2010

# Super Shedding of *Escherichia coli* O157:H7 by Cattle and the Impact on Beef Carcass Contamination

Terrance M. Arthur
*USDA-ARS*, terrance.arthur@ars.usda.gov

Dayna M. Brichta-Harhay
*USDA-ARS*, dayna.harhay@ars.usda.gov

Joseph M. Bosilevac
*USDA-ARS*, mick.bosilevac@ars.usda.gov

Norasak Kalchayanand
*USDA-ARS*

Steven D. Shackelford
*USDA-ARS*

***See next page for additional authors***

Meat Science 86 (2010) 32–37

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Review

# Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination

Terrance M. Arthur *, Dayna M. Brichta-Harhay, Joseph M. Bosilevac, Norasak Kalchayanand, Steven D. Shackelford, Tommy L. Wheeler, Mohammad Koohmaraie [1]

*U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA*

ARTICLE INFO

*Article history:*
Received 29 January 2010
Received in revised form 2 April 2010
Accepted 7 April 2010

*Keywords:*
*E. coli*
O157
Beef
Cattle
Super shedding
Carcass

ABSTRACT

Beef carcass contamination is a direct result of pathogen transfer from cattle hides harboring organisms such as enterohemorrhagic *Escherichia coli*. Hide contamination occurs from direct and indirect fecal contamination in cattle production and lairage environments. In each of these environments, individual animals shedding *E. coli* O157:H7 at high levels ($>10^4$ CFU/g of feces, hereafter referred to as "super shedders") can have a disproportionate effect on cattle hide and subsequent carcass contamination. It is not known what criteria must be met to cause an animal to shed at levels exceeding $10^4$ CFU/g. Understanding the factors that play a role in super shedding will aid in minimizing or eliminating the super shedding population. Interventions that would prevent supershedding in the cattle population should reduce *E. coli* O157:H7 transmission in the production and lairage environments resulting in reduced risk of beef carcass contamination and a safer finished product.

Published by Elsevier Ltd. on behalf of The American Meat Science Association.

**Contents**



## 1. Introduction

Beef carcass contamination with *Escherichia coli* O157:H7 has been the subject of research and intervention development for over twenty years. During that time great strides have been made in minimizing the transfer of pathogenic organisms, found on or in animals as they enter the processing plant, to the carcass and subsequently the finished product. In spite of these advances, *E. coli* O157:H7 contamination of red meat still occurs resulting in human illness and product loss. Recently, the ecology of *E. coli* O157:H7 colonization in cattle has been the focus of intense research (Chase-Topping et al., 2008; Fox, Shi, & Nagaraja, 2008; Naylor et al., 2003) with the goal of reducing the pathogen's prevalence and levels associated with cattle as they enter the processing plant.

A large amount of data has implicated the hide as the major source for pathogen contamination of beef carcasses at harvest (Arthur, Bosilevac, Brichta-Harhay, Guerini et al., 2007; Barkocy-Gallagher et al., 2003; Bosilevac et al., 2004; Nou et al., 2003). Chemical dehairing, in essence the sanitizing of the animal hide, was shown to reduce the prevalence of *E. coli* O157:H7 contamination on preevisceration carcasses from 50% to 1% (Nou et al., 2003). The use of dehairing was short-lived due to problems with waste disposal and modified hide tanning, but modification of the concept led to the development of a system for spray washing of the cattle hide to achieve reduced carcass contamination. Bosilevac, Nou, Osborn, Allen, & Koohmaraie (2005)

* Corresponding author. Tel.: +1 402 762 4227; fax: +1 402 762 4149.
*E-mail address:* terrance.arthur@ars.usda.gov (T.M. Arthur).
[1] Current address: IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155, USA.

0309-1740/$ – see front matter. Published by Elsevier Ltd. on behalf of The American Meat Science Association.
doi:10.1016/j.meatsci.2010.04.019

demonstrated the efficacy of hide-on carcass washers in reducing the transfer of pathogenic organisms from hide to carcass, while maintaining the value of the hide and minimizing waste disposal. Due to the high costs and large space requirements of these cabinets they were implemented by only one large beef processing company. For widespread use across the beef processing industry, the technology would need to be adapted to a small scale, low cost option. An evaluation of such an option by Arthur, Bosilevac, Brichta-Harhay, Kalchayanand, et al. (2007) described a minimal hide wash cabinet that would meet the financial and space needs of the majority in the beef processing industry while effectively reducing the *E. coli* O157:H7 load on incoming cattle hides.

Research on *E. coli* O157:H7 hide contamination and its effects on downstream processing leads to the hypothesis regarding process control, that if contamination on the hide can be controlled, then the process will be in control. This is best described in a model where *E. coli* O157:H7 harbored on the hide of cattle is the major source of carcass contamination during processing. Pathogens present on the hide may be transferred to the carcass during the hide opening and removal process. Pathogenic bacteria present on the carcass must be removed by the antimicrobial interventions in-place at the processing plant in order to have a wholesome final product. In the United States, multiple hurdle intervention strategies are utilized to reduce or remove pathogens from the carcass. These strategies employ several different antimicrobial mechanisms to kill or remove the target organisms from the carcass. Several research studies have reported effectiveness of these individual interventions at reducing various levels of pathogens (Gill, 2009; Koohmaraie et al., 2005; Sofos, 2009). However, each intervention has an upper limit of bacteria, which once exceeded; the final product will be contaminated. The same is true for multiple hurdles. A threshold would exist for the particular intervention system where incoming bacterial loads above the threshold will overwhelm the subsequent interventions and finished product contamination will result (Ahmadi et al., 2007). The critical threshold is a function of pathogenic bacterial load on the cattle hide. If the population of pathogens on the hide is kept below the cumulative capacity of the in-plant interventions, the bacteria transferred to the carcass can be addressed by the in-plant interventions and the process will be in control. One caveat to this hypothesis is that it is based on the assumption that all interventions are functioning at optimal levels and that processing personnel are working within the guidelines of the industry's best practices. Breakdowns in intervention application or hygienic technique may allow final product contamination in situations where the incoming load is below the critical threshold.

## 2. *E. coli* O157:H7 colonization of cattle

In controlling hide contamination coming into processing plants, one needs to understand the dynamics of transmission of *E. coli* O157:H7 between animals in cattle-associated environments. These cattle-associated environments include production (i.e. pastures or feedlots) and harvest (transportation and lairage prior to entry into the processing plant). While the hide is the main source of carcass contamination, it is the gastrointestinal tract of cattle that serves as the primary habitat for *E. coli* O157:H7 (Savageau, 1983). Therefore, the carcass is contaminated from the hide and the hide is contaminated by feces from *E. coli* O157:H7-colonized cattle. For many years it was believed that *E. coli* O157:H7 cells were ingested by cattle and these cells passively migrated through the GI tract, increasing in numbers until they were voided in the form of contaminated feces (Brown, Harmon, Zhao, & Doyle, 1997; Cray & Moon, 1995; Magnuson et al., 2000). This was shown not to be the case when Naylor et al. (2003) described a specific site of colonization in the distal colon near the recto-anal junction (RAJ).

The intestinal epithelial region 0 to 3 cm proximal to the RAJ was identified as a colonization site when content samples from various regions of the digestive tract failed to yield *E. coli* O157:H7, while samples of voided feces from the same animals did. This indicated that samples had not been collected from sites close enough to the intestinal terminus (Naylor et al., 2003). When samples were collected starting at the RAJ and working proximally, it was found that the concentration of *E. coli* O157:H7 was greatest in the 0 to 3 cm proximal region and decreased as sampling was moved further in the proximal direction (Naylor et al., 2003). In reporting the RAJ colonization site, Naylor et al. hypothesized that a subset of cattle (super shedders) may shed *E. coli* O157:H7 at high levels ($>10^4$ CFU/g of feces) and that colonization at the RAJ was necessary for the high-level shedding. Subsequent studies have described an association between RAJ colonization and super shedding status (Cobbold et al., 2007; Low et al., 2005).

## 3. Super shedders and *E. coli* O157:H7 transmission among cattle

Concomitantly with the discovery of RAJ colonization and the association of super shedder status other researchers were attempting to develop mathematical models to describe the transmission of *E. coli* O157:H7 among cattle (Matthews, McKendrick, et al., 2006; Robinson, Wright, Hart, Bennett, & French, 2004). In analyzing data of fecal shedding on multiple Scottish farms, Matthews, McKendrick, et al. (2006) found a large variability in the prevalence of *E. coli* O157:H7 among the different cattle populations. Potential explanations provided by the study's authors for the variable pathogen prevalence included differences in environment suitability for *E. coli* O157:H7 growth, animal movement onto and away from farms, and carriage levels and persistence in some animals. It was determined that the model providing the best fit of the data included variability in the transmission rates by allowing a proportion of the animals to have much higher transmission rates than the others (i.e. super shedders) (Matthews, McKendrick, et al., 2006). In support of the super shedder model, Matthews, Low, et al. (2006) presented data suggesting that 20% of the *E. coli* O157:H7 infections in cattle on Scottish farms were responsible for 80% of the transmission of the organism between animals. Another study showed that 9% of the animals shedding *E. coli* O157:H7 at harvest produced over 96% of the total *E. coli* O157:H7 fecal load for the group (Omisakin, MacRae, Ogden, & Strachan, 2003). Conversely, feedlot cattle that did not shed *E. coli* O157:H7 over the course of study were five times more likely to be housed in a pen that did not contain a super shedder (Cobbold et al., 2007). These results have led to the conclusion that if colonization could be prevented in 5% of individuals (namely those individuals that would shed $\geq 10^4$ *E. coli* O157:H7 CFU/g) the spread of infection could be controlled (Matthews, Low, et al., 2006).

## 4. Super shedder effects on hide contamination

Transmission of *E. coli* O157:H7 among cattle and the resulting cycles of animal colonization in production environments impact carcass contamination at processing via the cattle hide. As the hide presents the major source of carcass contamination it is the effects of super shedders on the hide bacterial load that must be understood in order to develop methods of mitigating the risk of final product contamination.

The effect of super shedders on hide contamination was recently simulated by placing inoculated fecal pats in pens containing naïve cattle (Stephens, McAllister, & Stanford, 2008). The inoculated strains were detected in hide samples from the high-level inoculum group one day after deposition of the fecal pats. Overall the pens receiving the high-level inoculated fecal pats had the highest hide prevalence (3%) with the low-level pens having only one positive hide sample (0.45%) and none for the control pens (Stephens et al., 2008). Similar findings were obtained by McGee, Scott, Sheridan, Earley, & Leonard (2004) as inoculated steers, each shedding *E. coli* O157:H7 at levels

greater than 500 CFU/g of feces, were placed in pens with five uninoculated, non-colonized cohorts. Hide samples from 66% of the cohort animals were found to be positive for the marked strains after 48 h of exposure to the high shedding animals (McGee et al., 2004). One pen had all occupants, the one inoculated animal and its five cohorts, harboring *E. coli* O157:H7 on their hides within 24 h of co-mingling (McGee et al., 2004).

The relationship between shedding animals and *E. coli* O157:H7 hide prevalence was investigated in a feedlot setting over a 9-month period (Arthur et al., 2009). In that study, cattle were categorized into four shedding classifications: not shedding, prevalence positive (fecal positive, but less than 200 CFU/g), high density fecal shedder ($\geq 2\times10^2$ CFU/g), and fecal super shedding ($>10^4$ CFU/g). The analysis of cattle hide prevalence as a function of fecal prevalence demonstrated a threshold response relationship between fecal prevalence and hide prevalence was identified. In this relationship, as fecal pen prevalence exceeded 20%, hide pen prevalence was usually greater than 80%. It was also noted that most of the feedlot pens housing one or more super shedders also were pens with fecal prevalence greater than 20%. The authors concluded that 20% fecal prevalence was a functional threshold marker of super shedder pens and super shedding cattle, but could not determine if the presence of super shedders or the increased prevalence was the causal factor in the scenario due to the interrelatedness of the factors (Arthur et al., 2009).

In the same study, hide carriage of *E. coli* O157:H7 also was categorized. Animals were described as either not carrying the pathogen on the hide, positive but <40 CFU/100 $cm^2$, or high-level hide concentration ($\geq$40 CFU/100 $cm^2$). Cattle classified in the high-level hide contamination category were thought to present a greater risk of carcass contamination at slaughter. The analysis showed that when >80% of cattle in a pen were hide positive, the high density hide prevalence increased rapidly. Conversely, the high density hide prevalence did not exceed 10% when the hide prevalence was <80%. Thus, 80% hide prevalence is a functional threshold marker of increased high density hide contamination. Most pens containing one or more super shedders were pens with hide prevalence greater than 80%. The high-level hide prevalence exceeded 20% in a majority of pens housing at least one super shedder. Based on these results, the authors recommended that the *E. coli* O157:H7 fecal prevalence should be maintained below 20% and levels of shedding need to be kept below 200 CFU/g to minimize the contamination of cattle hides (Arthur et al., 2009).

Interestingly, the fact that an animal is a super shedder does not seem to have a direct relationship to the contamination of that animal's carcass at the time of processing. Fox, Renter, et al. (2008) presented data showing that the probability of isolating *E. coli* O157 from a carcass was not significantly associated with the fecal level or fecal prevalence status of the animal from which the carcass was derived. In that study, the probability of carcass contamination was significantly associated with all truckload-level measures of fecal *E. coli* O157, particularly whether or not a high shedder was present within the truckload (Fox, Renter, et al., 2008). Similarly, Fegan, Higgs, Vanderlinde, & Desmarchelier (2005) also emphasized the importance of incoming pathogen load at the lot-level, as carcass contamination rates were highest for those lots with the highest pathogen load, including carcasses from animals that were not shedding the pathogen.

## 5. Lairage environment contamination

Until recently, the hide contamination occurring in the production environment was thought to account for the majority of carcass contamination at slaughter. Data collected during the transport to and lairage of cattle at processing plants has caused a shift in the model used to describe *E. coli* O157:H7 passage through the beef production pipeline from live animal to finished product. Some of the first work to identify processing plant lairage environments as a potential source of pathogens to contaminate cattle was conducted in the lab of Dr. Sava Buncic. Samples collected from the unloading-to-slaughter routes at abattoirs in southwest England harbored multiple foodborne pathogens including *E. coli* O157:H7 (Small et al., 2002. That work was expanded upon through the use of pulsed field gel electrophoresis (PFGE). PFGE analysis of *E. coli* O157:H7 isolates collected from the hides of multiple lots of cattle presented for processing identified common profiles (Avery, Small, Reid, & Buncic, 2002). The authors concluded that the lairage environment was the one common factor among the various groups of cattle and was suggested as the most probable source of the pathogen contamination (Avery et al., 2002).

In a separate study, PFGE analysis of *Xba*I-digested genomic DNA was performed for over 1000 *E. coli* O157:H7 isolates collected from cattle hide samples at nine commercial beef processing plants across North America (Arthur, Bosilevac, Nou, et al., 2007). This analysis resulted in 277 unique PFGE profiles resulting from a single restriction endonuclease digestion. Of the total 277 unique profiles, 19.5% contained isolates collected from multiple regions. In order to further distinguish the strains in each of the profile clusters, DNA digests using two additional restriction enzymes, *Bln*I and *Spe*I, were performed. After the two subsequent rounds of PFGE analysis, there was still a large population of isolates ($n=154$) that had indistinguishable patterns even though they were collected from different regions, separated by distances up to 1400 mi (2253 km). On multiple occasions, strains isolated from cattle hides in Canada had profiles that were indistinguishable from cattle hide isolates collected in Kansas and Nebraska (Arthur, Bosilevac, Nou, et al., 2007). The data led the authors to conclude that the cattle hides were being contaminated from the processing plant lairage environments. Multiple lots of cattle were sent from individual feedlots to different processing plants. Cattle originating from the same feedlot were thought to have a high likelihood of carrying *E. coli* O157:H7 isolates of identical PFGE profiles. Previous studies had described the predominance of certain PFGE profiles in feedlot settings (LeJeune et al., 2004; Sanderson et al., 2006). The cattle would contaminate the processing plant lairage environment upon arrival and holding prior to slaughter. Subsequent cattle passing through the lairage environment would now be contaminated with *E. coli* O157:H7 strains not found in their feedlot of origin, a scenario suggested by (Tutenel, Pierard, Van Hoof, & De Zutter, 2003).

The previously mentioned studies demonstrated that the lairage environment may be a source of cattle hide contamination, but failed to show any link to carcass contamination. It could be that while the lairage environment added to the *E. coli* O157:H7 load residing on cattle from the feedlot, the amount added would be lacking in comparison to pre-existing contamination and add little in the way of risk of carcass contamination. This question was answered through two studies that followed cattle from the feedlot through processing. The first study sampled multiple lots of cattle (hides and feces) at a feedlot immediately prior to loading onto transport trailers. The trailers were sampled prior to loading cattle. The cattle were sent to the processing plant and hide and carcass samples were collected on the process chain. Overall, only 29% ($n=764$) of the isolates collected post-harvest were found to match PFGE types of isolates collected prior to transport (Arthur, Bosilevac, Brichta-Harhay, Guerini, et al., 2007). The majority of post-harvest isolates could not be matched to any pre-transport PFGE types and were assumed to have come from sources in the lairage environment. The remaining 2% of post-slaughter isolates matched PFGE patterns of isolates collected from the truck trailers. When only the strains collected from carcass samples were analyzed, 80% ($n=80$) of the carcass isolates did not match pre-transport PFGE types, with one matching a truck isolate type and the other 66 being believed to come from the lairage environment (Arthur, Bosilevac, Brichta-Harhay, Guerini, et al., 2007).

Unfortunately, samples were not collected from the lairage environment in that study leaving the question not fully answered. Childs et al. (2006) also utilized PFGE and reported that isolates with similar genotypes were obtained from the lairage environment surfaces and on cattle hides during processing, but hide samples were not collected prior to cattle shipment to determine if the organisms in question were present before transport and carcass samples were not collected to determine the lairage impact on carcass contamination. A second study was performed by Arthur et al. (2008), again sampling multiple lots of cattle at a feedlot and on the processing chain, but samples from each lairage environment space also were collected just prior to the test animals passage through that space. This study confirmed that the lairage environment played a larger role in cattle hide and subsequent carcass contamination than the feedlot of origin. This phenomenon also was seen in Scottish cattle by Mather et al. (2008) and they concluded that intervention strategies needed to be focused on preventing contact with contamination sources or reducing hide prevalence after cattle had left the farm of origin. This is in contrast to work by Fegan, Higgs, Duffy, & Barlow (2009) and Barham, Barham, Johnson, Allen, Blanton, Jr., and Miller (2002), who found reductions in fecal and hide levels and prevalence for *E. coli* O157:H7 between the feedlot and the abattoir, although, Barham et al. (2002) did see increases in *Salmonella* prevalence. The reasons for the discrepancies in these studies are not know.

With the high animal density and confined spaces associated with processing plant lairage environments it is presumed that super shedding cattle would have a large impact on the overall contamination of animals currently in these environments and those that will enter these areas at subsequent times. In these areas, hundreds to thousands of animals traverse the same approximate path each day. Animals shedding over $10^4$ *E. coli* O157:H7 CFU/g of feces could readily deposit enough pathogen-laden material to contaminate a significant portion of cattle lots passing through the spaces for the remainder of the processing cycle. Presently, there are no data to determine the true impact of super shedders on lairage environment contamination.

## 6. Super shedders at processing

Evidence indicates that direct fecal contamination of the carcass at processing is a rare occurrence (Arthur, Bosilevac, Brichta-Harhay, Guerini, et al., 2007; Bosilevac et al., 2004; Nou et al., 2003) and as such, the significance of super shedders with regard to risk of carcass contamination is unknown. In commercial U.S. processing plants piercing of the intestinal tract is a rare event (personal observation). Hence following bunging and tying off of the esophagus, the intestinal tract is essentially sealed. Once the viscera are removed and separated into marketable sections, the contents are released and the material is again a potential source of contamination in the processing plant for offal products, some of which (weasand and heart) can be used in ground beef production.

*E. coli* O157:H7 has been found in multiple sections of the GI tract of harvested cattle. In a study using three inoculated adult cattle, *E. coli* O157:H7 was recovered from various GI tract sites from the colon to the rumen (Cray & Moon, 1995). Another study utilized cattle at commercial processing plants to screen for *E. coli* O157:H7 in four GI tract locations: rumen, cecum, colon, and rectum. The target pathogen was isolated from all four of the sample sites, with the rectum the most likely site of harborage (Walker, Shi, Sanderson, Sargeant, & Nagaraja, 2009), consistent with the RAJ being the colonization site for *E. coli* O157:H7. Neither of these studies identified any super shedders in the populations studied. Lim, Sheng, Besser, Potter, & Hovde (2007) utilized three animals that were persistently shedding *E. coli* O157:H7 for at least three months. At the time of sampling, all three animals were colonized, but at a low level with RAJ samples being ≤30 CFU/

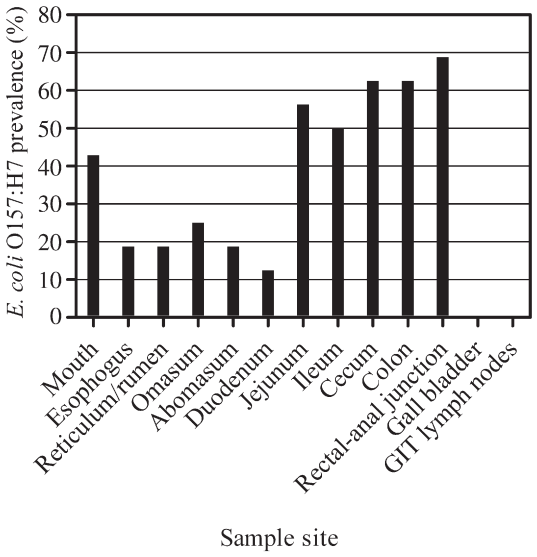


**Fig. 1.** *E. coli* O157:H7 prevalence for various samples from animals that were high or persistent shedders ($n = 15$). GIT = gastrointestinal tract.

swab. Only GI tract samples from the RAJ had detectable levels of *E. coli* O157:H7 (Lim et al., 2007). The authors also reported that samples from the gall bladder of each animal were negative for *E. coli* O157:H7.

In order to determine the distribution of *E. coli* O157:H7 associated with super shedding cattle, the authors of the work presented here identified fifteen animals that were either persistently shedding *E. coli* O157:H7 or were super shedders at some point in time. Samples of the GI tract included: mouth, esophagus, rumen, omasum, duodenum, jejunum, ileum, cecum, colon, and RAJ. Two non-GI tract sites (gall badder and mesenteric lymph nodes) also were sampled. Similar to previous reports, the RAJ was to site of most frequent *E. coli* O157:H7 detection (Fig. 1). Only two animals met the criteria of super shedders on the day of processing. Of particular note is that these two animals harbored *E. coli* O157:H7 at every sample site of the gastrointestinal tract with the exception that the mouth sample for one animal was negative. The *E. coli* O157:H7 concentration for those animals was highest at the RAJ and decreased as more proximal sites were sampled. None of the gall bladder or lymph node samples contained detectable levels of *E. coli* O157:H7, including those that came from the super shedding animals.

Not usually thought of as invasive, multiple studies have reported isolation of *E. coli* O157:H7 in lymph nodes harvested from inoculated calves (Alali, Sargeant, Nagaraja, & DeBey, 2004; Cray & Moon, 1995; Woodward et al., 1999). However, finding *E. coli* O157:H7 in lymph tissue of adult animals, has been rare. Bonardi, Foni, Chiapponi, Salsi, & Brindani (2007) recovered the pathogen from 1.1% of both tonsils and mesenteric lymph nodes (one positive sample for each) of adult cattle at slaughter in Italy. It is not known if these samples came from feedlot-reared animals or cull dairy cows as both were represented in the samples. Also, the risk to ground beef is questionable, since these items would be discarded and not included in materials destined for grinding.

As shown in Fig. 1, *E. coli* O157:H7 was recovered from over 40% of the mouth samples emphasizing the importance of proper head removal and cheek meat harvest. Other studies have reported a range of 1% to 75% for *E. coli* O157:H7 prevalence in samples of the oral cavity in non-inoculated cattle (Bach, Selinger, Stanford, & McAllister, 2005; Fegan et al., 2005; Keen & Elder, 2002; Stephens et al., 2007).

## 7. Hide-to-carcass transfer

Attention to detail and proper training of personnel are important elements in the maintenance of process control for a beef harvest

system. Effective interventions can be rendered ineffective when best practices are not taught, implemented, and maintained. One of the most critical steps in preventing beef carcass contamination is the hygienic removal of the cattle hide. Several studies have shown that carcasses become contaminated early in the process and that contamination is responsible for final product contamination unless removed or killed by an intervention procedure (Arthur et al., 2004; Barkocy-Gallagher et al., 2001; Brichta-Harhay et al., 2008; Elder et al., 2000). The earliest point for carcass contamination to occur is in the hide opening and subsequent skinning process. The effectiveness in preventing hide-to-carcass transfer has been shown to have a processing plant specific-component. A comparison presented by Koohmaraie et al. (2007) demonstrated that on multiple processing days, one plant had lower *E. coli* O157:H7 carcass prevalence rates than another even though its incoming hide prevalence was higher. A drawback to this study was that only prevalence data were collected. If the level of *E. coli* O157:H7 on the hides of incoming cattle differed significantly, it may have affected the ability to plant personnel to prevent transfer to the carcass. Brichta-Harhay et al. (2008) presented similar data showing a difference of over 40% in *Salmonella* carcass prevalence between two plants with approximately the same incoming hide prevalence. This dataset also included enumeration data for hide and carcass samples. The additional data showed that for the plant with the higher carcass prevalence, there was a slightly higher incoming *Salmonella* load on the hides, but there also was a disproportionately higher load on the carcasses. These results suggest that both incoming load and proficiency in hide removal impact carcass pathogen prevalence.

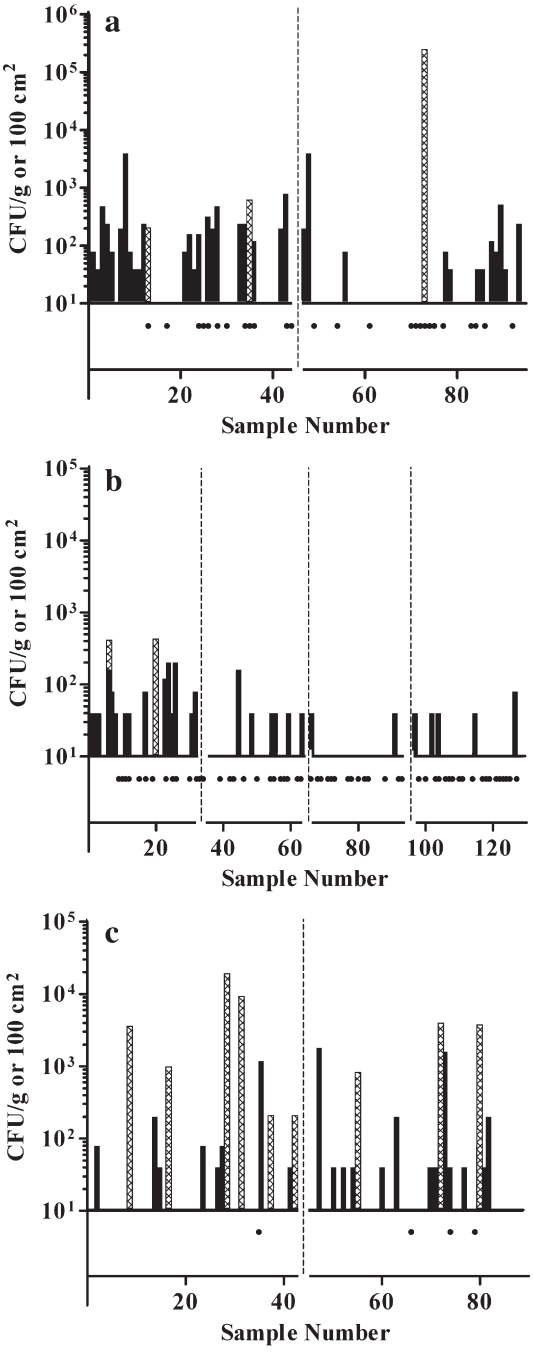


**Fig. 2.** Relationship between high-level fecal shedding (hatched bars, CFU/g) of *E. coli* O157:H7 and high density hide contamination (solid bars, CFU/100 cm$^2$) with *E. coli* O157:H7 carcass contamination (solid circles). Dashed vertical lines represent lot breaks. At each lot break, multiple lots of non-test cattle were processed. Cattle were sampled consecutively. Data for a and b come from different days at the same processing plant, which does not utilize a hide wash cabinet. Data for c comes from a processing plant that does employ a hide wash cabinet.

The contributions made by each of the factors can be seen in Fig. 2a and 2b. The data shown here represent *E. coli* O157:H7 prevalence and levels from fecal, hide, and preevisceration carcass samples collected during harvest. In Fig. 2a it can be seen that the carcasses (filled circles) found to have *E. coli* O157:H7 seem to be clustered around the super shedding animals (hatched bars) and the animals with high-level contamination on their hides (solid bars), representing the impact of high incoming load. In Fig. 2b the rate of carcass positives appears to be independent of incoming load, most likely representing inadequacies in hide removal technique. Hide wash cabinets can greatly reduce the impact of incoming load and improper technique, the results of which are dramatic reductions in carcass prevalence (Fig.2c).

## 8. Needed research

It is not known what criteria must be met to cause the super shedding phenomenon. The most likely contributing factors would be strain differences of *E. coli* O157:H7, animal genetics leading to the expression of suitable factors at the RAJ, cattle diet, and/or modulations of the resident microbial flora to allow *E. coli* O157:H7 to flourish in some animals for some period of time. Understanding how each of these factors play a role in super shedding will aid in minimizing or eliminating the super shedding population. Interventions that would prevent supershedding in the cattle population should reduce *E. coli* O157:H7 transmission in the production and lairage environments resulting in reduced risk of beef carcass contamination and a safer finished product.

## References

Ahmadi, B. V., Frankena, K., Turner, J., Velthuis, A. G. J., Hogeveen, H., & Huirne, R. B. M. (2007). Effectiveness of simulated interventions in reducing the estimated prevalence of *E. coli* O157: H7 in lactating cows in dairy herds. *Veterinary Research*, *38*(5), 755−771.

Alali, W. Q., Sargeant, J. M., Nagaraja, T. G., & DeBey, B. M. (2004). Effect of antibiotics in milk replacer on fecal shedding of *Escherichia coli* O157:H7 in calves. *Journal of Animal Science*, *82*(7), 2148−2152.

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Guerini, M. N., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & 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. *Journal of Food Protection*, *70*(2), 280−286.

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., King, D. A., Shackelford, S. D., Wheeler, T. L., & 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. *Journal of Food Protection*, *71*(9), 1752−1760.

Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2007). Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *Journal of Food Protection*, *70*(5), 1076−1079.

Arthur, T. M., Bosilevac, J. M., Nou, X., Shackelford, S. D., Wheeler, T. L., Kent, M. P., Jaroni, D., Pauling, B., Allen, D. M., & Koohmaraie, M. (2004). *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *Journal of Food Protection*, *67*(4), 658−665.

Arthur, T. M., Bosilevac, J. M., Nou, X., Shackelford, S. D., Wheeler, T. L., & 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. *Journal of Food Protection*, *70*(7), 1622−1626.

Arthur, T. M., Keen, J. E., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., Nou, X. W., & Koohmaraie, M. (2009). Longitudinal study of *Escherichia coli* O157:H7 in a beef cattle feedlot and role of high-level shedders in hide contamination. *Applied and Environmental Microbiology*, *75*(20), 6515−6523.

Avery, S. M., Small, A., Reid, C. A., & Buncic, S. (2002). Pulsed-field gel electrophoresis characterization of Shiga toxin-producing *Escherichia coli* O157 from hides of cattle at slaughter. *Journal of Food Protection*, *65*(7), 1172−1176.

Bach, S. J., Selinger, L. J., Stanford, K., & McAllister, T. A. (2005). Effect of supplementing corn- or barley-based feedlot diets with canola oil on faecal shedding of *Escherichia coli* O157:H7 by steers. *Journal of Applied Microbiology*, *98*(2), 464−475.

Barham, A. R., Barham, B. L., Johnson, A. K., Allen, D. M., Blanton, J. R., Jr., & Miller, M. F. (2002). Effects of transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. *Journal of Food Protection*, *65*(2), 280−283.

Barkocy-Gallagher, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *Journal of Food Protection*, *66*(11), 1978−1986.

Barkocy-Gallagher, G. A., Arthur, T. M., Siragusa, G. R., Keen, J. E., Elder, R. O., Laegreid, W. W., & 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. *Applied and Environmental Microbiology*, *67*(9), 3810−3818.

Bonardi, S., Foni, E., Chiapponi, C., Salsi, A., & Brindani, F. (2007). Detection of verocytotoxin-producing *Escherichia coli* serogroups O157 and 026 in the cecal content and lymphatic tissue of cattle at slaughter in Italy. *Journal of Food Protection*, *70*(6), 1493−1497.

Bosilevac, J. M., Arthur, T. M., Wheeler, T. L., Shackelford, S. D., Rossman, M., Reagan, J. O., & Koohmaraie, M. (2004). Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *Journal of Food Protection*, *67*(4), 646−650.

Bosilevac, J. M., Nou, X., Osborn, M. S., Allen, D. M., & Koohmaraie, M. (2005). Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *Journal of Food Protection*, *68*(2), 265−272.

Brichta-Harhay, D. M., Guerini, M. N., Arthur, T. M., Bosilevac, J. M., Kalchayanand, N., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2008). *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: An evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Applied and Environmental Microbiology*, *74*(20), 6289−6297.

Brown, C. A., Harmon, B. G., Zhao, T., & Doyle, M. P. (1997). Experimental *Escherichia coli* O157:H7 carriage in calves. *Applied and Environmental Microbiology*, *63*(1), 27−32.

Chase-Topping, M., Gally, D., Low, C., Matthews, L., & Woolhouse, M. (2008). Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nature Reviews. Microbiology*, *6*(12), 904−912.

Childs, K. D., Simpson, C. A., Warren-Serna, W., Bellenger, G., Centrella, B., Bowling, R. A., Ruby, J., Stefanek, J., Vote, D. J., Choat, T., Scanga, J. A., Sofos, J. N., Smith, G. C., & Belk, K. E. (2006). Molecular characterization of *Escherichia coli* O157:H7 hide contamination routes: Feedlot to harvest. *Journal of Food Protection*, *69*(6), 1240−1247.

Cobbold, R. N., Hancock, D. D., Rice, D. H., Berg, J., Stilborn, R., Hovde, C. J., & Besser, T. E. (2007). Rectoanal junction colonization of feedlot cattle by *Escherichia coli* O157:H7 and its association with supershedders and excretion dynamics. *Applied and Environmental Microbiology*, *73*(5), 1563−1568.

Cray, W. C., Jr., & Moon, H. W. (1995). Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, *61*(4), 1586−1590.

Elder, R. O., Keen, J. E., Siragusa, G. R., Barkocy-Gallagher, G. A., Koohmaraie, M., & Laegreid, W. W. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proceedings of the National Acadamy of Science USA*, *97*(7), 2999−3003.

Fegan, N., Higgs, G., Duffy, L. L., & Barlow, R. S. (2009). The effects of transport and lairage on counts of *Escherichia coli* O157 in the feces and on the hides of individual cattle. *Foodborne Pathogens and Disease*, *6*(9), 1113−1120.

Fegan, N., Higgs, G., Vanderlinde, P., & Desmarchelier, P. (2005). An investigation of *Escherichia coli* O157 contamination of cattle during slaughter at an abattoir. *Journal of Food Protection*, *68*(3), 451−457.

Fox, J. T., Renter, D. G., Sanderson, M. W., Nutsch, A. L., Shi, X., & Nagaraja, T. G. (2008). Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. *Journal of Food Protection*, *71*(9), 1761−1767.

Fox, J. T., Shi, X., & Nagaraja, T. G. (2008). *Escherichia coli* O157 in the rectoanal mucosal region of cattle. *Foodborne Pathogens and Disease*, *5*(1), 69−77.

Gill, C. O. (2009). Effects on the microbiological condition of product of decontaminating treatments routinely applied to carcasses at beef packing plants. *Journal of Food Protection*, *72*(8), 1790−1801.

Keen, J. E., & Elder, R. O. (2002). Isolation of shiga-toxigenic *Escherichia coli* O157 from hide surfaces and the oral cavity of finished beef feedlot cattle. *Journal of the American Veterinary Medical Association*, *220*(6), 756−763.

Koohmaraie, M., Arthur, T. M., Bosilevac, J. M., Brichta-Harhay, D. M., Kalchayanand, N., Shackelford, S. D., & Wheeler, T. L. (2007). Interventions to reduce/eliminate *Escherichia coli* O157: H7 in ground beef. *Meat Science*, *77*(1), 90−96.

Koohmaraie, M., Arthur, T. M., Bosilevac, J. M., Guerini, M., Shackelford, S. D., & Wheeler, T. L. (2005). Post-harvest interventions to reduce/eliminate pathogens in beef. *Meat Science*, *71*(1), 79−91.

LeJeune, J. T., Besser, T. E., Rice, D. H., Berg, J. L., Stilborn, R. P., & Hancock, D. D. (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. *Applied and Environmental Microbiology*, *70*(1), 377−384.

Lim, J. Y., Li, J., Sheng, H., Besser, T. E., Potter, K., & Hovde, C. J. (2007). *Escherichia coli* O157:H7 colonization at the rectoanal junction of long-duration culture-positive cattle. *Applied and Environmental Microbiology*, *73*(4), 1380−1382.

Low, J. C., McKendrick, I. J., McKechnie, C., Fenlon, D., Naylor, S. W., Currie, C., Smith, D. G., Allison, L., & Gally, D. L. (2005). Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. *Applied and Environmental Microbiology*, *71*(1), 93−97.

Magnuson, B. A., Davis, M., Hubele, S., Austin, P. R., Kudva, I. T., Williams, C. J., Hunt, C. W., & Hovde, C. J. (2000). Ruminant gastrointestinal cell proliferation and clearance of *Escherichia coli* O157:H7. *Infection and Immunity*, *68*(7), 3808−3814.

Mather, A. E., Reid, S. W., McEwen, S. A., Ternent, H. E., Reid-Smith, R. J., Boerlin, P., Taylor, D. J., Steele, W. B., Gunn, G. J., & Mellor, D. J. (2008). Factors associated with cross-contamination of hides of Scottish cattle by *Escherichia coli* O157. *Applied and Environmental Microbiology*, *74*(20), 6313−6319.

Matthews, L., Low, J. C., Gally, D. L., Pearce, M. C., Mellor, D. J., Heesterbeek, J. A., Chase-Topping, M., Naylor, S. W., Shaw, D. J., Reid, S. W., Gunn, G. J., & Woolhouse, M. E. (2006). Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proceedings of the Nation Academy of Sciences USA*, *103*(3), 547−552.

Matthews, L., McKendrick, I. J., Ternent, H., Gunn, G. J., Synge, B., & Woolhouse, M. E. (2006). Super-shedding cattle and the transmission dynamics of *Escherichia coli* O157. *Epidemiology and Infection*, *134*(1), 131−142.

McGee, P., Scott, L., Sheridan, J. J., Earley, B., & Leonard, N. (2004). Horizontal transmission of *Escherichia coli* O157:H7 during cattle housing. *Journal of Food Protection*, *67*(12), 2651−2656.

Naylor, S. W., Low, J. C., Besser, T. E., Mahajan, A., Gunn, G. J., Pearce, M. C., McKendrick, I. J., Smith, D. G., & Gally, D. L. (2003). Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infection and Immunity*, *71*(3), 1505−1512.

Nou, X., Rivera-Betancourt, M., Bosilevac, J. M., Wheeler, T. L., Shackelford, S. D., Gwartney, B. L., Reagan, J. O., & 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. *Journal of Food Protection*, *66*(11), 2005−2009.

Omisakin, F., MacRae, M., Ogden, I. D., & Strachan, N. J. (2003). Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Applied and Environmental Microbiology*, *69*(5), 2444−2447.

Robinson, S. E., Wright, E. J., Hart, C. A., Bennett, M., & French, N. P. (2004). Intermittent and persistent shedding of *Escherichia coli* O157 in cohorts of naturally infected calves. *Journal of Applied Microbiology*, *97*(5), 1045−1053.

Sanderson, M. W., Sargeant, J. M., Shi, X., Nagaraja, T. G., Zurek, L., & Alam, M. J. (2006). Longitudinal emergence and distribution of *Escherichia coli* O157 genotypes in a beef feedlot. *Applied and Environmental Microbiology*, *72*(12), 7614−7619.

Savageau, M. A. (1983). *Escherichia coli* habitats, cell-types, and molecular mechanisms of gene-control. *American Naturalist*, *122*(6), 732−744.

Small, A., Reid, C. A., Avery, S. M., Karabasil, N., Crowley, C., & Buncic, S. (2002). Potential for the spread of *Escherichia coli* O157, *Salmonella*, and *Campylobacter* in the lairage environment at abattoirs. *Journal of Food Protection*, *65*(6), 931−936.

Sofos, J. N. (2009). ASAS Centennial Paper: Developments and future outlook for postslaughter food safety. *Journal of Animal Science*, *87*(7), 2448−2457.

Stephens, T. P., Loneragan, G. H., Thompson, T. W., Sridhara, A., Branham, L. A., Pitchiah, S., & Brashears, M. M. (2007). Distribution of *Escherichia coli* O157 and *Salmonella* on hide surfaces, the oral cavity, and in feces of feedlot cattle. *Journal of Food Protection*, *70*(6), 1346−1349.

Stephens, T. P., McAllister, T. A., & Stanford, K. (2008). Development of an experimental model to assess the ability of *Escherichia coli* O157:H7-inoculated fecal pats to mimic a super shedder within a feedlot environment. *Journal of Food Protection*, *71*(3), 648−652.

Tutenel, A. V., Pierard, D., Van Hoof, J., & De Zutter, L. (2003). Molecular characterization of *Escherichia coli* O157 contamination routes in a cattle slaughterhouse. *Journal of Food Protection*, *66*(9), 1564−1569.

Walker, C., Shi, X., Sanderson, M., Sargeant, J., & Nagaraja, T. G. (2009). Prevalence of *Escherichia coli* O157:H7 in gut contents of beef cattle at slaughter. *Foodborne Pathogens and Disease*, *7*(3), 249−255.

Woodward, M. J., Gavier-Widen, D., McLaren, I. M., Wray, C., Sozmen, M., & Pearson, G. R. (1999). Infection of gnotobiotic calves with *Escherichia coli* O157:H7 strain A84. *Veterinary Record*, *144*(17), 466−470.