. Effects of transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *J. Food Prot.* 65:280–283.
7. 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.
8. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
9. 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.
10. Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* 67:646–650.
11. Bosilevac, J. M., X. Nou, M. S. Osborn, 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.
12. Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and fecal samples using direct plating methods. *J. Appl. Microbiol.* 103:1657–1668.
13. Childs, K. D., C. A. Simpson, W. Warren-Serna, G. Bellenger, B. Centrella, R. A. Bowling, J. Ruby, J. Stefanek, D. J. Vote, T. Choat, J. A. Scanga, J. N. Sofos, G. C. Smith, and K. E. Belk. 2006. Molecular characterization of *Escherichia coli* O157:H7 hide contamination routes: feedlot to harvest. *J. Food Prot.* 69:1240–1247.
14. Collis, V. J., C. A. Reid, M. L. Hutchison, K. P. A. Wheeler, A. Small, and S. Buncic. 2004. Spread of marker bacteria from the hides of cattle in a simulated livestock market and at an abattoir. *J. Food Prot.* 67:2397–2402.
15. Cuesta Alonzo, E. P., S. E. Gilliland, and C. R. Krehbiel. 2007. Incidence and toxin production ability of *Escherichia coli* O157:H7 isolated from cattle trucks. *J. Food Prot.* 70:2383–2385.
16. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
17. Hunter, S. B., P. Vauterin, M. A. Lambert-Fair, M. S. Van Duyne, K. Kubota, L. Graves, D. Wrigley, T. Barrett, and E. Ribot. 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *J. Clin. Microbiol.* 43:1045–1050.
18. Nou, X., T. M. Arthur, J. M. Bosilevac, D. M. Brichta, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2006. Improvement of immunomagnetic separation for *Escherichia coli* O157:H7 detection by PickPen magnetic particle separation device. *J. Food Prot.* 69: 2870–2874.
19. 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.
20. Rahn, K., S. A. DeGrandis, R. C. Clarke, S. A. McEwen, J. E. Galan, C. Ginocchio, R. Curtiss, 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. Reicks, A. L., M. M. Brashears, K. D. Adams, J. C. Brooks, J. R. Blanton, and M. F. Miller. 2007. Impact of transportation of feedlot cattle to the harvest facility on the prevalence of *Escherichia coli* O157:H7, *Salmonella,* and total aerobic microorganisms on hides. *J. Food Prot.* 70:17–21.
22. Robinson, S. E., E. J. Wright, C. A. Hart, M. Bennett, and N. P. French. 2004. Intermittent and persistent shedding of *Escherichia coli* O157 in cohorts of naturally infected calves. *J. Appl. Microbiol.* 97:1045–1053.
23. Small, A., C. James, S. James, R. Davies, E. Liebana, M. Howell, M. Hutchinson, and S. Buncic. 2006. Presence of *Salmonella* in the red meat abattoir lairage after routine cleansing and disinfection and on carcasses. *J. Food Prot.* 69:2342–2351.
24. Small, A., C. A. Reid, S. M. Avery, N. Karabasil, C. Crowley, and S. Buncic. 2002. Potential for the spread of *Escherichia coli* O157, *Salmonella,* and *Campylobacter* in the lairage environment at abattoirs. *J. Food Prot.* 65:931–936.
25. Small, A., C. A. Reid, and S. Buncic. 2003. Conditions in lairages at abattoirs for ruminants in southwest England and in vitro survival of *Escherichia coli* O157, *Salmonella* Kedougou, and *Campylobacter jejuni* on lairage-related substrates. *J. Food Prot.* 66:1570–1575.
26. Tutenel, A. V., D. Pierard, J. Van Hoof, and L. De Zutter. 2003. Molecular characterization of *Escherichia coli* O157 contamination routes in a cattle slaughterhouse. *J. Food Prot.* 66:1564–1569.
27. Wang, R. F., W. W. Cao, and C. E. Cerniglia. 1997. A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. *J. Appl. Microbiol.* 83:727–736.