

Escherichia coli O157:H7 Contamination of Raw Beef Products: High Event Periods Present a Challenge to the Current Model

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INTRODUCTION

Escherichia coli O157:H7 is a foodborne pathogenic bacterium that resides in the intestinal tract of cattle and has been associated with a variety of foodstuffs including raw beef products (Arthur et al. 2013, 2010, 2009; Ebel et al. 2004; Macdonald et al. 2000; Vogt and Dippold 2005; Wachtel et al. 2003). While most *E. coli* are harmless to humans, a handful of *E. coli* types can cause disease. *E. coli* O157:H7 is possibly the most virulent of all pathogenic *E. coli* capable of causing severe disease and even death. The pathogenesis of this organism is conveyed for the most part by excreted Shiga toxins. During an infection, the bacterium attaches to the epithelium of the human intestinal tract and secretes Shiga toxins, which are transported to the bloodstream (LeBlanc 2003; Mohawk et al. 2010). Circulation takes these toxins to multiple susceptible tissues causing damage to the kidneys, lungs and central nervous system.

It was through a major disease outbreak that *E. coli* O157:H7 came to the forefront of food safety concerns in the beef industry. The outbreak began in November of 1992 and included over 500 laboratory confirmed illnesses and four associated deaths (CDC 1993). The source of the outbreak was determined to be undercooked hamburgers from a commercial vendor. Shortly after this outbreak, the Food Safety Inspection Service (FSIS) declared raw non-intact beef products containing *E. coli* O157:H7 to be adulterated, requiring any such products to be excluded from commerce (FSIS 1999). This marked the first instance where bacterial contamination of a fresh meat product was considered adulteration in the U.S.

The declaration of *E. coli* O157:H7 as an adulterant has led to multiple recalls of non-intact beef products based on

the results of diagnostic testing conducted by the FSIS (CDC 2005, 2002b; Kramer et al. 2005; Robbins et al. 2014). FSIS testing is done on a relatively limited scale in terms of overall sample number when compared to the number of raw beef trim lots produced across the beef industry. The FSIS results are used to monitor the raw beef supply in its entirety, but do not have sufficient resolution to monitor the process control of any one facility. However, based on the results of FSIS testing or traceback from disease outbreaks, raw beef products have been recalled from the retail market (CDC 2005, 2002b; Kramer et al. 2005; Robbins et al. 2014). The beef processing industry conducts a much more thorough testing strategy. In the present state of the beef industry, most beef processing companies test all of their raw beef trim before release into the food supply, a procedure referred to as "test-and-hold" (Arthur et al. 2005; Guerini et al. 2006). The procedure entails sampling each 2,000-lb bin of raw beef trim, referred to as combos, and retaining control of those combos until the test results come back negative for *E. coli* O157:H7. This strategy has been implemented to maximize product safety, reduce the amount of product recalled and provide the industry with another means to monitor process control.

By testing all lots of raw beef trim at the time of production, the beef industry has been able to collect a great deal of data pertaining to finished product contamination. These data have shown that contamination of beef trim can manifest itself in two different forms. The first form could be characterized as chronic and occurs at low levels, but at a fairly regular basis. This contamination is typically confined to a few combos and does not impact a significant amount of product in a production shift, but may appear weekly or even daily during the peak *E. coli* O157:H7 season of summer. The other form would be characterized as acute and occurs on a much less frequent basis. Contamination of this form involves several lots of product within a shift or across multiple shifts and can even impact the entirety of product produced in a production day. These acute contamination occurrences have been termed high event periods or HEP. FSIS has defined HEP as production intervals during which slaughter

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establishments experience a high rate of positive results for *E. coli* O157:H7 (or STEC or virulence markers) in trim samples (FSIS 2012). HEP are rare phenomena that have not been well characterized and are problematic to study. The difficulty in trying to determine the source of contamination and the causal mechanism for HEP is that they occur without warning, resolve without notable corrective action and cannot be identified until approximately 24 to 48 h after they have occurred. The only way to determine if an HEP has occurred is through finished-product testing such as the test-and-hold programs employed by most beef processors. Test-and-hold results are obtained within 10 to 12 hours of sample collection, however, harvest of the original carcass occurred 1 to 2 days prior, meaning the plant has gone through 1 to 2 sanitation shifts. This results in little to no evidence for source identification or traceback to determine the cause of the HEP.

ANALYSIS OF HEP

In order to start gathering information on HEP, Arthur et al. utilized molecular fingerprinting to investigate the *E. coli* O157:H7 strains associated with HEP (Arthur et al. 2014). In that study, beef trim enrichments representing HEP were obtained from commercial processing plants. Each enrichment was processed to isolate *E. coli* O157:H7 strains. Once isolated, the *E. coli* O157:H7 strains were characterized by a novel, non-PulseNet protocol (PFGE). A novel protocol was utilized to prevent improper association with any human clinical isolates on the basis of pattern matching without employing the required epidemiology. The study analyzed beef trim enrichment samples (n=639, isolates recovered from 566) representing 21 HEP that originated from nine beef processing plants operated by multiple companies and management systems. The number of HEP sample sets received from individual plants ranged from one to seven with all processing plants participating in the study having harvest rates of over 200-head per hour.

The results of the study were surprising in the fact that within an HEP, there was very low diversity of *E. coli* O157:H7 strain types. In many cases there was no diversity as only one strain type was isolated within each HEP. The most extreme example came from a HEP where 166 beef trim combos had tested positive for *E. coli* O157:H7. Arthur et al. (2014) were able to isolate *E. coli* O157:H7 bacteria from 157 of those samples. When analyzed by genetic fingerprinting, there was no difference observed among all 157 strains indicating they were from the same contamination source. Because the 157 positive samples came from individual 2000-lb combos, this HEP consisted of at least 314,000 lbs of beef trim. Given the typical carcass yield of trim is \approx 140 lb per carcass, 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.

CHALLENGE TO CURRENT MODEL FOR FINISHED PRODUCT CONTAMINATION

While this was a completely novel finding and represented the first data set collected for HEP, it also presented a challenge to a long-standing model for beef trim contamination. The existing model was based on a great deal of data that followed bacterial contamination from feedlot to the final processed carcass. Based on this model, finished product contamination was a function of contamination on incoming animals exceeding the threshold capacity of in-plant antimicrobial interventions. Therefore, the diversity of strains contaminating the finished product should mimic the diversity of strains associated with cattle hides as they enter the plant, which was not the case for the isolates from HEP. To fully comprehend this dichotomy, one needs to have a thorough knowledge of *E. coli* O157:H7 populations at different stages of beef processing as well as the genetic diversity of those populations.

E. coli O157:H7 routes of contamination: In the production environment such as a feedlot, *E. coli* O157:H7 transmission among cattle is impacted greatly by certain animals shedding the pathogen at very high levels, supershedders (Arthur et al. 2010, 2009). This transmission causes cohort animals to become colonized with *E. coli* O157:H7, both in their intestinal tracts and on their hides. As the cattle leave the feedlot and arrive at the processing plant, the cattle hides are exposed to additional *E. coli* O157:H7 contamination as they pass through the lairage environment (Arthur et al. 2007a, 2008; Barham et al. 2002; Dewell et al. 2008). The hides are the main focal point for contamination studies, as the contamination model was built on several data sets that established that the main source of carcass contamination during processing was the cattle hide (Arthur et al. 2007a, 2007b; Barkocy-Gallagher et al. 2003, 2001; Bosilevac et al. 2004; Nou et al. 2003). Nou et al. (2003) used chemical dehairing to show that if the hide of the carcass was sanitized before removal the occurrence of *E. coli* O157:H7 contamination on the carcass was reduced from 50% to 1%. Also, Gallagher et al. (2001) tracked strains using molecular fingerprinting by pulsed field gel electrophoresis (PFGE) to show that the majority of *E. coli* O157:H7 isolates obtained from finished carcasses were indistinguishable from those found on cattle hides and on the carcasses immediately after the hide was removed, indicating that carcass contamination occurs early in the harvest process. This work shows that as the hide is removed, bacterial contamination that is transferred to the carcass and must be removed, reduced or killed by the antimicrobial interventions utilized by the processing plant to prevent contamination of the finished carcass (Koochmaraie et al. 2007, 2005). Hence, the existing model for raw beef trim contamination by *E. coli* O157:H7 posited that contamination of beef trim would occur when

contamination levels on incoming cattle hides was such that contamination of the carcass overwhelmed the threshold capacity of the antimicrobial interventions to eliminate all of the bacterial contamination on the carcass leading to a contaminated final product.

E. coli O157:H7 strain diversity through harvest: To date, most studies of *E. coli* O157:H7 contamination at different points in the farm-to-finished product continuum have shown a great deal of *E. coli* O157:H7 strain diversity at each step. While there has been research showing various *E. coli* O157:H7 strains emerging 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. (2004) 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. (2009) 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. (1999) 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 (Arthur et al. 2007a; Barham et al. 2002; Childs et al. 2006). Arthur et al (2007a) 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 the animals were transported on, but were different from the genotypes found in the feedlot the cattle originated from. It must be noted that large scale processing plants will process around 4,000-head of cattle per day. Hence, such plants will receive between 100 to 150 truckloads of cattle each day, representing upwards of twenty production lots and significant *E. coli* O157:H7 diversity.

As cattle are placed in lairage at the processing plant, further contamination of the hide by *E. coli* O157:H7 occurs. The source of this additional contamination is the lairage environment. The lairage environment consists of the pens and alleyways connecting the unloading dock to the plant entrance. Fecal matter is deposited in these spaces as cattle are processed throughout the day. As new cattle arrive and pass through these spaces, they are quickly exposed to further contamination of the hide, which results in increased strain diversity in the incoming load (Arthur et al. 2008; Childs et al. 2006; Dewell et al. 2008). Hide contamination has been shown to be the source of carcass contamination and as such the diversity observed on hides is subsequently transferred to the carcass (Figure 1). Arthur et al. (2007a) reported that 80% (67 of 80 representing 10 genotypes) of the isolates recovered from carcasses sampled prior to evisceration did not come from the feedlot of origin for those cattle, but were attributed to hide contamination acquired in the lairage environment.

Similarly, Dodd et al. (2010) also reported high levels of diversity (17 subtypes from 39 positive carcasses out of 1503 total carcass samples) among *E. coli* O157:H7 isolates from pre-evisceration carcasses. At every step from feedlot to processed carcass, researchers have observed heterogeneous *E. coli* O157:H7 populations.

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

DATA GAP IN CURRENT MODEL

From this discussion it becomes apparent, that the current model for beef trim contamination may not be suitable for HEP. One reason for this may be that the current model for contamination of raw beef trim, while being built upon a good deal of supporting data, did not have any data sets connecting carcass contamination directly to contamination of raw beef trim. This gap exists due to the logistics of the harvest process. While the early steps of processing, from incoming animal to chilled carcass, are linear and easily studied by sampling, once the carcasses are chilled, they are sorted into different product groups based on carcass quality traits. This means that carcasses from cattle that were harvested as a group will be comingled with carcasses from other harvest groups prior to further processing into wholesale meat cuts. This comingling results in combos containing beef trim from multiple lots. Due to the low frequency with which beef trim is contaminated (<1%), the carcass sorting process impedes the traceback from beef trim to carcass by necessitating an implausibly high number of samples to find enough *E. coli* O157 isolates for useful tracking.

At this point in time there are many more questions than answers regarding finished product contamination and especially HEP. It is quite likely that there are multiple mechanisms for beef trim contamination. If the chronic and acute forms of contamination are viewed as separate phenomena, the current model would serve as a logical explanation for the chronic form of contamination. The scenario where either the antimicrobial interventions are overwhelmed or plant employees fail to follow best practices resulting in intermittent bouts of low level carcass and subsequent trim contamination occur would be quite plausible.

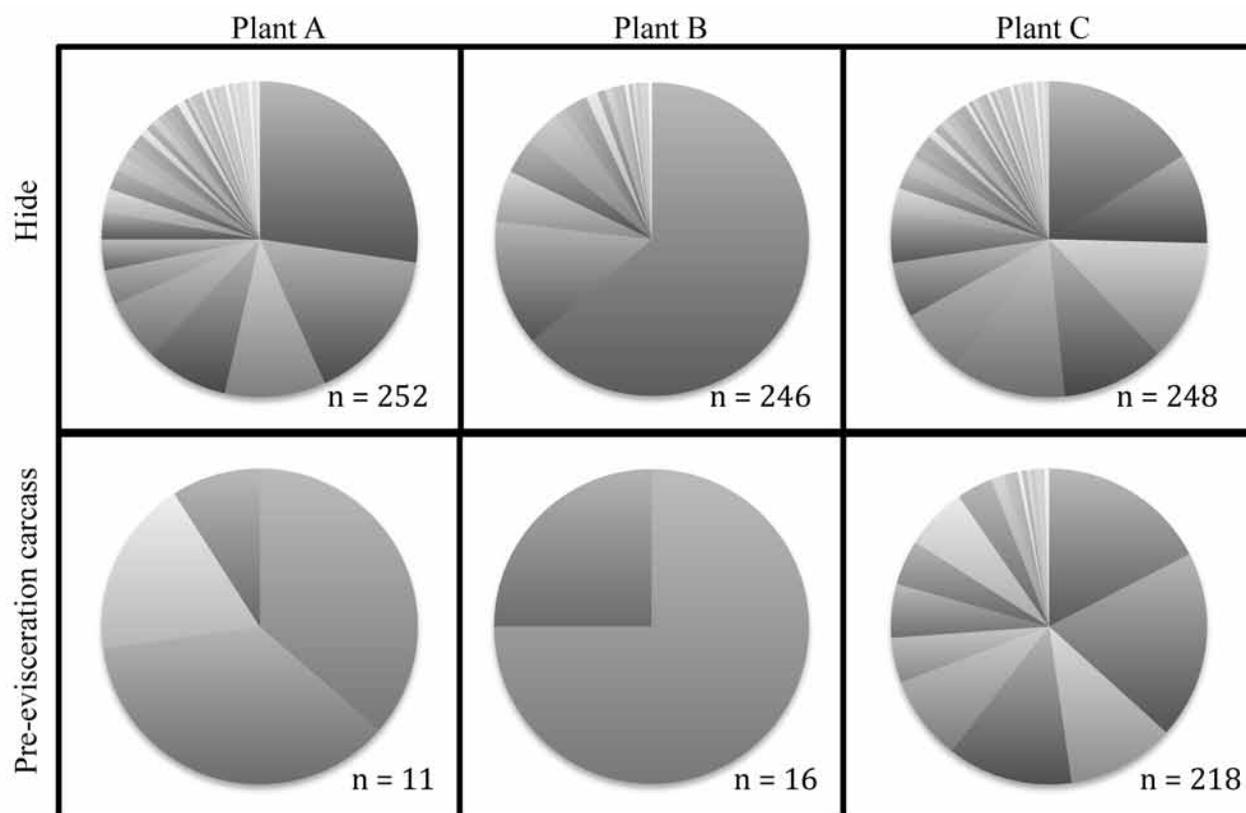


Figure 1. Pie charts showing the diversity of *E. coli* O157:H7 strains on cattle hides entering beef processing plants and the associated pre-visceration carcasses. Each chart represents PFGE types for isolates from hide or carcass samples collected from one lot of cattle at three different processing plants (Arthur et al. 2008).

Since the diversity of *E. coli* O157:H7 in HEP does not match the diversity observed on incoming cattle, it is logical to conclude that HEP contamination is not resulting as a direct result of hide-to-carcass transfer of bacterial contamination. Hence, a new model must be developed for HEP. Building this model will have to account for the practice of sorting carcasses during the chilling process prior to downstream processing of carcasses into products. It can be hypothesized that because beef trim contamination during HEP is homogeneous across sorted lots, the contamination may be occurring after the chilled carcass sorting process rather than during hide removal. This would present a completely new challenge to the beef industry, as the potential sources for such contamination as well as possible interventions are unknown at this time.

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