Journal of Food Protection, Vol. 67, No. 2, 2004, Pages 295–302

Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* in Two Geographically Distant Commercial Beef Processing Plants in the United States†

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MS 03-293: Received 30 June 2003/Accepted 18 September 2003

ABSTRACT

For two large beef processing plants, one located in the southern United States (plant A) and one located in the northern United States (plant B), prevalence of *Escherichia coli* O157:H7, *Listeria* spp., *Listeria monocytogenes,* and *Salmonella* was determined for hide, carcass, and facility environmental samples over the course of 5 months. The prevalence of *E. coli* O157:H7 (68.1 versus 55.9%) and *Salmonella* (91.8 versus 50.3%) was higher ($P < 0.05$), and the prevalence of *Listeria* spp. (37.7 versus 75.5%) and *L. monocytogenes* (0.8 versus 18.7%) was lower ($P < 0.05$) for the hides of cattle slaughtered at plant A versus plant B. Similarly, the prevalence of *Salmonella* (52.0 versus 25.3%) was higher ($P < 0.05$) and the prevalence of *Listeria* spp. (12.0 versus 40.0%) and *L. monocytogenes* (1.3 versus 14.7%) was lower ($P < 0.05$) for the fence panels of the holding pens of plant A versus plant B. The prevalence of *E. coli* O157:H7 (3.1 versus 10.9%), *Listeria* spp. (4.5 versus 14.6%), and *L. monocytogenes* (0.0 versus 1.1%) was lower ($P < 0.05$) for preevisceration carcasses sampled at plant A versus plant B. *Salmonella* (both plants), *Listeria* spp. (plant B), and *L. monocytogenes* (plant B) were detected on fabrication floor conveyor belts (product contact surfaces) late during the production day. For plant B, 21 of 148 (14.2%) late-operational fabrication floor conveyor belt samples were *L. monocytogenes* positive. For plant B, *E. coli* O157:H7 and *L. monocytogenes* were detected in preoperational fabrication floor conveyor belt samples. Overall results suggest that there are regional differences in the prevalence of pathogens on the hides of cattle presented for harvest at commercial beef processing plants. While hide data may reflect the regional prevalence, the carcass data is indicative of differences in harvest practices and procedures in these plants.

In the United States, foodborne pathogens have been estimated to cause 7 million illnesses and up to 9,000 deaths annually, with a resulting economic loss of six billion dollars *(14).* Also, bacterial pathogens account for 60% of hospitalizations attributable to foodborne transmission and 67% of estimated food-related deaths. Mead et al. *(28)* have reported that 90% of the estimated food-related deaths involve the pathogens *Salmonella* (31%), *Listeria monocytogenes* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 (3%).

Two major foodborne bacterial pathogens, *E. coli* O157:H7 and *Salmonella,* have an animal reservoir and have been associated with the contamination of meat and meat products *(7, 9, 38).* Recently, *L. monocytogenes* has also been identified as a serious foodborne pathogen *(38)* and has been demonstrated to be a contaminant of beef carcasses *(23).* These pathogens have been associated with the hide, the intestinal tract of healthy animals, and the environment *(7, 11, 15, 31, 32, 36).* Recent studies have shown high prevalence of *E. coli* O157:H7 and *Salmonella* on hides *(3, 4, 5, 11, 22),* which serve as a potential source of indirect carcass contamination during hide removal. Generally, the muscle surfaces of the carcasses are sterile and contamination occurs as a result of microbial transfer during hide removal and dressing defects during the slaughtering process.

Sources of in-plant carcass microbial contamination during slaughter include those associated with the processing practices, slaughter plant facilities, and plant personnel *(7, 15, 36).* The slaughter plants in the United States have developed Hazard Analysis Critical Control Points plans to decrease the risk of foodborne illnesses by intervening at stages of processing that pose a plausible risk of carcass contamination *(40).* These plans require adequate microbiological data to be able to assess the effectiveness of control programs for foodborne pathogens.

Carcass contamination is a function of incoming bacterial load on the cattle hides and in-plant harvest practices and procedures. Therefore, the present study was conducted to determine differences in the prevalence of *E. coli* O157:

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† Brand names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standards of any product mentioned, 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.

TABLE 1. *Environmental sampling sites for each processing plant (plant A and plant B)*

Site	Number of samples per plant per month	Area sampled (cm^2) (plant A/plant B)
Before operation[a]		
Slaughter floor		
Floor drains	10	413–1,006/162–366[b]
PCS[c]	10	206.5/318–488
Brisket saws	4	205.5/83
Split saws	4	205.5/194
Fabrication floor		
PCS-conveyor belts	20	1,463/660–828.5
Anytime during operation[d]		
Locker room		
Floor drains	6	642/32–180
Knobs on external doors	6	826/195–320
Knobs on toilet stalls	6	58/125–137
Knobs on lockers	6	148/32
Knobs on soap dispensers	6	77/41
Holding pen fences		
Panels	15	774/549
Late in operation[d]		
Slaughter floor		
Trolleys	15	535.5/19
Floor drains	15	413–1,006/162–366
PCS	15	200/244–488
Brisket saws	6	206.5/83
Split saws	6	206.5/194
Fabrication floor		
PCS-conveyor belts	30	1,463/600–828.5

[a] Samples were taken on the first and third week of the month.
[b] Area sampled was a function of the size and shape of the item to be sampled. Thus, the area for a given sample type varied within and between plants.
[c] Product contact surface.
[d] Samples were taken on the first, second, and third week of the month.

H7, *L. monocytogenes,* and *Salmonella* between incoming bacterial load on the cattle's hides (index of regional differences) and carcasses (index of the plant's practices and procedures) in two geographically distant commercial beef processing plants in the United States.

MATERIALS AND METHODS

Sampling procedure. Samples were collected for a period of 5 months (April, May, July, August, and October) in three trips per month to each of the two large steer/heifer commercial beef processing plants. One plant was located in the southern (plant A) United States while the other was located in the northern (plant B) United States. For both of these plants, slaughter chain speed ranged from 250 to 300 animals per hour. The carcass decontamination treatments used by both plants is a combination of available interventions including but not limited to (i) steam vacuuming, (ii) knife trimming, (iii) preevisceration carcass washing, and (iv) final postevisceration carcass rinses.

Depending on which environmental site was to be sampled, samples were taken either before operation, anytime during operation, or late in operation (Table 1). At the same time that the environmental sampling was being conducted (anytime during operation), 35 samples each of hides, preevisceration carcasses (before any application of antimicrobial treatments), and postintervention (after full complement of antimicrobial intervention) carcasses were taken at each plant. Thus, a total of 105 animals per month were sampled and the sampling of hides and carcasses was performed as previously described *(5, 11),* with the modification that all hides and carcass samples were collected using a HydraSponge (cat. no. HS-10BPW/2G, International BioProducts, Bothell, Wash.) premoistened with 10 ml of buffered peptone water.

Environmental samples were taken by swabbing the site area (Table 1) using a HydraSponge (cat. no. HS-10NB/2G, International BioProducts) premoistened with 10 ml of neutralizing buffer. Environmental, hide, and carcass samples were transported on ice packs *(1)* to the corresponding laboratories and processed within 20 to 24 h.

Preenrichment of the samples. In the laboratory, once the samples were received, 90 ml of Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.) was added to each sponge sample, stomached for 1 min, and incubated at 25°C for 2 to 3 h. The resulting preenrichment culture was divided into three portions for three enrichment procedures, which were handled as fol-

lows. For the enrichment of *E. coli* O157:H7, 10 ml of the preenriched sample was transferred into a sterile 15-ml conical tube, incubated for 6 h at 42°C, and held at 4°C overnight *(6).* These samples were then shipped on ice packs to the Roman L. Hruska U.S. Meat Animal Research Center via overnight mail *(1)* for the isolation and characterization of *E. coli* O157:H7. For the enrichment of *Listeria* spp., 4 ml of the preenriched sample was added to 36 ml of Fraser broth (Difco) and incubated at 30°C for 18 to 24 h. Finally, the remaining 76 ml of the preenriched sponge sample was incubated at 35°C for 22 to 24 h for the screening and recovery of *Salmonella.*

Recovery and confirmation of *E. coli* O157:H7. Upon arrival of the samples at the Roman L. Hruska U.S. Meat Animal Research Center, *E. coli* O157:H7 was isolated from the enriched samples using immuno-magnetic separation as previously described *(6).* Characteristic colonies on either ctSMAC or nt-Rainbow plates were tested (up to 6 colonies per sample) using the Dryspot *E. coli* O157 latex test (Oxoid, Inc., Ogdensburg, N.Y.). Latex-positive colonies were streaked onto ctSMAC plate for purity and single colonies were stored in nutrient agar stabs *(33)* for further testing. Subsequently, broth cultures of each isolate were stored at −70°C as glycerol stocks. Suspect colonies were confirmed using an indirect ELISA (O157 and H7 antigens) *(11)* and multiplex polymerase chain reaction to detect the presence of stx_1, stx_2, *eaeA,* $fliC_{H7}$, and $rfbE_{O157}$ gene fragments *(19).* Isolates were further verified as *E. coli* species using the Sensititre Gram-negative AutoIdentification (AP80) system (Accumed International, Westlake, Ohio). Samples were considered positive if at least one isolate recovered in the sample met the following requirements: either expressed the O157 antigen or carried the $rfbE_{O157}$ gene, expressed the H7 antigen or carried the $fliC_{H7}$ gene, carried at least one *stx* gene, and was determined to be *E. coli* by the AP80 system.

Enrichment, recovery, and confirmation of *Listeria* spp. After incubation of the samples in Fraser broth, a 1-ml aliquot of each sample was transferred into 9 ml of buffered *Listeria* enrichment broth (BLEB, Difco) containing 8.5 g of 3-(N-morpholino) propanesulfonic acid (MOPS), and 13.7 g of MOPS sodium salt in 1 liter purified water. These secondary enrichment (MOPS-BLEB) samples were incubated at 30 ± 2°C for 20 ± 2 h. Thereafter, 1 ml was removed from each sample and the detection of *Listeria* spp. was performed according to the AOAC Official Method 996.14 *(2)* using the Assurance EIA *Listeria* enzyme immunoassay test kit (BioControl System, Inc., Belleview, Wash.). The remaining enrichment broth was refrigerated and used for confirmation of presumptive positives, as described elsewhere *(10).* Typical hemolytic colonies of *Listeria* were streaked to a purity plate (Trypticase soy agar–yeast extract or bilayer horse blood plates) to obtain a pure isolate. If no hemolysis was observed, the typical nonhemolytic *Listeria* colonies were also streaked to a purity plate. The pure culture was then confirmed for *Listeria* spp. and/or *L. monocytogenes* using the biochemical identification method (AOAC official method 992.19) *(2),* the semisolid motility agar for motility *(41),* or the API-*Listeria* (bioMérieux-Vitek, Hazelwood, Miss.) rapid test strip.

Enrichment, recovery, and confirmation of *Salmonella.* After the 22 to 24 h incubation at 35°C of the samples in TSB, selective enrichment and detection of motile and nonmotile *Salmonella* was performed following the AOAC Official Method 992.11 protocol *(2).* The M-broth cultures as well as the selective media cultures were retained for confirmation of presumptive positives on the enzyme immunoassay test kit. Presumptive-positive samples were confirmed using culture, biochemical, and serological methods as previously described *(10).* Isolates giving typical *Salmonella* reactions were tested serologically for the presence of Poly (O) and Poly (H) antigens using the *Salmonella* O Antiserum Poly A-I and Vi (Difco 2264-47) and H Antiserum Poly a-z (Difco 2406-47) following the manufacturer's instructions.

Statistical analysis. For each type of sample (hide, carcass, and environmental), prevalence of each pathogen was estimated for both processing plants by dividing the number of positive samples by the total number of samples tested. The exact binomial 95% confidence intervals (CI) were calculated for each prevalence point estimate using PEPI *((13)*; ‹http://www.sagebrushpress.com/pepibook.html›). In order to test for pathogen- and sample type-specific prevalence differences between plants, DIFFER procedure of PEPI was used to calculate a continuity-adjusted chi-square for the difference between plants.

For hide data, the interaction of processing plant with month of sampling was also tested by multiple continuity-adjusted chi-square tests. To avoid Type I error rates due to multiple comparisons, the pair-wise *P* values were adjusted using Hommel's modification of the Bonferroni procedure *(18)* using PEPI software.

RESULTS AND DISCUSSION

The data collected and reported in this study is unique in that a number of key pathogens were simultaneously isolated from the same sample. This is in contrast with taking individual samples for each pathogen. The nonrandom distribution of pathogens on sites examined in these experiments and in general requires simultaneous isolation *(30).* A potential drawback to this approach could be that the prevalence of pathogens at sampling sites with low cell density may be underestimated. However, the advantages (elimination of nonrandom distribution as a source of error) in our assessment far outweigh the potential disadvantage.

Prevalence of pathogenic bacteria on hides, carcasses, and holding pen environment. The sample period that was chosen to carry out this study was based on previous data on fecal shedding and prevalence on hide of *E. coli* O157:H7, which has been shown to peak during summer and early fall *(5, 11, 24).* Overall prevalence of the three pathogens tested on hides was statistically different ($P < 0.001$) between plants (Table 2). The overall prevalence of Shiga toxin-producing *E. coli* O157:H7 and *Salmonella* on hides was significantly higher for plant A, whereas the prevalence of *Listeria* spp. and *L. monocytogenes* on hides was higher for plant B. The data suggest that hide is a principal source of *E. coli* O157:H7, *Listeria* spp., *L. monocytogenes,* and *Salmonella.* To the best of our knowledge, the prevalence of *Listeria* spp. and *L. monocytogenes* on cattle hides has not been determined in the United States. A limited documentation on the prevalence of these pathogens in cattle feces has been done in Europe *(12, 34)* and Brazil *(17).*

The prevalence of *Listeria* spp. and *L. monocytogenes* on fence panels in the holding pens was significant between plants, with plant B showing higher prevalence (40.0 and 14.7%, respectively), while the prevalence of *Salmonella* on fence panels was significantly higher ($P < 0.01$) for plant A (Table 2). There were no significant differences between plants in the prevalence of *E. coli* O157:H7 for

TABLE 2. *Prevalence (%) of pathogens on the holding pen fences and on animals' hides and carcasses in plant A and plant B*

	Salmonella			*Listeria* spp.			*L. monocytogenes*			*E. coli* O157:H7		
Site/plant	*n*	%	95% CI	*n*	%	95% CI	*n*	%	95% CI	*n*	%	95% CI
Hides												
Plant A	510	91.8[a]	89.0–94.0	512	37.7[a]	33.5–42.1	510	0.8[a]	0.2–7.0	511	68.1[a]	63.9–72.1
Plant B	523	50.3	45.9–54.7	523	75.5	71.6–79.2	523	18.7	15.5–22.4	522	55.9	51.6–60.2
Preevisceration carcasses												
Plant A	511	23.3	19.7–27.2	511	4.5[a]	2.9–6.7	511	0.0[b]	0.0–0.7	510	3.1[a]	1.8–5.0
Plant B	522	26.8	23.1–30.9	522	14.6	11.7–17.9	522	1.1	0.4–2.5	523	10.9	8.4–13.9
Postintervention carcasses												
Plant A	499	0.0	0.0–0.7	499	0.0	0.0–0.7	499	0.0	0.0–0.7	497	0.0[c]	0.0–0.7
Plant B	520	0.8	0.2–2.0	520	0.2	0.0–0.1	520	0.0	0.0–0.7	520	1.0	0.3–2.2
Fence panels in the holding pens												
Plant A	75	52.0[d]	40.2–63.7	75	12.0[a]	5.6–21.6	75	1.3[d]	0.0–7.2	75	13.3	6.6–23.2
Plant B	75	25.3	16.0–36.7	75	40.0	28.9–52.0	75	14.7	7.6–24.7	75	5.3	1.5–13.1

[a] Within an organism and sample type, prevalence differed between plants ($P < 0.001$).
[b] Within an organism and sample type, prevalence differed between plants ($P < 0.05$).
[c] Within an organism and sample type, prevalence differed between plants ($P < 0.10$).
[d] Within an organism and sample type, prevalence differed between plants ($P < 0.01$).

this site. The incidence of these pathogens on fence panels was lower than on hides with the exception of *L. monocytogenes* in plant A, which is in agreement with a previous study *(35)*. Furthermore, the lower incidence on the fence panels in our study may be a function of the difference in sampling area between hides (~1,700 cm^2) and the fence panels (774 or 549 cm^2; Table 1). The data in this study shows that all three pathogens tested were recovered from the fence panels in the holding pens (Table 2). Others have reported similar observations in the lairage environment at abattoirs in Europe *(3, 12, 35)*.

There was an interaction ($P < 0.05$) between plant and sampling months in the presence of all three pathogens on hides (Table 3). Whereas prevalence of *E. coli* O157:H7 on hides was higher for plant A during May, July, and August, the plants did not differ ($P < 0.05$) during the months of April, July, August, and October. The seasonal onset of high *E. coli* O157:H7 prevalence occurred sooner for plant A. The prevalence of *Salmonella* was higher for plant A during all sampling months. However, the magnitude of the difference between plants was lowest during August. The prevalence of *L. monocytogenes* was ≤2.0% during each sampling month for plant A, but for plant B, the prevalence of *L. monocytogenes* ranged from 3.8 to 48.6% during the 5 sampling months. The cattle presented for slaughter usually come from approximately 150 miles radius of a plant. Because plant A and plant B are located far from each other, the prevalence of pathogens on the animal's hide represent an index of regional differences in the prevalence of these microorganisms.

Despite the higher incidence of *E. coli* O157:H7 and *Salmonella* on hides of cattle harvested at plant A, the prev-

TABLE 3. *Interaction of plant (plant A versus plant B) and sampling month on the prevalence (%) of pathogens on hides*

	Prevalence by month				
Organism/plant	April	May	July	August	October
E. coli O157:H7[a]					
Plant A	46.5 C	79.0 AB	72.5 AB	81.8 A	60.6 BC
Plant B	40.4 C	44.8 C	71.4 AB	82.7 A	40.4 C
Listeria spp.[a]					
Plant A	39.6 CD	42.9 CD	32.7 D	13.9 E	58.7 C
Plant B	82.9 B	93.3 AB	46.7 CD	57.7C	97.1 A
L. monocytogenes[a]					
Plant A	2.0 C	1.0 C	0.0 C	0.0 C	1.0 C
Plant B	16.2 B	48.6 A	9.5 BC	3.8 BC	15.4 B
Salmonella[a]					
Plant A	98.0 AB	87.6 BC	95.0 AB	99.0 A	79.8 C
Plant B	26.7 E	49.5 D	45.7 DE	77.9 C	51.9 D

[a] Within an organism, means with different letters are significantly ($P < 0.05$) different.

TABLE 4. *Differences between plant A and plant B for prevalence (%) of pathogens in slaughter floor environmental samples*

Site/plant[a]	Microorganism			
	Salmonella	*Listeria* spp.	*L. monocytogenes*	*E. coli* O157:H7
Floor drains, before operation				
Plant A	2/50 (4.0)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)
Plant B	2/50 (4.0)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)
Floor drains, late during operation				
Plant A	17/74 (23.0)	2/74 (2.7)	0/74 (0.0)	0/75 (0.0)
Plant B	10/75 (13.3)	4/75 (5.3)	1/75 (1.3)	0/75 (0.0)
Product contact surfaces, before operation				
Plant A	0/49 (0.0)	0/49 (0.0)	0/49 (0.0)	0/49 (0.0)
Plant B	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)
Product contact surfaces, late during operation				
Plant A	0/74 (0.0)	0/74 (0.0)	0/74 (0.0)	1/74 (1.4)
Plant B	1/75 (1.3)	1/75 (1.3)	0/75 (0.0)	0/75 (0.0)
Brisket saw, before operation				
Plant A	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)	1/20 (5.0)
Plant B	0/11 (0.0)	1/11 (9.1)	1/11 (9.1)	0/11 (0.0)
Brisket saw, during break/lunch				
Plant A	0/30 (0.0)	0/30 (0.0)	0/30 (0.0)	0/30 (0.0)
Plant B	0/17 (0.0)	0/17 (0.0)	0/17 (0.0)	0/16 (0.0)
Splitting saw, before operation				
Plant A	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)
Plant B	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)	0/20 (0.0)
Splitting saw, during break/lunch				
Plant A	0/27 (0.0)	0/27 (0.0)	0/27 (0.0)	0/27 (0.0)
Plant B	0/29 (0.0)	0/29 (0.0)	0/29 (0.0)	0/29 (0.0)
Trolleys, late during operation				
Plant A	0/73 (0.0)	1/73 (1.4)	0/73 (0.0)	0/73 (0.0)
Plant B	0/73 (0.0)	2/73 (2.7)	0/73 (0.0)	0/73 (0.0)

[a] Plants did not differ for any comparison ($P > 0.10$).

alence of these two pathogens on preevisceration and postintervention carcasses was significantly lower (Table 2). Similar trends were observed for plant B. More important, for both plants, the antimicrobial interventions used during carcass processing substantially reduced the prevalence of *E. coli* O157:H7, *Salmonella, Listeria* spp., and *L. monocytogenes* on postintervention carcasses (Table 2). Hide removal and evisceration are considered to be procedures in which pathogens are transferred onto carcasses *(15, 36)*. *Escherichia coli* O157:H7, *Salmonella, Listeria* spp., and *L. monocytogenes* have been found to be contaminants of carcasses after dehiding and other slaughtering/dressing processes *(4, 5, 11, 12, 20, 21, 23, 26, 27, 37, 39, 42)*.

An overall (both plants, see Table 6) evaluation of the bacterial profile of the samples collected from hides, preevisceration carcasses, and from panels in the holding pens

TABLE 5. *Differences between plant A and plant B for prevalence (%) of pathogens in fabrication floor environmental samples*

Site/plant	Microorganism			
	Salmonella	*Listeria* spp.	*L. monocytogenes*	*E. coli* O157:H7
Product contact surfaces, before operation				
Plant A	0/100 (0.0)	0/100 (0.0)[a]	0/100 (0.0)[a]	0/100 (0.0)
Plant B	0/99 (0.0)	6/99 (6.1)	6/99 (6.1)	2/98 (2.0)
Product contact surfaces, late during operation				
Plant A	4/150 (2.7)	0/150 (0.0)[b]	0/150 (0.0)[b]	0/150 (0.0)
Plant B	3/148 (2.0)	25/148 (16.9)	21/148 (14.2)	0/148 (0.0)

[a] $P < 0.05$.
[b] $P < 0.001$.

TABLE 6. *Number of hide, carcass, and plant environmental samples containing one or more bacterial species*[a]

Site	Number of pathogens			
	0[b]	1	2	3
Before operation				
Slaughter floor				
Drains	96	4	0	0
Product contact surfaces	99	0	0	0
Brisket saws	28	2	0	0
Splitting saws	40	0	0	0
Fabrication floor				
Product contact surfaces	190	8	0	0
Anytime during operation				
Slaughter floor				
Hides	38	274	479	241
Carcasses (pre-evisceration)	657	324	46	5
Carcass cooler				
Carcass (postevisceration)	1,007	10	0	0
Locker room				
Drains	47	9	1	0
Knobs on external doors	59	1	0	0
Knobs on lockers	58	0	0	0
Knobs on soap dispensers	58	0	0	0
Pens				
Panels	68	57	21	4
Late in operation				
Slaughter floor				
Trolleys	143	3	0	0
Drains	117	31	1	0
Product contact surfaces	146	3	0	0
Brisket saws	46	0	0	0
Split saws	56	0	0	0
Fabrication floor				
Product contact surfaces	268	28	2	0

[a] *E. coli* O157:H7, *Salmonella, Listeria* spp., and *L. monocytogenes.*
[b] Negative by method.

revealed that a proportion of the samples contained more than one bacterial species.

Prevalence of pathogenic bacteria on environmental samples. A total of 812 and 797 samples were obtained from the 11 environmental sites (not including pens) sampled at plant A and plant B, respectively, representing the slaughter floor, fabrication floor, and locker rooms at different times during operation (Tables 4 and 5). A total of 417 and 400 samples (plant A and plant B, respectively) from the slaughter floor were tested for all three pathogens (Table 4). Only two positive samples of *E. coli* O157:H7 were obtained from plant A and were identified on the product contact surfaces (PCS), late during operation, and on the brisket saw (before operation). As for *Salmonella,* the floor drains were positive for both plants (before and late during operation); plant B also tested positive for the PCS (late during operation). *L. monocytogenes* was also found in plant B floor drains (late during operation) and on the brisket saw (before operation). There were no significant differences ($P > 0.10$) between plants for all the pathogens tested. Overall, the data indicates that sources of pathogen contamination were identified on the processing equipment and surfaces (brisket saw and PCS) in addition to the floor drains at different time points. Moreover, the pathogen contamination observed on the slaughter floor of each plant emulates the prevalence of these pathogens on hides and preevisceration carcasses.

On the fabrication floor, prevalence of pathogenic bacteria was only tested on conveyor belts (Table 5). *E. coli* O157:H7 and *L. monocytogenes* were recovered on this site for plant B before operation. Interestingly, late during operation, the incidence of *L. monocytogenes* on conveyor belts was found in 14.2% of the samples tested. Also, *Salmonella* was found on the conveyor belts late during operation in both plants, which suggests that there was a source of contamination during processing. Once more, this

difference in the prevalence of these pathogens on the conveyor belts in each plant is consistent with the preevisceration carcass data.

An evaluation of a total of 145 and 150 samples, from plant A and plant B, respectively, for the presence of pathogens on different sites within the locker room environment reveals that *Salmonella* (10%), *L. monocytogenes* (3.3%), and *E. coli* O157:H7 (3.3%) were present on the floor drains from plant B, whereas in plant A, only *Salmonella* (22.2%) was present on this site. Furthermore, the locker rooms' door knobs in plant A tested positive for *E. coli* O157:H7 and the toilet stalls' door knobs in plant B tested positive for this pathogen. There were no significant differences ($P > 0.10$) between plants for all the pathogens tested. An assessment of the pathogenic microbial profile in the overall environmental samples from both plants showed that pathogens recovered from 5.5% of the samples contained only one bacterial species whereas four samples contained two species (Table 6). The data suggests that contamination can occur in the environment of the plant as well as by the personnel anytime during processing.

Few studies have addressed the prevalence of multiple pathogenic bacteria species in commercial beef processing plants; therefore, reported data regarding the prevalence of *E. coli* O157:H7, *Salmonella,* and *Listeria* species within the environment of beef slaughtering plants in the United States at different time points of beef processing is scarce or nonexistent. Studies conducted in beef abattoirs in Europe have revealed that the points of pathogen contamination include the processing equipment *(8, 12, 16, 20, 25, 29),* plant operation personnel *(8, 16, 20),* and the plant's environment *(16, 20, 25).* These findings, as well as those of the present study, suggest that bacterial contaminants can occur on mechanical equipment and PCS and that human factors can contribute to contamination of beef carcasses.

In conclusion, this research suggests that hides and fence panels in the holding pens are sources of contamination in the preslaughter environment and can be used to determine the contamination pattern of the carcasses. For instance, in plant A, *Salmonella* and *E. coli* O157:H7 were predominant in this environment whereas *Listeria* spp. and *L. monocytogenes* were predominant in plant B, and similar patterns of the incidence of the specific pathogens were observed on carcasses (Table 2) and in-plant environment (Tables 4 and 5). Furthermore, seasonality is a factor that can be used as predictors of these pathogens because the prevalence of *E. coli* O157:H7 and *Salmonella* on hides was sustained for a longer period of time in plant A (May, July, August, and October; Table 3).

ACKNOWLEDGMENTS

This project was funded in part by beef and veal producers and importers through their $1-per-head checkoff and was produced for the Cattlemen's Beef Board and state councils by the National Cattlemen's Beef Association. We thank Patty Beska, Julie Dyer, Troy Gramke, Sue Hauver, Bruce Jasch, Dee Kucera, Kim Kucera, Frank Reno, Audrey Smidt, and Pat Tammen for their technical assistance and Marilyn Bierman and Carol Grummert for secretarial assistance. Also, we thank Mindy Brashears, Jason Mann, and Mark Miller from Texas Tech University, Lubbock; and Dennis Buege, Steve Ingham, Jill Losinski, Cara Cross, Kole Ewoldt, and Lauren Sammel from the University of Wisconsin, Madison, for their assistance in sampling.

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