2012 Beef Industry Safety Summit

Research Update

Pre-Harvest Safety Research

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Characterization of E. coli *O157:H7 strains associated with super-shedding cattle*

Terry Arthur, U.S. Meat Animal Research Center

Cattle are the principal animal reservoir of *E. coli* O157:H7, and the rectal-anal junction (RAJ) has been shown to be the predominant colonization site. Once colonized, an animal can shed various amounts of *E. coli* O157:H7 in the feces. Super-shedders (RAJ colonized at greater than 10^4 CFU/g) are reported to be responsible for increased transmission of *E. coli* O157:H7 within production environments. Therefore, it is critical to identify and reduce the number of super-shedders in the cattle population in order to reduce *E. coli* O157:H7 transmission and beef carcass contamination.

Approaches previously tested for reducing *E. coli* O157:H7 colonization of the cattle gastrointestinal tract (GIT) have been inconclusive or treatments have only modestly affected colonization. None of these interventions have tested the effectiveness of reducing the prevalence of super-shedding in cattle populations. This is significant, as modeling studies suggest that possibly as high as 96% of *E. coli* O157:H7 isolates originate from super-shedding animals. It is evident that a more thorough understanding of the factors promoting super-shedding is needed before we can design effective evidence-based methods of reducing transmission of STEC from cattle populations to the food supply. This project was designed to determine the contribution of *E. coli* O157:H7 strain type to the development of super-shedding.

In this study, researchers characterized up to twenty *E. coli* O157:H7 strains from super-shedder samples for genotype and phenotype. Analyses included phage typing, curli production, PFGE, *stx* typing, lineage determination, and comparative genomic fingerprinting. Of the 4,500 swab samples from the recto-anal junction of cattle in feedlots and at slaughter, the overall average of super-shedders was 2%. Among the 103 super-shedder strains collected, no common genotype or phenotype was identified. These results show that strain type will no longer be a variable in super-shedding modeling.

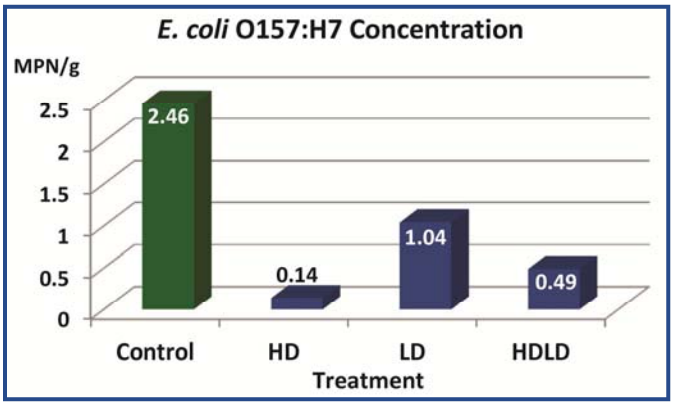
Prevalence and concentration of Escherichia coli *O157:H7 and non-O157 O types in beef feedlot cattle after feeding Bovamine® and detection through the real time PCR BAX® system from DuPont Qualicon*

M. Alexandra Calle, Texas Tech University

Recent reports on the prevalence of non-O157 STEC in the U.S. show 19.4% in bovine feces and 58% pre-evisceration. Studies have shown using intervention measures can reduce the prevalence of STECs. Bovamine® is a patented and proven combination of *Lactobacillus acidophilus* NP51 and a lactic acid-utilizing bacterium *Propionibacterium freudenreichii* NP24. Researchers evaluated the effect of Bovamine® rumen culture feeding programs, low dose (LD), high dose (HD), and a combination of LD and HD on prevalence and concentration of *E.*

coli O157:H7 and non-O157 STEC. An additional objective of the research was to adapt a protocol for the detection of non-O157 O types from bovine fecal samples using the BAX® system.

The prevalence of *E. coli* O157:H7 isolated from manure samples was low at 8.2% - 13.3% and no significant difference was found between treatments in terms of prevalence. However, the quantitative amount of O157 in the controls was significantly higher than in the treated samples at 2.46 MPN/g.



In animals fed the high dose, the prevalence of O26 was significantly reduced from 61.7% in the controls to 36.3%, and the prevalence of O103 from 55.6% in the controls to 40%. Serotype O45 reduced from 67.9% in the controls to 45% in the animals given the high dose. The prevalence of the other STECS was less than 10% with no differences detected among treatments. The protocol adapted for preparation of samples to run BAX® to detect non-O157 O groups was found to be very efficient since fewer steps were required to obtain consistent results. This method is less time consuming than the recommended protocol for beef samples but yields identical results.

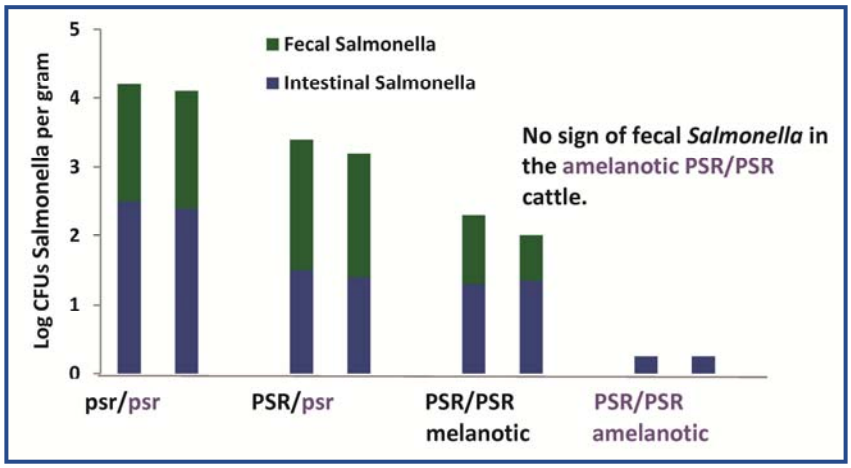
Identification of a bigenic genotype conferring resistance to Salmonella *and* E. coli *O157:H7 in beef cattle*

Steve A. Carlson, Iowa State University

Researchers tested the hypothesis that the combination of the non-black coat color genotype and a proprietary genotype confers *Salmonella* and *E. coli* O157:H7 resistance to beef cattle. The biological rationale for this approach is the fact that bacterial infection or colonization depends on some very specific interactions between the pathogen and the host. Single nucleotide polymorphisms (SNPs) have been implicated in numerous disease resistances.

Four experimental treatments were used to identify SNPs that produce alternate proteins conferring genetic resistance: 1) Screening *of Salmonella* resistance genotypes in beef cattle; 2) *In vitro* studies evaluating *Salmonella* resistance in blood obtained from cattle; 3) *In vivo* studies evaluating *Salmonella* resistance in cattle challenged with *Salmonella*; and 4) *In vivo* studies evaluating *E. coli* O157:H7 resistance in *PSR/PSR::mcr/mcr* cattle challenged with *E. coli* O157:H7.

The SNP relevant to the *Salmonella* infection process was designated the Phenotype *Salmonella* Resistant (*PSR*) SNP. The resistance was maximized in non-black cattle (genotype *mcr/mcr*) with the *PSR/PSR* genotype. The study results showed significant resistance to *Salmonella* infection in white blood cells obtained from *PSR/PSR::mcr/mcr* cattle. In addition, *PSR/PSR::mcr/mcr* cattle harbored 15- to 20-fold fewer *E. coli* O157:H7 when compared to other cattle.



The results of this study can be used to establish genetic lines of beef cattle that exhibit remarkable and significant natural resistance to: (a) clinical and subclinical salmonellosis; (b) lymph node contamination by *Salmonella*; and (c) intestinal colonization by *E. coli* O157:H7.

An outcomes model to evaluate risks and benefits of Escherichia coli *vaccination in beef cattle*

H. Scott Hurd, Iowa State University

Although the *E. coli* O157:H7 pathogen originates solely on the farm and lives within the food animal, the percentage of positive farms is relatively low. The post-harvest methods for reducing the prevalence of *E. coli* O157:H7 on beef are generally "maxed-out." The law of diminishing returns comes into play when considering further intervention strategies. Primarily, the industry is dealing with outlier events, which may culminate in an "event day." According to the Poisson distribution curve, an event day is likely the result of a high *E. coli* O157:H7 prevalence on the cattle entering the packing house coinciding with high *E. coli* O157:H7 prevalence on the carcass along with a high concentration. The event day is most likely to be avoided if the *E. coli* O157:H7 prevalence on the cattle entering the plant is reduced.

Using a stochastic simulation model, researchers evaluated the impact of *E. coli* O157:H7 vaccination on a reduction in the O157 prevalence in feedlot cattle as well as concentration in cattle feces and on outcomes at various points in the ground beef supply chain. Ultimately, this mathematical modeling method predicts the relationship between the reduction in O157:H7 shedding in cattle and a reduction in human illness.

The results show vaccination can have a significant benefit with respect to relevant outcomes such as: 1) the number of human O157 illnesses due to the consumption of ground beef, 2) the number of production lots with high O157 contamination levels, 3) the likelihood of detection by USDA Food Safety and Inspection Service testing, and 4) the probability of multiple illnesses due to ground beef servings from the same lot. The positive impact on public health is more a function of adoption of the use of the vaccine than the vaccine's efficacy. For example, if the vaccine is used to reduce the prevalence of *E. coli*-shedding cattle by 80% and if all U.S. steers and heifers were vaccinated, the expected number of human illnesses from ground beef-associated O157 would be reduced by almost 60%. If the vaccine is 60% or 40% effective, the illness rate would be reduced to about 45% or 40%, respectively.

The results of this study show that a reduction in the number of shedding animals and a reduced concentration of *E. coli* on carcasses can combine to reduce human illnesses and cost to beef packers by reducing the frequency and magnitude of "event days."

Molecular mechanisms of chitosan microparticles to reduce Escherichia coli *O157:H7 shedding in cattle*

K.C. Jeong, University of Florida

Excessive *E. coli* shedding by cattle at the end-point of pre-harvest and during lairage to the packing facility may challenge the post-harvest interventions currently in place. Chitosan has been used to make chitosan microparticles (CM) and has been an effective oral delivery agent of drugs and vaccines to the intestinal tract. To evaluate the influence of chitosan-supplemented feeding on *E. coli* O157 shedding, researches conducted a randomized, controlled, crossover study using seven weaned Holstein bull calves. Shedding was monitored by testing feces and the recto-anal junction.

Results of the study show that CM has a profound effect at reducing colonized *E. coli* O157:H7 in cattle. Not only did CM feeding significantly shorten the duration of *E. coli* O157:H7 shedding from 13.8 days to 3.8 days, it also significantly reduced the total number of *E. coli* O157:H7 in cattle. The pathogen was completely removed from the intestinal tracts of 60% of the cattle.

Duration of Shedding (days)				
Animal #	- CM feeding		+ CM feeding	
	Fecal	Swab	Fecal	Swab
12	15	13	11	14
13	12	12	1	0
14	15	15	0	15
15	13	14	5	8
17	11	13	0	0
18	14	15	2	0
22	17	7	8	8
Shedding ± SE	13.8 ± 2.0	12.7 ± 2.7	3.85 ± 4.2	2

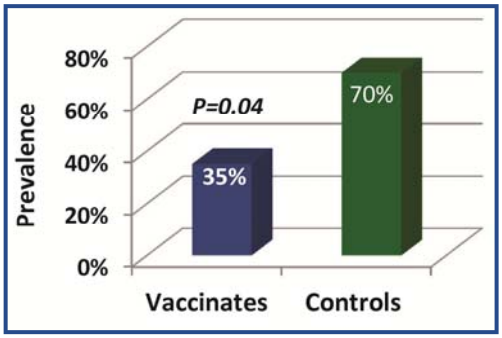
Direct ionic interactions between CM and STEC O157 are probably a key mechanism by which CM may interfere with colonization of STEC O157 and scrubbing off colonized bacteria on the colonization sites. In addition, CM binds to non-O157 *E. coli* and *Salmonella*, suggesting that CM can be effective against a broad spectrum of pathogens to reduce prevalence in cattle.

Commercial evaluation of a 3-dose regimen of an SRP-containing Escherichia coli *bacterial extract vaccine*

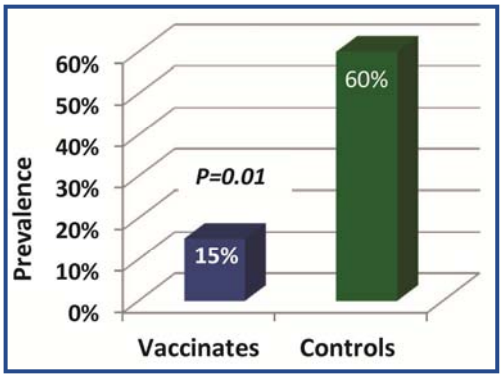
Guy H. Loneragan, Texas Tech University

The objective of this study was to characterize the efficacy of a 3-dose regimen of a vaccine containing siderophore receptors and porin proteins (SRP®) as an aid in the control of *E. coli* O157 and non-O157 serogroups and its impact on animal health and performance. Included in the study after the elimination of two feedlots were 6,803 animals in 62 pens (31 replicates of 2 pens each) in six feedlots which were assigned to either the SRP® vaccinate cohort or the control cohort which was routine management.

E. coli O157 was 2 and 4 times more likely to be detected in any swab (70% vs 35%; *P*=0.04)



or a random pool of 5 swabs (60 vs 15%; *P*=0.01) of control cattle relative to vaccinates, respectively.



For the feedlots that supplied most of the data, vaccine efficacy was 56%. For *E. coli* O157, a treatment*time interaction was detected with more than 60% efficacy in May/June and July while no vaccine effect was observed in August or September/October. Although the study was not designed to control non-O157 serotypes, evidence supporting a protective effect against O121, O111, and O45 was detected. However, a 23% increase in the gene encoding O26 was detected among vaccinates. The researchers advise cautious interpretation of the non-O157 serogroup results. Non-O157 serogroups are not as straightforward as O157 and, in fact the majority may not actually be STEC. Approximately 80% of fecal O26 are *stx* negative. No effect of the vaccine regimen was detected on animal health, performance, or carcass characteristics.

The treatment effect varied across a completely confounded source/feedlot variable. Within this feedlot, meaningful opportunities for improvement in drainage and pen maintenance were evident. The researchers suggest this highlights the need to consider best-practice prerequisites prior to implementing pre-harvest interventions.

Applicability of a multiplex PCR to detect the "top seven" shiga toxin-producing Escherichia coli *in cattle feces*

T. G. Nagaraja, Kansas State University

Shiga toxin-producing *E. coli* (STEC) are major foodborne pathogens and cattle are considered to be a primary reservoir. The STEC reside in the hindgut and are shed in the feces, which serve as a major source of food and water contamination for human infections. Among STEC, the O157 serogroup has long been recognized as a major pathogen. Recently, six additional O groups, O26, O45, O103, O111, O121, and O145, were classified as adulterants by FSIS and are considered to be of growing public health concern. According to the CDC, these six O groups are the cause of 71% of non-O157 infections. Methodologies for detection and isolation of non-O157 STEC in cattle feces have not been established. Researchers designed an 11-gene multiplex PCR (mPCR), based on genes that code for serogroup-specific O-antigens and four major virulence factors (intimin, enterohemorrhagic

hemolysin, and Shiga toxins 1 and 2), to identify the "top seven" (O157, O26, O45, O103, O111, O121, and O145) STEC.

The applicability of the assay to detect STEC in fecal samples (n=50), before and after enrichment, was evaluated by comparing with culture-based methods for O26, O111, and O157. The mPCR assay of 50 fecal samples showed seven positive (14%) before enrichment and 23 positive (46%) after enrichment for one or more of the seven O-groups. Overall, 17 O157 isolates from 17 fecal samples and 27 non-O157 isolates from 19 fecal samples (4 for O26, 3 for O45 and 20 for O103) were obtained by culture-based methods. Interestingly, none of the 27 non-O157 isolates possessed the *stx* genes, suggesting the prevalence of Shiga toxin-negative non-O157 serogroups in cattle feces.

The major application for this technology is to identify putative colonies of STEC obtained by culture-based methods. Further testing is required to determine the applicability of the assay to detect the "top seven" STEC in cattle feces. The usefulness of the assay is limited in that the detection of virulence genes does not necessarily associate those genes with the prevalent serogroups in the sample.

Evaluation of a commercially-available vaccine on preventing uptake of Salmonella *by the non-mesenteric lymph nodes of experimentally-infected dairy cattle*

Tom S. Edrington, USDA, Agricultural Research Service

Recent research suggests that non-mesenteric lymph nodes in cattle may be a significant source of the *Salmonella* contaminating ground beef. The objective of this research was to determine whether a commercially available *Salmonella* vaccine protects calves from lymph node colonization following significant oral challenge with two strains of *Salmonella* frequently isolated from dairy cattle, *Salmonella* Newport and Montevideo.

The first experiment included four groups of eight calves each. Two different strains of *Salmonella* were tested with a treatment group and a control group for each strain, *Salmonella* Newport and *Salmonella* Montevideo. At the end of the two different treatment periods (14 days and 21 days post inoculation), calves were euthanized and necropsied. The following lymph nodes were collected and cultured for the challenge strains of *Salmonella*: subiliac (left and right), popliteal (left and right), retropharangeal, superficial cervical (left and right), and mesenteric (ileo-cecal).
At the end of the experiment, very few lymph nodes contained quantifiable populations of *Salmonella*. In the group on the 21-day treatment period for *Salmonella* Newport, a tendency for the control-Newport calves to shed lower concentrations of *Salmonella* compared to the vaccine-Newport animals was seen.

Prevalence and characterization of Salmonella *recovered from lymph nodes and feces of cattle at harvest in Mexican slaughter facilities*

Sara E. Gragg, Texas Tech University

Previous research detected the prevalence of *Salmonella* in the subiliac lymph nodes of feedlot cattle and cull cattle at 15.5% and 1.8% respectively. It is not yet known how *Salmonella* reaches the lymph nodes (transdermal and/or gastrointestinal) or if other lymph nodes are contaminated. Researchers hypothesized that certain *Salmonella* serotypes have adapted to survive within lymph nodes. An analysis of their genetic fingerprints could explain how these serotypes disseminate throughout the lymphatic system and if similar serotypes reside in the various types of lymph nodes and feces. This study was conducted to quantify the prevalence of *Salmonella* in the feces and within the lymph nodes (subiliac, mediastinal, mesenteric and mandibular) of cattle presented for harvest in slaughter facilities throughout Mexico. A second objective was the characterization of a subset of recovered Mexican *Salmonella* isolates by Pulsed-Field Gel Electrophoresis (PFGE) to evaluate similarity across various lymph nodes within animals.

Results of the PFGE indicate that certain *Salmonella* fingerprints are more likely to colonize specific lymph nodes suggesting various routes of entry into the body. For example, the presence of the same *Salmonella* fingerprint in the mediastinal lymph node of different

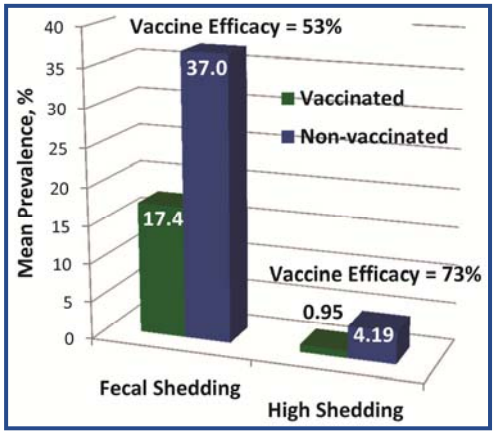
animals suggests the ability of these isolates to enter the body by the same route. Trends observed in the prevalence of *Salmonella* colonization of certain types of lymph nodes indicate a potential for *Salmonella* to enter the food supply. These research results highlight the need to evaluate factors that may mitigate contamination such as preventing lymph node contamination from occurring, removing lymph nodes from trim, or downstream lethality treatment of beef products.

Efficacy of the SRP® vaccine and/or low-dose Bovamine® against fecal shedding of E. coli *O157:H7 in a randomized field trial of commercial feedlot cattle*

David G. Renter, Kansas State University

E. coli O157:H7 inhabits the hindgut of cattle and is commonly shed in the feces, though a high level of variability exists. Less than 5% of animals shed at high levels (>10^4 cfu/g). Previous research shows feeding distiller's grains (DG) can increase fecal shedding of *E. coli* O157:H7 in cattle. This study was designed to determine the efficacy of a protein-based vaccine containing siderophore receptors and porin (SRP®) and/or a direct-fed microbial (DFM) against fecal shedding of *E. coli* O157:H7 in pens of commercial feedlot cattle fed a corn-based diet with >/=25% DG (dry matter basis) during the summer. The O157:H7 SRP® vaccine inhibits iron uptake by the bacteria while Bovamine® direct-fed microbial has probiotic effects. Both are commercially available in the United States.

The study design included two pen-level measures of fecal shedding: overall fecal prevalence of O157:H7 and the prevalence of high-shedding animals. Of all treatment protocols, only the two-dose SRP® significantly reduced overall fecal prevalence and the percentage of high shedders, with no significant interactions between treatment and time of sampling.



The administration of the DFM had no significant effect on the prevalence of fecal O157:H7 or the prevalence of high shedders. With > 50% reduction of *E. coli* O157:H7 shedding and > 75% reduction in high shedders, this two-dose regimen of the *E. coli* SRP® vaccine appears to be an efficacious pre-harvest intervention in a commercial feedlot setting.

Post-Harvest Safety Research

Analysis of E. coli *O157:H7 strains isolated from raw beef contamination events*

Terry Arthur, U.S. Meat Animal Research Center

Contamination of beef trim in commercial establishments can usually be described as occurring at a very low baseline level. However, spurious peaks in contamination rates occur where multiple positive lots are clustered in a short time frame, often across multiple product types and multiple production days. These peaks have been referred to as contamination "events," and result in large amounts of product diverted to a cooked process or destroyed. Frequently a cause/source is not identified, and the contamination will be resolved without visible corrective action being taken.

To gain insight into the cause of contamination events, researchers employed molecular typing of the *E. coli* O157:H7 isolates collected from events. By typing organisms from multiple lots and time points within an event, and across multiple events, information was available to determine if the contamination derived from a single point source or from multiple sources as would be expected if the incoming load were exceeding the capacity of in-plant interventions. It also allowed for tentative determinations regarding where in the process (slaughter floor vs. fab) contamination is occurring and if particular strains are more commonly associated with events.

The findings of this study indicate that most contamination events in raw beef products consist of a singular dominant O157 strain type. In these cases, the dominant strains were found across multiple product types and separated by substantial spans of time. This would be in disagreement with the current model of beef contamination, which states that the finished

product contamination occurs when incoming load (hide and feces carriage of the pathogen) exceeds the capacity of the in-plant interventions to remove carcass contamination. The data obtained in this project lead to the conclusion that contamination events may be the result of contamination occurring after carcasses exit the kill floor. This information should aid the industry in finding solutions to mitigate contamination events.

Escherichia coli *O157:H7 and non-O157 STEC survival and reduction on intact beef subprimal and non-intact beef trim and steaks by intervention and cooking processes*

W. Evan Chaney, Texas Tech University

The USDA-FSIS announcement declaring an additional six non-O157 shiga toxin-producing *E. coli* (STEC) serotypes in ground beef as adulterants warrants a reassessment and validation of current interventions and cooking processes to evaluate their efficacy against these organisms. Researchers conducted three independent studies to 1) evaluate reduction efficacy of 4.4% and 5% lactic acid, 2% hypobromous acid, and 2% peroxyacetic acid on beef subprimals or beef trim by spray or dip intervention and 2) evaluate cooking survival/inactivation and STEC survival in needle-tenderized steaks.

In study one, both the lactic acid and water spray significantly reduced *E. coli* STEC on brisket samples and no significant difference was observed between the two interventions. In study 2, the lactic acid dip was the only treatment to significantly reduce all STECs on beef trim and in ground beef. In study three, lactic acid and hypobromous acid reduced *E. coli* STEC by 2.30 and 1.00 $\log_{10}$ /50cm^2, respectively, on strip loin samples after 14 days. Cooking to 50°C or 70°C reduced *E. coli* O157:H7 and non-O157 STECs in study three; however, needle-tenderized cooked strip loins remained positive for STEC serotypes despite the inoculum concentration. *E. coli* O103 was the most prevalent serotype recovered from the cooked steaks.

Data from these study results indicate the potential of current interventions and cooking processes for effectively reducing the prevalence of non-O157 STECs on beef. However, the data suggests that heat resistance among the STEC serotypes may vary.

Electrolyzed oxidizing water (EO water) and levulinic acid plus sodium dodecyl sulfate (LA -SDS) - potential interventions to reduce Escherichia coli *O157:H7 on spiked beef trims*

Ravirajsinh Jadeja, University of Georgia

Not only is *Escherichia coli* O157:H7 the most frequently isolated STEC in North America, it is also the STEC most often associated with hemolytic-uremic syndrome (HUS). As fecal contamination of beef products can be a source of *E. coli* O157:H7, it is important to evaluate new intervention steps that have the potential to reduce the prevalence of *E. coli* O157:H7. This study evaluated the efficacy of two potential interventions, electrolyzed oxidizing water (EO water) and levulinic acid plus sodium dodecyl sulfate (LA-SDS), on artificially contaminated beef trim. LA-SDS has been approved as GRAS by the FDA.

Both interventions were found to effectively reduce *E. coli* O157:H7 from beef trim. After five minutes, the EO water was less effective than the LA-SDS but yielded greater reduction during the first sixty seconds. Depending on the availability of time and the bacterial load, either intervention could be used to reduce *E. coli* O157:H7 contamination of beef trim.

Efficacy of EO water and LA-SDS treatments to reduce *E. coli* O157:H7 on beef trim

Treatment	Time (min)	Effective reduction* (log CFU/cm$^{2)}$	Pathogen survival in treatment solution**
LA-SDS	1	0.3	ND
	2	1.9	ND
	3	<5.1	ND
	5	<5.1	ND
EO water	1	0.7	ND
	2	0.9	ND
	3	1.0	ND
	5	1.8	ND

*Effective reduction = Treatment (EO water or LA-SDS) - Control (deionized water)
**ND - No detection after 24h enrichment

Molecular serotyping of non-O157 shiga toxin-producing Escherichia coli *by interrogation of single nucleotide polymorphisms in* gnd

J.R. Elder, Texas Tech University

In general, current assays to screen beef trim for *E. coli* O157:H7 and non-O157 serotypes of clinical importance, including O23, O45, O103, O111, O121, and O145, begin with an initial screen for genes encoding the Shiga toxins and other key virulence factors carried by pathogenic STEC. This test is followed by PCR reactions to detect targets specific to the O serogroups of concern. The initial screening is useful to identify samples devoid of pathogenic STEC and, therefore, acceptable as product. The purpose of this study was to develop a rapid and high-throughput molecular serotyping method to group Shiga toxin-producing *E. coli* (STEC) isolates into the seven serotypes identified as adulterants in raw ground beef by interrogating single nucleotide polymorphisms (SNPs) in *gnd*, which encodes 6-phosphogluconate dehydrogenase.

Researchers were able to identify serotype-specific allelic types for each of the seven STEC serotypes declared as adulterants, which allowed the differentiation of the "big six" non-O157 serotypes and O157 from other STEC serotypes. One isolate of serotype O20 was misclassified as O111 and an isolate belonging to serotype O26 and another of serotype O103 shared allelic types with STEC not considered adulterants.

The serotype-specific allelic types identified for O157 and the "big six" were confirmed with DNA sequence data. Using this molecular serotyping method, ground beef samples can be rapidly screened for *E. coli* O157:H7 and the six non-O157 serotypes identified as adulterants by FSIS.

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