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Characterization of *E. coli* O157:H7 Strains Resulting from Contamination of Raw Beef Trim during High Event Periods

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ABSTRACT

The development and implementation of effective antimicrobial interventions by the beef processing industry in the United States has dramatically reduced the incidence of beef trim contamination by *Escherichia coli* O157:H7. However, individual processing plants still experience sporadic peaks in contamination rates where multiple *E. coli* O157:H7-positive lots are clustered in a short time frame. These peaks have been referred to as "High Event Periods" (HEP) of contamination. The results reported here detail the characterization of *E. coli* O157:H7 isolates from twenty-one HEP across multiple companies and processing plants to gain insight regarding the mechanisms causing these incidents. Strain genotypes were determined by pulsed field gel electrophoresis and isolates were investigated for characteristics linking them to human illness. Through these analyses, it was determined that individual HEP show little to no diversity of strain genotype. Hence, each HEP has one strain type that makes up most if not all of the contamination. This is shown to differ from the genotypic diversity of *E. coli* O157:H7 found on the hides of cattle entering processing plants. In addition, it was found that a high proportion (81%) of HEP are caused by strain types associated with human illness. These results pose a potential challenge to the current model for finished product contamination during beef processing.

41 The development and implementation of effective antimicrobial interventions by the beef
42 processing industry in the United States has reduced the incidence of beef trim contamination by
43 *Escherichia coli* O157:H7. These improvements have resulted in decreased contamination rates of raw
44 beef trim by the bacterial pathogen *E. coli* O157:H7 to an estimated national prevalence of 0.39% (1).
45 However, individual processing plants experience sporadic peaks in contamination rates where multiple
46 positive lots are clustered in a short time frame. These peaks have been referred to as "High Event
47 Periods" (HEP) of contamination. The Food Safety and Inspection Service (FSIS) of the USDA has
48 defined HEP as production intervals during which slaughter establishments experience a high rate of positive
49 results for *E. coli* O157:H7 (or STEC or virulence markers) in trim samples (2). Typically, a cause/source
50 for a HEP is not identified, and the contamination event will be resolved before notable correction of the
51 process can be performed.

52 The current model of finished product contamination during beef processing starts with the
53 pathogen load on the hides of cattle entering the processing plant. Several studies (3-5) have identified
54 the hide as the major source of *E. coli* O157:H7 contamination of carcasses during processing. Once
55 contamination has been transferred from the hide to the carcass during dehiding, it must be removed or
56 destroyed through antimicrobial interventions to prevent finished product contamination. However,
57 research has indicated that interventions or even systems of multiple interventions can be overwhelmed
58 by high concentrations of bacteria and fail to prevent finished product contamination (6). In addition to
59 exceeding the threshold of properly functioning interventions, the model assumes that finished product
60 contamination will occur when interventions are not functioning at optimal levels or processing
61 personnel are not working within the guidelines of the industry's best practices.

62 It has been assumed that HEPs would follow the basic premise of this contamination model and
63 be a function of incoming pathogen load. However, there is a large knowledge gap regarding the

mechanism of HEPs. Due to the intricacies of the beef harvest process, most studies of beef processing can only follow contamination from the incoming animal, through the killfloor, to the point where the carcasses are chilled after all interventions have been applied. Following the chilling process, carcasses are graded and sorted into similar weight/grade categories to facilitate marketing prior to further processing of the carcass into primal and subprimal cuts and the production of beef trim. Due to the sorting of carcasses into groups that were harvested at different times, combined with the typically low levels of *E. coli* O157 contamination, sample numbers too high to be feasible are required to track contamination beyond the chilled carcass to the finished product.

To gain insight into the cause of HEP contamination events, we employed molecular typing of *E. coli* O157:H7 isolates collected from beef trim produced during HEP. Organisms from multiple trim lots and time points within a HEP, and across multiple HEPs, were typed to gain information regarding the source of contamination, specifically whether HEP contamination is derived from a single point source or from multiple sources. The latter would be expected if the incoming load were exceeding the capacity of in-plant interventions. Genetic typing of HEP strains also would provide information regarding where in the process (slaughter floor vs. fabrication) HEP contamination may be occurring and if particular strains are more commonly associated with events.

The objectives of this work were to (1) describe the diversity of strains within and among individual HEP, (2) determine if HEP occurring in the same processing plant are caused by the same strains, and (3) characterize HEP strains for attributes related to human disease.

MATERIALS AND METHODS

Experiment design. Beef trim enrichment samples (n=639, isolates recovered from 566) representing 21 HEP (referred to as HEP-A through HEP-U, Table 1) were received from nine beef processing plants operated by multiple companies and management systems. The

processing plants were located in Beef Industry Food Safety Council (BIFSCo) regions #1, northwest (WA, OR, ID); #3, southwest (AZ, NM, TX); #5, upper Midwest (NE, ND, SD, MN, WI); and #8, northeast (IL, IN, KT, MS, ME, MD, MI, NJ, NY, NH, CN, RI, OH, WV, VA, VT, PA, DE). The number of HEP sample sets received from individual plants ranged from one to seven. All processing plants participating in this study harvest over 200-head per hour.

All samples had been determined previously to harbor *E. coli* O157:H7 and product represented by each sample was either diverted to a cooking process or destroyed. Upon arrival at the lab, enrichments were cultured to recover *E. coli* O157:H7. Pure strains recovered from each culture were analyzed by a novel, non-PulseNet PFGE method. In addition, strain lineages and *tir* alleles were determined to identify commonalities between strains causing contamination events. For HEP-A, B, and C, two *E. coli* O157:H7 isolates per sample were selected for PFGE analysis, while 4 isolates per sample were analyzed for HEP-Q. It was determined that multiple isolates from the same enrichment yielded the same PFGE pattern. For the remaining HEP, when *E. coli* O157:H7 was recovered from an enrichment, a single isolate was used to represent that sample for characterization.

In order to determine the diversity of *E. coli* O157:H7 on incoming cattle hides for comparison to HEP, PFGE analyses conducted for previous studies (3, 7) were utilized. Incoming load diversity for *E. coli* O157:H7 hide isolates was evaluated from two sampling designs: consecutive animal sampling within a lot and sampling across an 8-hour shift. Hide samples collected to represent an 8-hour shift and were thought to simulate the total incoming load that would contribute to the widespread contamination issues observed in HEP. Incoming hide isolates were obtained from 100 head per day for three days each at three different processing plants.

Alternatively, consecutive sampling of individual cattle within a lot was used to determine the incoming diversity associated with single source animals. When sampling consecutively, the number of cattle sampled per trip ranged from 56 to 149 for six different lots (Table 3). All processing plants from which hide samples were collected operated in excess of 200-head per hour. Hide samples were not associated with HEP. Hide samples were processed as described previously (3). When positive, a single isolate was used to represent each sample for PFGE.

Isolation of *E. coli* O157:H7 from HEP samples. Beef trim samples were collected by processing plant personnel and analyzed in accordance with each plant's routine trim testing program. Aliquots of each enrichment were typically sent to the U.S. Meat Animal Research Center within one week following the determination of a HEP having occurred, however one set of samples was stored at 4°C for 10 months following the HEP. Upon arrival at the lab, the enriched HEP sample aliquots were vortexed vigorously for 30 sec, allowed to set for 1 min, then 10 ul was removed to streak for isolated colonies onto ntCHROMagar (CHROMagar-O157 [DRG International, Mountainside, NJ] supplemented with novobiocin [5 mg/liter; Sigma, St. Louis, MO] and potassium tellurite [2.5 mg/liter; Sigma]). Simultaneously, the samples were processed by immunomagnetic separation, in which 1 ml from each enrichment was subjected to immunomagnetic bead-cell concentration using 20 µl of anti-*E. coli* O157 beads (Invitrogen, Carlsbad, CA). The beads were extracted from enrichment samples and washed two times in phosphate buffered saline-Tween 20 (PBS-Tween, Sigma) using an automated magnetic particle processor (KingFisher 96, Thermo Fisher Scientific, Inc. Waltham, MA). The beads were resuspended in 100 µl of PBS-Tween. Fifty microliters of the final bead-bacteria complexes were spread-plated onto ntCHROMagar. All plates were incubated at 37°C for 18 to 20 h. After

133 the plates were incubated, up to three presumptive positive colonies were picked for
134 confirmation. Multiplex PCR (8) was used to confirm that each *E. coli* isolate harbored genes
135 for the O157 antigen, H7 flagella, gamma intimin, and at least one of the Shiga toxins. All
136 isolates were maintained as frozen stocks in 15% glycerol (Sigma) for later use in PFGE.
137 **Pulsed Field Gel Electrophoresis (PFGE).** In order to obtain *E. coli* O157:H7 isolates
138 from commercial processors, an agreement was reached that HEP isolates would not be analyzed
139 by *Xba*I-PFGE and therefore would not be inappropriately connected to human disease isolates
140 simply by inference from similar PFGE patterns. To satisfy this requirement, a novel PFGE
141 technique was developed. Isolates from HEP (n=743) were analyzed by PFGE using separation
142 of *Spe*I-digested genomic DNA. To validate the resolution of *Spe*I-PFGE, a comparison was
143 performed between *Spe*I-PFGE and *Xba*I-PFGE. The PFGE comparison utilized 77 *E. coli*
144 O157:H7 isolates previously collected from cattle hides (7) that represented the breadth of *Xba*I-
145 PFGE diversity in the USMARC strain collection. The indices of discrimination for the resulting
146 dendrograms were calculated as described by Hunter and Gaston (9).
147 *E. coli* O157:H7 *Xba*I fingerprints were generated for cattle hide isolates to describe the
148 incoming diversity. This analysis utilized the PFGE separation of *Xba*I-digested genomic DNA,
149 as currently used by members of PulseNet (10). Briefly, pulsed-field gel certified agarose
150 (SeaKem Gold Agarose) was obtained from Cambrex Bio Science Rockland Inc. (Rockland,
151 ME) and Tris-borate-EDTA running buffer and Proteinase K were purchased from Sigma. *Xba*I
152 was purchased from New England Biolabs (Beverly, MA). *Salmonella* serotype Braenderup
153 strain H9812 was used as a control and for standardization of gels (11). Banding patterns were
154 analyzed and comparisons made using Bionumerics software (Applied Maths, Sint-Martens-
155 Latem, Belgium), employing the Dice similarity coefficient in conjunction with the unweighted

pair group method using arithmetic averages for clustering. Position tolerance settings used 1.5% optimization and 1.5% band tolerance.

*Spe*I-PFGE analysis was carried out as for *Xba*I with the following modifications. Genomic DNA was digested with *Spe*I (Promega, Madison, WI). The *Spe*I electrophoresis conditions utilized an initial switch time value of 1.79 sec, a final switch time of 18.66 sec at a gradient of 6 V/cm and an included angle of 120°. Run time was 17.5 h in 0.5 TBE (Sigma).

Lineage-specific polymorphism assay (LSPA). The LSPA was carried out as previously reported (12) with the modifications described by Hartzell et al. (13). Reference strains for lineage I (FRIK 523) and lineage II (FRIK 920) were generously provided by Dr. Andrew Benson at the University of Nebraska-Lincoln.

A set of 75 *E. coli* O157:H7 isolates obtained from routine ground beef and beef trim testing was kindly provided by the FSIS. The strain set consisted of a random collection of isolates collected between 2009 and 2012. These isolates were analyzed by LSPA for comparison to HEP isolates.

***tir* SNP genotyping.** *E. coli* O157:H7 HEP isolates were genotyped for either the *tir* 255 T>A allele by real time PCR genotyping as described previously (14). Each reaction consisted of TaqMan Universal PCR Master Mix (2X) (Applied Biosystems), 0.5 ng of genomic DNA, 1X Assay mix (0.9 uM of each primer & 0.2 uM of each fluorescent probe) and molecular grade water to a final volume of 25 ul. Amplification and detection were carried out in optical-grade 96 well plates, sealed with optical film in a Chromo4 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The reactions were cycled at 50°C for 2 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min with optical reading of the fluorophore taken after

178 the extension step. Opticon 3.0 application software (Bio-Rad Laboratories) was used to
179 determine the *tir* allele for each strain.

180 **RESULTS**

181 **Comparison of *Spe*I- and *Xba*I-PFGE.** The 77-strain *E. coli* O157:H7 diversity panel was
182 analyzed by *Spe*I- and *Xba*I-PFGE. Panel isolates produced 51 unique restriction digest patterns (RDP)
183 by *Spe*I and 54 unique RDP by *Xba*I (Fig. 1). The diversity indices were calculated for both resulting
184 dendrograms. The diversity index for the *Spe*I-digested panel was 0.967 and 0.972 for the *Xba*I-digested
185 panel (Fig. 1).

186 **PFGE analysis of individual HEP.** Isolates from twenty-one HEP were analyzed by *Spe*I-
187 PFGE. Typical PFGE results are shown in Figures 2A to 2C. In all cases but one, HEP were found to
188 consist of a predominant strain. That is not to say that for all HEP the same strain was isolated, but
189 within each HEP there was little to no strain diversity. For nine HEP, all isolates analyzed within an
190 HEP were indistinguishable by PFGE (Table 1). An additional six HEP would be considered to have
191 essentially the same strain throughout the HEP using the definition of "closely related" strains put
192 forward by Tenover et al. (15). Overall, with the exception of HEP-N, the predominant
193 indistinguishable strain within each HEP represented ≥ 72% of the samples, while closely related strains
194 represented ≥ 86% of the isolates within an HEP (Table 1).

195 **Diversity of incoming *E. coli* O157:H7.** The PFGE analysis of cattle hide isolates collected in
196 previous sampling projects (3, 7) was utilized to determine the typical diversity of *E. coli* O157:H7
197 associated with incoming cattle. Sponge samples for cattle hides, analyzed by individual trip and
198 overall, showed much more diversity of isolate genotypes on incoming cattle than that observed for
199 HEP.

Hide samples characterizing an 8-hour shift were analyzed and the results are presented in Table 2 and Figure 3. From 100 head per day sampled for three days at each of three processing plants, the number of *E. coli* O157:H7 isolates obtained per day ranged from 22 to 76. The number of unique RDP obtained per day ranged from 6 to 24.

When sampling consecutively across individual lots of cattle, the number of isolates obtained from each trip ranged from 34 to 134 per lot (Table 3, Fig 4). Lot 1 produced the fewest unique RDP with 63 isolates being categorized by six unique RDP. Lot 6 had the most unique RDP (n=29) from 98 isolates.

Indistinguishable isolates across multiple HEP. When analyzing the HEP isolates as a whole, one indistinguishable strain type was found to be the predominant strain in five different HEP (HEP-A, C, G, K, and M). In addition, this strain type was indistinguishable from the minority strain in HEP-H, which was closely related to the predominant strain in that HEP. These HEP were from three different plants, operated by two different companies, but they were located within the same BIFSCo region. HEP-A and C occurred in the same plant and HEP-G, H, and M occurred in another. Both of these plants had additional HEP associated with unrelated strains. Aside from this strain type, there were no other HEP that shared a common strain.

Lineage and *tir* alleles for HEP isolates. Seventeen of the twenty-one (81%) HEP consisted of strain lineages typically associated with human disease, lineages I and I/II (Table 1). Of those 17 HEP, seven HEP had only lineage I strains and ten HEP contained only lineage I/II strains. Only, HEP-E, Q, R, and T yielded strains of lineage II. While HEP-Q, R, and T were populated by lineage II strains in every sample, HEP-E consisted of indistinguishable lineage II strains for 6 of 7 samples and a lineage I strain in the remaining sample (Table 1).

The *tir* allele results for HEP strains were similar to the lineage determinations. All lineage I and I/II strains harbored the human illness-associated *tir*T allele, while the lineage II strains carried the *tir*A allele. Hence, *tir*T-containing strains were found to be the predominant constituents for the vast majority of HEP (81%, Table 1). The predominant strains in HEP-E, Q, R, and T were the only strains found to harbor the *tir*A allele. HEP-E was the only HEP that consisted of strains differing in lineage or *tir* allele. For all other HEP, even when different PFGE patterns were identified within a HEP, all strains within the HEP were of the same lineage and *tir* type.

Lineage determination for non-HEP beef trim and ground beef isolates. Lineages I and I/II had 31 and 30 isolates, respectively, out of the 75 total beef trim and ground beef isolates provided by FSIS. This resulted in a combined prevalence of 81.3% human-biased lineages (data not shown). The remaining 14 (18.7%) isolates were lineage II.

DISCUSSION

The findings of this study indicate that most HEP from large commercial beef processing plants consist of a singular dominant *E. coli* O157:H7 strain type within each HEP (Table 1). In these cases the dominant strains were found across multiple product types (trim from multiple lines originating from different sections of the carcass) and spread over substantial spans of time (occasionally more than one 8-h shift) and product (tens of thousands of pounds or greater). These findings would appear to be in disagreement with the current model of beef contamination, which states that finished product contamination originates on the kill floor and occurs when interventions malfunction, dressing practices are improper, or incoming load (hide carriage of the pathogen inadvertently transferred to the carcass surface) exceeds the capacity of the in-plant interventions to remove carcass contamination (3-5). In this model, one would expect to observe a diversity of *E. coli* O157:H7 isolates in the finished product

similar to that on the hides of incoming cattle. The results obtained herein do not appear to support that hypothesis.

It should be noted that the hide samples presented herein provide a snapshot of the typical *E. coli* O157:H7 diversity entering beef processing plants and were not linked to HEP. The determination that a HEP has taken place occurs at least 48 h after the cattle have been harvested. Therefore, it is not possible to collect hide samples for a HEP, the occurrence of which cannot be determined a priori.

The conversion of live animal to finished product for human consumption is a complicated process and should not be thought of as a linear progression through a system, but rather as a complex network of pathways and branch points based on the assignment of product grades and the sorting of carcasses into like marketing groups to facilitate production and packaging of final products. The tracking of *E. coli* O157:H7 through this network is further complicated due to numerous sources inputting multiple pathogen types throughout the system. A group of cattle exit a production setting such as a feedlot and enter the processing plant as a lot. Typically this lot will have a shared diet and management regiment and previous reports indicate that as a lot, cattle may share a predominant *E. coli* O157:H7 strain (16, 17) in the feedlot environment. Our group and others (3, 18-20) have shown that upon arrival at the beef processing plant, the lairage environment can result in significant pathogen contamination of the cattle hide. This additional contamination adds many new strain types to the hide microflora, which may be subsequently transferred to the dehided carcass (3, 7).

The carcasses are maintained as a lot as they progress through the abattoir kill floor where multiple antimicrobial interventions are applied, followed by entry into the cooler. Following the 24 to 48 h carcass chilling period, carcasses are graded and sorted such that lots are no longer maintained together. Sorting carcasses by grades results in carcasses from multiple sources being intermingled before further processing. During further processing, called fabrication, the carcasses are broken down

into primal and subprimal cuts with individual carcass sections being routed to specific cutting lines to achieve the multitude of final products from each carcass.

At essentially every step in the fabrication process small portions of meat are trimmed away from the main product. These trim pieces, consisting of lean and fat, are collected in 2,000 lb lots referred to as beef trim combos and are ultimately used in the production of ground beef. With a typical feedlot-produced steer or heifer, one would estimate that ≈ 140 lbs of beef trim would be produced per carcass, which would be distributed among several combos depending on a variety of factors (original primal and subprimal source, desired fat:lean ratios, etc.). The filled combo is the endpoint in this process and the point where most beef processors conduct pathogen testing prior to release of the trim material for ground beef production.

A detailed understanding of the breakdown of carcasses into final products is necessary to give context to the results of the study described herein. It is easy to see through this description why the hypothesis of this study was that HEP would contain a diverse array of strain types originating from the hides of incoming cattle. As seen in Figure 3 and Table 2, many different strain types can be found on incoming cattle over a time frame consistent with many HEP. Most plants of the capacity sampled herein will process in excess of 1,500 cattle in separate lots originating from multiple sources over an 8-h shift. Aside from the *E. coli* O157:H7 diversity presented by multiple incoming lots, there also is a continuous deposition of *E. coli* O157:H7-laden feces in the lairage environment (3) that will contribute to the within lot diversity of hide contamination as seen in Figure 4 and Table 3. In light of the incoming diversity and the intermingling of carcasses as well as carcass products it was surprising to observe such a high degree of homogeneity in *E. coli* O157:H7 strain types when HEP occurred.

The most striking example comes from HEP-U. This HEP had the largest number of positive samples for any HEP studied herein and all *E. coli* O157:H7 isolates were of the same PFGE type. The

157 positive samples all came from 2000-lb combos totaling 314,000 lbs of beef trim. Given the typical carcass yield of trim is ≈ 140 lb, the minimum number of carcasses represented by this HEP would be estimated to be 2,243. The actual number of carcasses contributing to this HEP was likely much higher because the trimmings from individual carcasses are not contained as discrete units within a combo, but are dispersed into multiple combos. It is difficult to imagine a mechanism of contamination for such an event. The scenario would require a source containing a single *E. coli* O157:H7 genotype and be of sufficient concentration and volume to be spread over such a large amount of product.

While there has been research showing various *E. coli* O157:H7 strains will emerge as predominant over time within a group of cattle in a production setting, the exclusivity is not nearly to the degree seen for HEP. LeJeune et al. (16) used PFGE to show that 230 isolates obtained from eight feedlot pens consisted of 56 unique genotypes. Isolates belonging to a group of four closely related genetic subtypes made up 60% of all isolates collected over the sampling period. Carlson et al. (17) collected 132 *E. coli* O157:H7 isolates representing 32 different PFGE subtypes from 788 feedlot cattle in five pens. A single, predominant PFGE subtype accounted for 53% of the 132 isolates. In addition, Rice et al. (21) found up to 11 PFGE subtypes per farm with up to 7 subtypes/farm identified from a single date.

Upon exiting the production environment, cattle are exposed to additional *E. coli* O157:H7 contamination during transportation to the processing plant (18, 19, 22). Arthur et al (18) found that up to 10% of the *E. coli* O157:H7 isolates obtained from carcasses within a lot during processing matched genotypes found in the trucks they were transported on, which were different from the genotypes found in the feedlot the cattle originated from.

As cattle are placed in lairage at the processing plant, further contamination of the hide by *E. coli* O157:H7 occurs, which results in further increased strain diversity in the incoming load (3, 19, 20).

313 This diversity can be observed in the hide sampling results presented in Table 3. As many as 23 unique
314 *E. coli* O157:H7 genotypes could be identified within as few as 56 head from the same lot sampled
315 consecutively. Hide contamination has been shown to be the source of carcass contamination and as
316 such the diversity observed on hides is subsequently transferred to the carcass. Arthur et al. (18)
317 reported that 80% (67 of 80 representing 10 genotypes) of the isolates recovered from carcasses sampled
318 prior to evisceration did not come from the feedlot of origin for those cattle, but were attributed to hide
319 contamination acquired in the lairage environment. Similarly, Dodd et al. (23) also reported high levels
320 of diversity (17 subtypes from 39 positive carcasses out of 1503 total carcass samples) among *E. coli*
321 O157:H7 isolates from pre-evisceration carcasses.

322 While the homogeneity in genotypes within HEP appears to differ with respect to the diversity of
323 the incoming load and what is found on the carcass during processing, there does seem to be agreement
324 with genotypic profiles obtained from beef recalls and disease outbreaks. Investigations into beef-
325 related outbreaks of disease due to *E. coli* O157:H7 have found a similar high degree of strain
326 homogeneity. Most of the isolates (16 of 18) from a 1997 outbreak and associated recall were
327 determined to have indistinguishable PFGE patterns, while the remaining two isolates differed from the
328 predominant pattern by one band (24). In a 2002 outbreak/recall, 354,200 lbs of ground beef were
329 implicated and illnesses spanned seven states. The genotypes of all isolates (19 of 19) collected from
330 human illness cases (n=18) and one ground beef sample were determined to be indistinguishable by
331 PFGE analysis (25).

332 At this point in time it is difficult to resolve the dichotomy that *E. coli* O157:H7 contamination
333 on cattle hides and carcasses consists of a high degree of diversity, while HEP show little to no strain
334 diversity. One argument would state that there is no dichotomy and that the current model of incoming
335 load overwhelming that antimicrobial interventions remains applicable through one of three possible

scenarios. The first of these scenarios would focus on animals shedding *E. coli* O157:H7 at extremely high levels, supershedders. It is plausible that a lot containing multiple supershedders not only would contaminate themselves and their cohorts, but also deposit large amounts of a particular strain type in the lairage environment to contaminate subsequent cattle lots. It can be speculated that this would provide a large concentration and volume of strain-specific contamination that would need to be reduced through proper dressing and functional interventions. However, this scenario seems unlikely because supershedders make up approximately 2% of the cattle population (26) and multiple supershedders are likely entering processing plants on a daily basis during high shedding season. As shown in Tables 3 and 4, there is little evidence of incoming cattle hides being predominantly contaminated with one strain type. Even acknowledging the lack of data in this regard, it is unreasonable to conclude that HEP only occur when a singular genotype dominates the incoming load.

The second scenario also pertains to supershedder-derived contamination. The basis for this scenario would be the gross contamination of a small group of carcasses with very high concentrations of *E. coli* O157:H7. Cross-contamination of workers and contact surfaces would occur to transmit the contamination to multiple lots of finished product. This scenario relies on poor dressing practices and the inability of antimicrobial interventions to reduce the contamination load. There are two main concerns with this model. It is difficult to imagine contamination of a few carcasses providing enough material to be spread across large HEP such as HEP-U. Secondly, it seems just as likely to achieve gross contamination of carcasses with a mixed strain population leading to HEP with multiple genotypes. If scenarios 1 or 2 were occurring, it seems likely that one would observe HEP with one dominant strain and HEP with multiple strains, which was not the case in this study.

In the third scenario, the diversity seen in carcass contamination is reduced by multi-hurdle intervention schemes employed by the processing plants, but through this reduction a selection of robust

strains is facilitated. This would seem unlikely for a variety of reasons. First, while there was one strain found in multiple HEP, most of the HEP were caused by unique strains indicating there are multiple strain types that can survive this selection, which should be manifest in more diverse HEP. Secondly, there are limited data from previous studies comparing the effects of antimicrobial interventions on multiple *E. coli* O157:H7 genotypes and no significant differences in their survival were observed (27, 28). However, many more strain types need to be evaluated to validate this point.

An opposing argument would suggest that HEP contamination is occurring post-kill floor. While it is unknown at this time what the mechanism for such contamination would be, this would explain why beef trim from carcasses harvested several hours apart would share a common contaminant genotype. Currently, there is little to no additional data to support or refute this model, but it is difficult to imagine a source of wide spread contamination post-kill floor. It does not appear to be plant-specific endemic contamination as several plants had multiple HEP caused by differing strains of *E. coli* O157:H7.

Another significant finding of this work is the bias towards human illness-related *E. coli* O157:H7 strains among those isolated from HEP. Seventeen of the 21 (81%) HEP consisted exclusively of strains associated with human illness (*tir* allele T). This was significant as previously the *tir* alleles were found in cattle populations at rates of 55% T and 44% A, but were heavily biased toward the T allele (99% T vs 1% A) among *E. coli* O157:H7 strains isolated from human illness cases (14). To further investigate the potential bias towards *tir*T in HEP strains, a set of *E. coli* O157:H7 isolates were obtained from the raw beef-sampling program conducted by FSIS for *tir* analysis. The FSIS isolates had a similar high rate (81.3%) of human illness-associated strain types indicating that the *tir*T allele may not be associated specifically with HEP, but rather with beef trim in general. It should be noted that *tir*T was recently found to have a prevalence among *E. coli* O157:H7 isolated from supershedding cattle of

71% (26). More data will be needed to determine if human illness-associated strains are associated with beef trim and if supershedding plays a role in such an association.

In conclusion, much more work needs to be done to determine the mechanism responsible for HEP. The difficulty in such work is that there is no way to know when HEP are going to occur and HEP are not detected until approximately 24 to 48h after the contamination has taken place. It may be and is quite likely that both models are correct and contamination events can occur from both kill floor and post-kill floor contamination. The data reported herein suggest that whatever the mechanism, HEP occurring at large beef processing plants typically show little to no diversity of *E. coli* O157:H7 genotype and the majority consist of human-illness related strains.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. USDA is an equal opportunity provider and employer.

REFERENCES

1. **FSIS** 2011. National Prevalence Estimate of Pathogens in Domestic Beef Manufacturing Trimmings (Trim). www.fsis.usda.gov/wps/wcm/connect/f07f5e1d-63f2-4ec8-a83a-e1661307b2c3/Baseline_Data_Domestic_Beef_Trimmings_Rev.pdf?MOD=AJPERES
2. **FSIS** 2012. Compliance Guideline for Establishments Sampling Beef Trimmings for Shiga Toxin-Producing *Escherichia coli* (STEC) Organisms or Virulence Markers. www.fsis.usda.gov/wps/wcm/connect/e0f06d97-9026-4e1e-a0c2-1ac60b836fa6/Compliance_Guide_Est_Sampling_STEC_0512.pdf?MOD=AJPERES
3. **Arthur TM, Bosilevac JM, Brichta-Harhay DM, Kalchayanand N, King DA, Shackelford SD, Wheeler TL, Koohmaraie M.** 2008. Source tracking of *Escherichia coli* O157:H7 and *Salmonella* contamination in the lairage environment at commercial

U.S. beef processing plants and identification of an effective intervention. J Food Prot **71:**1752-1760.

4. **Barkocy-Gallagher GA, Arthur TM, Siragusa GR, Keen JE, Elder RO, Laegreid WW, Koohmaraie M.** 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.
5. **Nou X, Rivera-Betancourt M, Bosilevac JM, Wheeler TL, Shackelford SD, Gwartney BL, Reagan JO, Koohmaraie M.** 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.
6. **Vosough Ahmadi B, Velthuis AG, Hogeveen H, Huirne RB.** 2006. Simulating *Escherichia coli* O157:H7 transmission to assess effectiveness of interventions in Dutch dairy-beef slaughterhouses. Prev Vet Med **77:**15-30.
7. **Arthur TM, Bosilevac JM, Nou X, Shackelford SD, Wheeler TL, Koohmaraie M.** 2007. Comparison of the molecular genotypes of *Escherichia coli* O157:H7 from the hides of beef cattle in different regions of North America. J Food Prot **70:**1622-1626.
8. **Hu Y, Zhang Q, Meitzler JC.** 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. J Appl Microbiol **87:**867-876.
9. **Hunter PR, Gaston MA.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol **26:**2465-2466.
10. **Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ.** 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and Shigella for PulseNet. Foodborne Pathog Dis **3:**59-67.
11. **Hunter SB, Vauterin P, Lambert-Fair MA, Van Duyne MS, Kubota K, Graves L, Wrigley D, Barrett T, Ribot E.** 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.
12. **Yang Z, Kovar J, Kim J, Nietfeldt J, Smith DR, Moxley RA, Olson ME, Fey PD, Benson AK.** 2004. Identification of common subpopulations of non-sorbitol-fermenting, beta-glucuronidase-negative *Escherichia coli* O157:H7 from bovine production environments and human clinical samples. Appl Environ Microbiol **70:**6846-6854.
13. **Hartzell A, Chen C, Lewis C, Liu K, Reynolds S, Dudley EG.** 2011. *Escherichia coli* O157:H7 of genotype lineage-specific polymorphism assay 211111 and clade 8 are common clinical isolates within Pennsylvania. Foodborne Pathog Dis **8:**763-768.
14. **Bono JL, Keen JE, Clawson ML, Durso LM, Heaton MP, Laegreid WW.** 2007. Association of *Escherichia coli* O157:H7 tir polymorphisms with human infection. BMC Infect Dis **7:**98.
15. **Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **33:**2233-2239.
16. **LeJeune JT, Besser TE, Rice DH, Berg JL, Stilborn RP, Hancock DD.** 2004. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle:

predominance and persistence of specific clonal types despite massive cattle population turnover. Appl Environ Microbiol **70:**377-384.

17. **Carlson BA, Nightingale KK, Mason GL, Ruby JR, Choat WT, Loneragan GH, Smith GC, Sofos JN, Belk KE.** 2009. *Escherichia coli* O157:H7 strains that persist in feedlot cattle are genetically related and demonstrate an enhanced ability to adhere to intestinal epithelial cells. Appl Environ Microbiol **75:**5927-5937.

18. **Arthur TM, Bosilevac JM, Brichta-Harhay DM, Guerini MN, Kalchayanand N, Shackelford SD, Wheeler TL, Koohmaraie M.** 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.

19. **Childs KD, Simpson CA, Warren-Serna W, Bellenger G, Centrella B, Bowling RA, Ruby J, Stefanek J, Vote DJ, Choat T, Scanga JA, Sofos JN, Smith GC, Belk KE.** 2006. Molecular characterization of *Escherichia coli* O157:H7 hide contamination routes: feedlot to harvest. J Food Prot **69:**1240-1247.

20. **Dewell GA, Simpson CA, Dewell RD, Hyatt DR, Belk KE, Scanga JA, Morley PS, Grandin T, Smith GC, Dargatz DA, Wagner BA, Salman MD.** 2008. Impact of transportation and lairage on hide contamination with *Escherichia coli* O157 in finished beef cattle. J Food Prot **71:**1114-1118.

21. **Rice DH, McMenamin KM, Pritchett LC, Hancock DD, Besser TE.** 1999. Genetic subtyping of *Escherichia coli* O157 isolates from 41 Pacific Northwest USA cattle farms. Epidemiol Infect **122:**479-484.

22. **Barham AR, Barham BL, Johnson AK, Allen DM, Blanton JR, Jr., Miller MF.** 2002. Effects of the 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.

23. **Dodd CC, Renter DG, Fox JT, Shi X, Sanderson MW, Nagaraja TG.** 2010. Genetic relatedness of *Escherichia coli* O157 isolates from cattle feces and preintervention beef carcasses. Foodborne Pathog Dis **7:**357-365.

24. Centers for Disease Control and Prevention. 1997. *Escherichia coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers--Colorado. JAMA **278:**891.

25. Centers for Disease Control and Prevention. 2002. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef--United States, June-July. JAMA **288:**690-691.

26. **Arthur TM, Ahmed R, Chase-Topping M, Kalchayanand N, Schmidt JW, Bono JL.** 2013. Characterization of *Escherichia coli* O157:H7 strains isolated from supershedding cattle. Appl Environ Microbiol **79:**4294-4303.

27. **Arthur TM, Kalchayanand N, Bosilevac JM, Brichta-Harhay DM, Shackelford SD, Bono JL, Wheeler TL, Koohmaraie M.** 2008. Comparison of effects of antimicrobial interventions on multidrug-resistant *Salmonella*, susceptible *Salmonella*, and *Escherichia coli* O157:H7. J Food Prot **71:**2177-2181.

28. **Berry ED, Barkocy-Gallagher GA, Siragusa GR.** 2004. Stationary-phase acid resistance and injury of recent bovine *Escherichia coli* O157 and non-O157 biotype I *Escherichia coli* isolatest. J Food Prot **67:**583-590.

501 **FIGURE LEGENDS**

502 Figure 1. Comparison of the discriminatory power of *Spe*I-digest vs *Xba*I-digest PFGE analysis.
503 Dendrograms for each enzyme digest are shown. The number of indistinguishable groups is
504 provided below along with the calculated discriminatory power (D) for each method.
505
506 Figure 2. Typical HEP PFGE profiles. Cluster analysis and dendrogram for (A) HEP I, (B) HEP
507 J, and (C) HEP O. Each cluster analysis and dendrogram is the result of *Spe*I-digested PFGE
508 analysis.
509
510 Figure 3. Diversity of incoming load on cattle throughout production shift. Dendrograms,
511 produced by *Xba*I restriction digests, represent the genotypic diversity of *E. coli* O157:H7 strains
512 during an 8-hour production shift each day for three days. Three separate processing plants are
513 represented: (A) Plant 1, (B) Plant 2, and (C) Plant 3. Each dendrogram combines isolates
514 collected on three separate days: Day1 – green, Day 2 – Red, Day 3 – blue.
515
516 Figure 4. Diversity of incoming *E. coli* O157:H7 on cattle hides by individual lots. Each image
517 depicts the *Xba*I restriction digest patterns for *E. coli* O157:H7 isolates in sequential order for
518 each animal in a lot. The number of unique genotypes for each lot can be found in Table 3: (A)
519 Lot 2, (B) Lot 4, and (C) Lot 6.

520 Table 1. Distribution of PFGE type, lineage, and *tir* alleles of strains isolated from HEP[a]

HEP	No. of positive enrichments received	No. of enrichments from which an isolate was obtained	No. of isolates identical to predominant RDP	No. of isolates closely related to predominant RDP	LSPA lineage	*tir* allele
A	8	8	8 (100)[b]	8 (100)	I/II	T
B	16	9	9 (100)	9 (100)	I	T
C	11	10	9 (90)	9 (90)	I/II	T
D	9	9	9 (100)	9 (100)	I/II	T
E	7	7	6 (86)	6 (86)	I & II	T & A
F	12	8	7 (88)	8 (100)	I	T
G	7	6	6 (100)	6 (100)	I/II	T
H	21	18	13 (72)	18(100)	I/II	T
I	20	20	15 (75)	20 (100)	I	T
J	20	17	16 (94)	16 (94)	I	T
K[c]	32	10	10 (100)	10 (100)	I/II	T
L	9	9	9 (100)	9 (100)	I	T
M	13	12	11 (92)	11 (92)	I/II	T
N	18	18	9 (50)	16 (89)	I/II	T
O	44	44	43 (98)	44 (100)	I	T
P	65	61	61 (100)	61 (100)	I	T
Q	50	50	50 (100)	50 (100)	II	A
R	50	35	33 (94)	35 (100)	II	A
S	44	43	42 (98)	42 (98)	I/II	T
T	17	15	15(100)	15 (100)	II	A
U	166	157	157 (100)	157 (100)	I/II	T

521 [a]abbreviations: PFGE – pulsed field gel electrophoresis, HEP – high event period, RDP –
522 restriction digest pattern.
523 [b]Number of isolates (percentage of total)
524 [c]Low recovery of isolates attributed to enrichments received after 10 mos. of storage at 4°C.

525 Table 2. *E. coli* O157:H7 PFGE types from 100 cattle hide samples collected each day for three
526 days.
527

Processing plant	Day	No. of isolates	No. of unique RDP
1	1	36	18
	2	76	24
	3	26	12
2	1	29	6
	2	30	12
	3	48	9
3	1	38	10
	2	22	7
	3	26	7

528 [a]abbreviations: PFGE – pulsed field gel electrophoresis, RDP – restriction digest pattern.
529

530 Table 3. *E. coli* O157:H7 PFGE types from consecutive cattle hide samples[a]
531

Lot	No. of head sampled	No. of isolates	No. of unique RDP
1	81	63	6
2	149	134	15
3	56	56	23
4	87	81	19
5	88	34	11
6	127	98	29

532 [a]abbreviations: PFGE – pulsed field gel electrophoresis, RDP – restriction digest pattern.
533