Project Title: Identification and Evaluation of Cattle Persistently Shedding vs. Cattle Non-Persistently Shedding *Escherichia coli* O157:H7

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Background

It is generally accepted that cattle are a major reservoir of *E. coli* O157:H7, a zoonotic pathogen that has become a significant cause of human hemorrhagic colitis and hemolytic uremic syndrome (Mead et al., 1999). It is poorly understood if animals become colonized with *E. coli* O157:H7, or if they transiently shed the organism after ingestion of environmental contamination. Khaitsa et al. (2003) reported that fecal shedding of *E. coli* O157:H7 from feedlot steers ranged from 1% to 80% over a 136 day sampling period. Based on that finding, it is reasonable to believe that at least some animals transiently shed *E. coli* O157:H7 at some point in their lives. Besser et al. (2001) showed that when calves are orally infected with *E. coli* O157:H7, they shed the organism consistently for 70 d and intermittently thereafter. Furthermore, *E. coli* O157:H7 could be cultured from feces, colonic and cecal contents 118 d after infection. Several other studies have shown evidence of persistence of *E. coli* O157:H7 infection in individual cattle, providing support that at least some animals can become colonized with *E. coli* O157:H7 (Besser et al., 2001; Brown et al., 1997 and Grauke et al., 2002). It seems reasonable to assume that infected animals can disseminate the organism into the environment, subsequently contaminating pen mates and facilities.

There has been, to a degree, varying evidence of the primary site of *E. coli* O157:H7 colonization in infected animals. Grauke et al. (2002) found the colon to show the most persistence and proliferation of *E. coli* O157:H7. More specifically, Naylor et al. (2003) reported that the recto-anal junction of the gastrointestinal (G.I.) tract was the primary site of *E. coli* O157:H7 colonization. These findings contradicted those who found the rumen to be the major site of propagation (Brown et al 1997; Laven et al., 2003; Rasmussen et al., 1993 and Tkalcic et al., 2000). Additionally, Besser et al. (2001) reported that cecal contents also were contaminated with *E. coli* O157:H7. *E. coli* also has been shown to have the ability to translocate to different segments of the mesenteric lymph node complex in laboratory mouse models (Gautreaux et al., 1994).

This study was designed to identify those cattle that are persistently colonized with *E. coli* O157:H7 and to determine the site of colonization. In addition, animals found to be persistently colonized with *E. coli* O157:H7 will be compared, both microbiologically and physiologically to animals that intermittently shed and never shed the organism. Results will be used to design pre-harvest *E. coli* O157:H7 interventions and application parameters to reduce *E. coli* O157:H7 numbers and prevalence in slaughter - ready animals.

The stated objectives for this work were:

1. To identify cattle persistently shedding *E. coli* O157:H7.
2. To identify physiological and microbiological differences in the intestinal tract between persistently shedding cattle and controls (non-persistently shedding cattle).

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3. 3. To identify sites, areas or locations at which pre-harvest food safety interventions should be targeted to preclude persistent shedding in finishing cattle.

Methodology

Animals

Seven hundred eighty-eight Holstein steers were fed at a commercial feedlot in eastern Kansas. The steers were fed a high-concentrate finishing ration during the sampling period. The steers were housed in five pens with three on the pens sharing fence lines and the remaining two pens independently located in other areas of the feedlot. Microbial sampling occurred every 20 to 22 d during the final 120 d (June – October) of the finishing period. Every animal was sampled during the initial and first subsequent sample periods to establish each animal's *E. coli* O157:H7 shedding status. The next consecutive four sample periods only evaluated animals that were either consistently *E. coli* O157:H7 positive or *E. coli* O157:H7 negative during the prior sample periods. Furthermore, a few animals that varied in their *E. coli* O157:H7 shedding status during the first two sample periods were randomly selected and sampled again to verify their shedding status. Animals were removed from the sample population if ever they received any treatment for clinical illness.

Fecal sample collection

At every pre-determined collection period, steers were processed through conventional processing facilities to accommodate fecal sample collection. While animals were restrained, approximately 30 g of feces were aseptically obtained through rectal palpation utilizing a clean shoulder-length plastic glove. Feces were transferred to a pre-labeled Whirl-Pak bag (Nasco, Modesto, CA) and subsequently placed in a cooler with ice packs. Following completion of each entire sample collection, samples were transported to the Pathogen Reduction Laboratory, Center for Red Meat Safety at Colorado State University where they were stored at 4°C until microbiological analysis.

Fecal E. coli O157:H7 analysis

Fecal samples were individually weighed into 10 - g Filter-Pak bags (Nasco) with 90 ml of phosphate buffered tryptic soy broth (Becton, Dickinson and Company, Sparks, MD; TSB - P04) as described by Barkocy-Gallagher et al. (2005). Fecal slurries were homogenized by hand massaging and incubated for 2 h at room temperature (25 ± 2°C), followed immediately by 6 h incubation at 42°C. Following incubation, fecal slurries were stored at 4°C until they were subjected to immunomagnetic bead separation (IMS). IMS was conducted as described by Barkocy-Gallagher et al. (2002), and ultimately 50 µl were plated onto Rainbow agar (Biolog, Inc, Hayward, CA) supplemented with 10 mg/L of novobiocin (Sigma-Aldrich, St. Louis, MO) and 0.8 mg/L of potassium tellurite (Sigma) and Soribitol MaConkeys agar (Becton) supplement with 20 mg/L of novobiocin and 2.5 mg/L of potassium tellurite (mSMAC). Rainbow plates were incubated for 24 ± 2 h at 37°C and mSMAC plates were incubated for 36 ± 2 h at 37°C. After incubation, up to 3 colonies displaying *E. coli* O157:H7 morphology were selected from each media and initially screened for the O157 antigen with RIM *E. coli* O157:H7 latex agglutination tests (Remel, Lenexa, KS). All agglutination positive colonies were cultured into 5 ml of TSB and incubated for 24 ± 2 h at 37°C and streaked onto mSMAC (36 ± 2 h; 37°C) for purity. Colonies were confirmed to be *E. coli* O157:H7 by a multiplex PCR assay as previously described by Hu et al. (1999).

PCR was performed in 96-well format with a volume of 25 µl containing 3.18 µl of a primer solution containing rfb (encodes the O157 antigen), flich7, (encodes the H7 antigen) eaeA (encodes intimin), stx-I (encodes Shiga toxin I) and stx-II (encodes Shiga toxin II), 12.5 µl Go Taq Green (Promega, Madison, WI), 8.08 µl of nuclease-free water and 1 – 2 µl of DNA template. Each PCR consisted of 20 cycles (2 min at 94°C; 30 s at 94°C; 1 min at 59°C and 1 min at 72°C) and 20 additional cycles with annealing temperature (7 min for 72°C) in a thermal cycler (GeneAmp 2720, Applied

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Biosystems, Foster City, CA). Every 96-well plate also included an *E. coli* O157:H7 isolate known to be positive for all five of the targeted genes as a positive control and a well containing only Go Taq Green, nuclease free water and primers as the negative control. PCR products were separated by electrophoresis in 2% agarose gels and visualized with UV illumination following staining with ethidium bromide. Isolates with a positive PCR reaction for rfb, flich7, eaeA, stx-I and stx-II were designated as *E. coli* O157:H7. All *E. coli* O157:H7 positive isolates were stored in 15% glycerol at –80oC for further characterization.

Because initial *E. coli* O157:H7 prevalence was believed to be low, and in order to save time and materials, samples from the first fecal collection were analyzed using the Pathatrix (Matrix MicroScience, Inc, Golden, CO) pooling method. After samples were weighed and individually enriched as described above, groups of 5 individual samples were created and recorded as a pooled sample. After pooled sample assignment, 50 ml was removed from each of the 5 individual fecal slurries and combined together into a Filter-Pak bag to ultimately create a fecal slurry consisting of 250 ml. The new pooled samples were placed in the Pathatrix's warming pots (preheated to 37°C). The Pathatrix apparatus was inserted into each pooled sample bag, and 50 µl of Matrix anti-O157 immunomagnetic beads was added to the connector tubing. The samples were recirculated for 30 min at 37°C, and then the beads were washed and directly plated onto mSMAC and nt-Rainbow agar plates. Plates were incubated as described previously. After incubation, up to 3 colonies displaying *E. coli* O157:H7 morphology were selected from each media and subjected to latex agglutination. Once a colony was determined to be O157 positive with latex agglutination, each of the 5 original samples that formed positive pooled sample were individually subjected to IMS as described above. The pooling method was abandoned after the first collection as *E. coli* O157:H7 prevalence was found to be at levels that eliminated feasibility.

Five-tube MPN assays (Food and Drug Administration) were used to enumerate *E. coli* O157:H7 levels from fecal samples of animals found to be shedding *E. coli* O157:H7 during the last sample collection. A 10 g sample (this was the amount remaining after IMS for several samples) was mixed with 90 ml of Butterfield's phosphate buffer and pummeled in a stomacher for 2 min. Three serial 1:10 dilutions were prepared from each sample in Lauryl tryptose broth (Becton; LTB) in a total of 15 tubes for each sample. Inoculated LTB tubes were incubated for 24 h at 37°C. After incubation, all turbid tubes were subjected to IMS and confirmed by multiplex PCR as previously described.

<u>Gastrointestinal tissue and content sample collection</u>
All animals identified as persistent shedders (PS) and non-shedders (NS), as well as a sub-sample of animals identified as transient shedders (TS), were harvested at a commercial harvesting facility in the upper Midwest. Full gut sets (esophagus, reticulum, rumen, omasum, abomasum, small intestine, large intestine, colon, bung and gall bladder) were collected from each animal and transported to a vacant area of the facility to allow for sample collection. Additionally, livers corresponding to the identified animals were examined for the presence of abscesses. Gut sets were laid out individually, on a concrete floor so that all sections to be sampled were exposed. Tissue and content samples (only tissue samples from the lymph nodes) were collected aseptically from the reticulum, rumen, omasum, abomasum, duodenum (proximal to the anterior side of the first loop), ileocecal valve, distal colon (~ 60 cm proximal to the anus), rectal-anal junction and two mesenteric lymph nodes (from a position ~ 30 cm proximal to the anterior root of the mesentery and the ileal cecal colic node) for microbiological analysis. Tissue samples were washed with sterile phosphate buffered saline with Tween 20 (PBS) to remove visible organic matter before placement into a Whirl-Pak bag. All microbial samples were placed in ice pack-filled coolers and shipped to Pathogen Reduction Laboratory, Center for Red Meat Safety at Colorado State University.

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Gastrointestinal content samples were processed as previously described. Ten gram aliquots of epithelial lining of each tissue was aseptically removed and placed into a Whirl-Pak bag containing 90 ml of TSB - P04. Lymph nodes were first, aseptically, trimmed of excess adipose tissue and had 10 g aliquots removed for *E. coli* O157:H7 analysis and placed into Whirl-Pak bags containing 90 ml of TSB –P04. All tissue samples were pummeled (IUL Instruments, Barcelona, Spain) for 2 min before incubation and ultimately processed as previously described.

Histologic sections of tissues (except from the lymph nodes) from the locations described above were fixed in 4% paraformaldehyde for 24 h. After fixation, excess paraformaldehyde was removed and samples were shipped to the Colorado State University Pathology Diagnostic Laboratory where they were evaluated histopathologically; paraffin embedded by automated tissue processor, sectioned at 5 micrometers on a microtome and stained with hematoxylin and eosin.

Pulsed-Field Gel Electrophoresis

PFGE typing of *E. coli* O157:H7 isolates was performed using the standardized Centers for Disease Control and Prevention PulseNet protocol (Centers for Disease Control and Prevention, 2001). Briefly, isolates were grown on tryptic soy agar (Becton; TSA) plates and incubated at 37°C for 18 hours. Bacterial cultures were imbedded in 1% agarose (SeaKem Gold Agarose, Cambrex Bio Science Rockland, Inc., Rockland, ME), lysed, washed, and digested with XbaI overnight at 37°C. Restricted agarose plugs were then placed into 1% agarose gels and electrophoresed on a CHEF Mapper XA (BioRad Laboratories, Hercules, CA) for 21 h with switch times of 2.16 s to 54.17s. XbaI digested *Salmonella* ser. Braenderup (H9812) DNA was used as a reference size standard (Hunter et al., 2005). Agarose gels were stained in ethidium bromide and resultant images were captured with a FOTO/Analyst Investigator System (FOTODYNE, Inc., Hartland, WI). PFGE patterns then were analyzed and compared using the Applied Maths Bionumerics (Applied Maths, Saint-Matins-Latem, Belgium) (v3.5) software package. Similarity clustering analyses were performed with Bionumerics software using the unweighted pairs group matching algorithm and the Dice correlation coefficient (Hunter et al., 2005).

Cell attachment assay

The attachment efficiency of up to four *E. coli* O157:H7 isolates selected to represent each common PFGE type was determined by performing attachment assays using the Caco-2 human intestinal epithelial cell line. Briefly, caco-2 cells were seeded into 24-well plates at a density of 5 x 104 cells/well in media without antibiotics and grown to confluency (approximately 48 h). *E. coli* O157:H7 overnight cultures were prepared by inoculating a single well-isolated colony into a 10 ml tube of TSB and incubating at 37ºC for 18 h without shaking. Overnight *E. coli* O157:H7 cultures (1 ml) were pelleted by centrifugation and re-constituted in 1 ml of phosphate buffered saline. Confluent Caco-2 monolayers were infected with approximately 2 x 107 *E. coli* O157:H7/well. After infection for 30 min at 37ºC, non-adherent bacteria were removed by washing three times with phosphate buffered saline (PBS). Caco-2 cells were lysed by addition of 0.5 ml of sterile ultrapure water, followed by vigorous pipetting and vortexing. Adherent *E. coli* O157:H7, along with overnight *E. coli* O157:H7 cultures, were enumerated by spread plating appropriate serial dilutions in duplicate onto TSA plates. TSA plates were incubated at 37oC for 24 h and resultant colony forming units were enumerated. The attachment efficiency of each *E. coli* O157:H7 isolate was determined as a percentage of the initial inoculum that was recovered as adherent *E. coli* O157:H7 cells.

Findings

For the purposes of this study, we chose to focus on isolates shown to carry all five genetic markers for molecular confirmation of *E. coli* O157:H7 (i.e. rfb, flich7, eaeA, stx-I and stx-II) using

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a previously described multiplex PCR assay (Hu et al., 1999). Specifically, persistent shedding (PS) were defined as animals for which an *E. coli* O157:H7 isolate was obtained that carried rfb, flich7, eaeA, stx-I and stx-II at each of the six collection periods. Overall, based on these selection criteria, eight (1.0%) of the 788 animals were classified as PS. Additionally, 11 (1.4%) animals were never found to shed an isolate carrying any of the *E. coli* O157:H7 genetic markers described above. The remaining 769 animals were classified as intermittent or transient shedding (TS) as these animals sporadically shed *E. coli* O157:H7 isolates meeting our criteria during the sampling period. The distribution of both PS and non-shedding (NS) was balanced over the five pens as each pen contained at least one animal of each *E. coli* O157:H7 shedding status.

Over the first two collection periods, animals were found to shed *E. coli* O157:H7 isolates with a variety of virulence genotypes (Table 1). Prevalence of these genotypes could not be determined after the first two collection periods as only animals that were initially deemed as PS or NS, along with a small random selection of TS animals, were sampled during subsequent collection periods. Over the first two collection periods, *E. coli* O157:H7 isolates possessing the rfb, flich7, eaeA, stx-I and stx-II genes were most frequently shed, as 39.8% and 33.6% of animals shed isolates with those genotypes for the first and second collection, respectively. Animals shedding *E. coli* O157:H7 with both stx I and stx II decreased by 3.5% between collection 1 and 2.

In general, *E. coli* O157:H7 with only stx I were more prevalent than *E. coli* O157:H7 carrying only stx II. However, *E. coli* O157:H7 with both Shiga toxin encoding genes were more frequently observed in the sample population. Contrary to our results, Cobbold and Desmarchelier (2001) reported that prevalence of *E. coli* isolates carrying only stx II was greater than prevalence of isolates carrying only stx I or the combination of both Shiga toxin genes in their sample population. The high percentage of *E. coli* O157:H7 carrying all five virulence genes is expected as the isolates were, genetically, very similar.

Fecal samples from animals that were classified as PS from the final collection period were enumerated with the MPN procedure. PS-7 was the only animal to shed a level (46 cells per 10 g of feces) of *E. coli* O157:H7 that was detectable by our MPN enumeration scheme. The remaining seven animals shed levels of *E. coli* O157:H7 below the detection limit of 1.8 cells per 10 g of feces. Enumeration of *E. coli* O157:H7 during the final collection period was limited by an insufficient amount of feces to allocate 10 g of fecal material to both IMS and MPN analysis.

Escherichia coli O157:H7 was detected on tissue and in content samples of both upper and lower sites of the gastrointestinal tract (Table 2). Persistent shedding animals were more likely to have lower G.I. tissue and content samples that were positive for *E. coli* O157:H7 as compared to TS animals. Only PS-7 had an upper G.I. tissue (omasum) samples and one anterior root lymph node sample that were positive for *E. coli* O157:H7. Interestingly, PS-7 was the only animal shedding levels of *E. coli* O157:H7 that were detectable by MPN analysis. There were two upper G.I. tissue (reticulum and omasum) samples positive for *E. coli* O157:H7 in three TS animals (TS-2, TS-4 and TS-16). The remaining positive TS positive tissue and content samples were collected from the lower G.I. tract.

These results were consistent with previous studies that identified the lower G.I. tract as the primary site for *E. coli* O157:H7 colonization (Gautreaux et al., 1994; Grauke et al., 2002; Naylor et al., 2003). No gall bladder samples were positive for *E. coli* O157:H7 regardless of previous shedding status (Table 2). Additionally, all pH measurements obtained from content samples were similar across animals representing PS, TS and NS groups (data not shown).

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It previously was reported that cattle colonized with *E. coli* O157:H7 do not express any pathological symptoms (Besser et al., 1997). Histological samples of tissues from this study resulted in no notable differences between tissues of PS vs. NS animals (Figure 5, 6 and 7). All tissue samples from PS and NS animals were characterized as normal or having minor lesions commonly found in G.I. tissues obtained from fed cattle. Our results were consistent with Cray and Moon (1995) who reported no significant lesions in tissues obtained from cattle clinically infected with *E. coli* O157:H7. In addition, livers from animals representing PS, TS and NS groups did not show visible surface lesions (data not shown).

At least one isolate was selected to represent each collection period for PS animals (n = 8), and a random selection of isolates was obtained from TS animals (n = 16) were characterized by PFGE molecular subtyping. As illustrated in Table 3, the 138 *E. coli* O157:H7 isolates analyzed resulted in 34 different PFGE molecular subtypes (Figure 4). Subtype F was the most common, accounting for 50.7% of the isolates characterized. Subtype F was similarly distributed among PS and TS animals, as well as at each collection period. While 24 of 34 unique PFGE types were observed among *E. coli* O157:H7 isolates from PS animals, 19 PFGE subtypes only were observed among isolates obtained from PS animals. Unexpectedly, there were only five PFGE subtypes that overlapped between PS and TS animals. All eight PS animals were found to shed subtype F at least twice over the entire collection period and for at least two consecutive collection periods. However, only one PS animal (PS-4) shed subtype F over the entire collection period (Table 4). During ante-mortem sample collection, PS animals shed 17 subtypes in addition to subtype F, with 12 of those subtypes only differing from F by three or fewer bands. Tenover et al. (1995) characterized differences of less than three bands to the reference strain to be closely related. The remaining six subtypes differed by more than 4 bands and were not closely related to PFGE type F. The remaining six PFGE subtypes were obtained from post mortem collection of PS animals. Three of these six subtypes were genetically distinct from subtype F, as they differed by more than three bands. PS animals were more likely to shed *E. coli* O157:H7 isolates with varying PFGE subtypes than were TS animals. TS animals were more likely to shed *E. coli* O157:H7 isolates that were genetically similar to the *E. coli* O157:H7 strain that was most prevalent in the cattle sample population (subtype F).

Post-mortem collection yielded ten unique PFGE subtypes that were not present during ante-mortem collection, with four subtypes genetically similar to subtype F. It is possible that animals may have been contaminated with these PFGE subtypes during transit or during holding at the processing plant (Childs et al., 2006).

The attachment efficiency of up to four *E. coli* O157:H7 isolates selected to represent the five most common PFGE subtypes (i.e. subtypes F, H, I, G and J) was determined using the Caco-2 human intestinal epithelial cell line. There were not a sufficient amount of repetitions to produce statistically significant results, however graphical representation of the data are illustrated in Figure 1. Although no statistically significant differences in attachment efficiency were observed, *E. coli* O157:H7 isolates representing the most common PFGE subtype, subtype F, demonstrated the highest attachment efficiency. Interestingly, the two PFGE subtypes with the lowest average attachment efficiency represented subtypes that were unique to only two PS animals and never were isolated during ante-mortem sampling. Further work is needed to more completely characterize the attachment efficiency of *E. coli* O157:H7 isolates representing the most common PFGE subtypes observed here, as well as isolates belonging to more genetically diverse but less commonly observed PFGE subtypes. However, preliminary results suggest that *E. coli* O157:H7 molecular subtypes associated with enhanced attachment efficiency may be more likely to colonize the intestinal tract of healthy cattle.

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Results from this study support that, within a population of healthy feedlot cattle, (i) a small subpopulation of animals appear to be persistently colonized by *E. coli* O157:H7, (ii) PS and TS animals did not show physiological differences based on our observations of animal health status and post-mortem histopathology, (iii) PS animals do not necessarily shed high levels of *E. coli* O157:H7, (iv) PS and TS animals may be colonized by a predominant *E. coli* O157:H7 molecular subtype along with multiple other distinct but closely-related molecular subtypes, (v) PS and TS animals may be more likely to be colonized by *E. coli* O157:H7 molecular subtypes that demonstrate a greater ability to attach to intestinal epithelial cells. The underlying factors associated with persistent colonization of healthy cattle by *E. coli* O157:H7 are likely multi-factorial and remain to be fully elucidated.

Implications

We found that within a population of healthy feedlot cattle, there were a small percentage of animals persistently colonized with *Escherichia coli* O157:H7. The animals identified as persistent shedders did not exhibit any physiological ante-mortem symptoms or any post-mortem histopathology gastrointestinal tissue differences when compared to animals that never shed *E. coli* O157:H7. Molecular characterization of *E. coli* O157:H7 isolates demonstrated that a closely related group of *E. coli* O157:H7 molecular subtypes colonized the gastrointestinal tract of persistent shedders. Molecular subtypes commonly isolated from persistently colonized cattle displayed an increased ability to attach to intestinal epithelial cells than *E. coli* O157:H7 isolates less commonly found in the sample population. The underlying factors associated with persistent colonization of healthy cattle by *E. coli* O157:H7 are likely multi-factorial and remain to be fully elucidated.

Table 1. Distribution of *E. coli* O157:H7 virulence genes [*rfb* (encodes the O157 antigen), *flic*$_{h7}$ (encodes the H7 antigen), *eaeA* (encodes intimin), *stx*-I (encodes Shiga toxin I) and *stx*-II (encodes Shiga toxin II)] among animals collected during the first two ante-mortem collections periods.

	Collection 1		Collection 2	
Genotype	Animals Shedding	% of Animals	Animals Shedding	% of Animals
Flic$_{h7}$, *stx* II, *eaeA*, *rfb*, *stx* I	314	39.8	265	33.6
rfb	6	0.7	95	12.1
rfb, *stx* I	32	4.1	156	19.8
Stx II, *rfb*, *stx* I	3	0.3	0	0.0
Stx I	3	0.3	7	0.9
Stx II	1	0.1	0	0.0
Stx II, *eaeA*, *rfb*, *stx* I	9	1.1	42	5.3
Flic$_{h7}$, *stx* II, *rfb*, *stx* I	1	0.1	0	0.0
Flic$_{h7}$, *stx* II, *eaeA*, *stx* I	14	1.8	7	0.9
Total	383	48.6	572	
Verotoxin Isolates				72.6
Stx I	36	4.6	163	20.7
Stx II	1	0.1	0	0.0
Stx I and *stx* II	341	43.3	314	39.8

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Table 2. Distribution of post-mortem gastrointestinal tissues and contents positive for E. coli O157:H7 among persistent and transient shedders.

	Persistent Shedder		Transient Shedder	
Site	Tissue	Content	Tissue	Content
Reticulum	-	-	1	-
Omasum	1	-	1	-
Rumen	-	-	-	-
Abomasum	-	-	-	-
Duodenum	-	-	-	-
Ilieal-Cecal Junction	1	1	1	-
Colon	-	1	-	-
Rectal-Anal Junction	1	1	1	2
Ilieal Cecal Colic Node	-	-	-	-
Anterior Root Node	1	-	-	-
Gall Bladder	-	-	-	-

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Table 3. PFGE subtypes, number of bands differing from subtype F and distribution of isolates obtained from ante-mortem and (post-mortem) collection and number of animals shedding for each PFGE type for both persistent and transient shedders.

		Persistent Shedder		Transient Shedder	
PFGE Type	Band Difference from Type F	Isolates	Animals	Isolates	Animals
A	3	4(0)	3	0(0)	0
B	4	0(0)	0	0(1)	1
C	4	1(0)	1	0(0)	0
D	2	0(3)	1	0(0)	0
E	1	0(2)	1	1(0)	1
F	-	40(0)	8	24(6)	11
G	2	0(5)	2	0(0)	0
H	3	2(0)	2	3(0)	1
I	2	8(0)	3	2(0)	1
J	4	0(4)	2	0(0)	0
K	1	1(0)	1	0(0)	0
L	1	1(0)	1	0(0)	0
M	1	0(0)	0	2(0)	1
N	6	1(0)	1	1(0)	1
O	4	0(0)	0	0(1)	1
P	1	3(0)	1	0(0)	0
Q	2	0(0)	0	1(0)	1
R	3	1(0)	1	0(0)	0
S	1	0(0)	0	3(0)	1
T	1	1(0)	1	0(0)	0
U	1	1(0)	1	0(0)	0
V	2	0(0)	0	1(0)	1
W	1	1(0)	1	0(0)	0
X	3	0(0)	0	0(1)	1
Y	1	0(0)	0	1(0)	1
Z	4	1(0)	1	0(0)	0
AA	1	1(0)	1	0(0)	0
AB	>6	2(0)	1	0(0)	0
AC	>6	2(0)	1	0(0)	0
AD	>6	1(0)	1	0(0)	0
AE	>6	0(1)	1	0(0)	0
AF	>6	0(0)	0	0(1)	1
AG	3	0(1)	1	0(0)	0
AH	>6	0(0)	0	0(1)	1
Total		72(16)		39(11)	

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Table 4. Distribution of PFGE subtypes for each persistent shedder during each ante-mortem collection period.

	Collection					
Animal	1	2	3	4	5	6
PS-1	AA	F, AC, AD	F	F, I	AC	I
PS-2	F	F, I	F	I	I	I
PS-3	F	F	C	T, K	Z	N
PS-4	F	F	F, U	F	F	F
PS-5	F	F	F	H	I	A
PS-6	F	F	F	L	R	H
PS-7	F	F, W	F	F, A	P	F
PS-8	F	F	F	A	F	AB

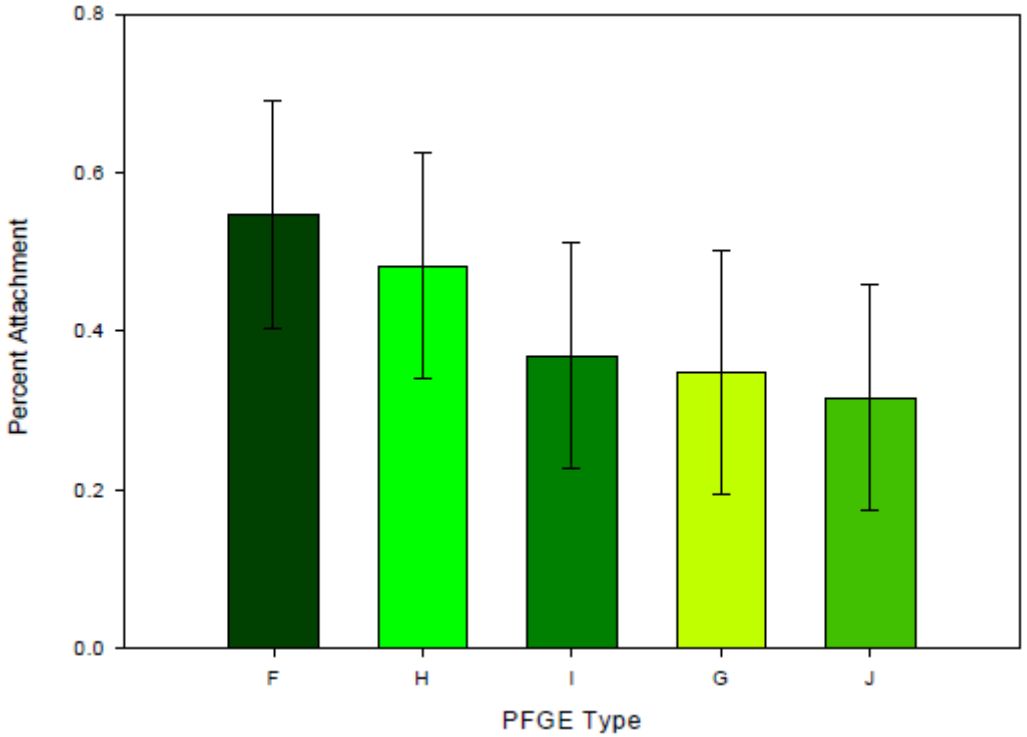

Figure 1. Percent attachment of five PFGE (F, H, I, G, and J) subtypes of *E. coli* O157:H7.

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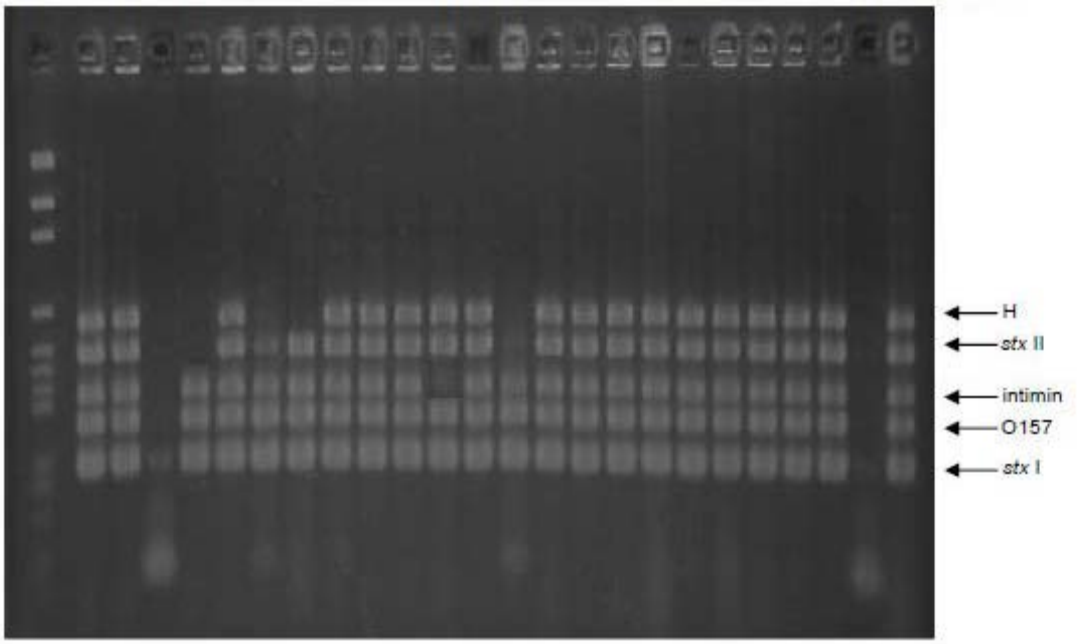
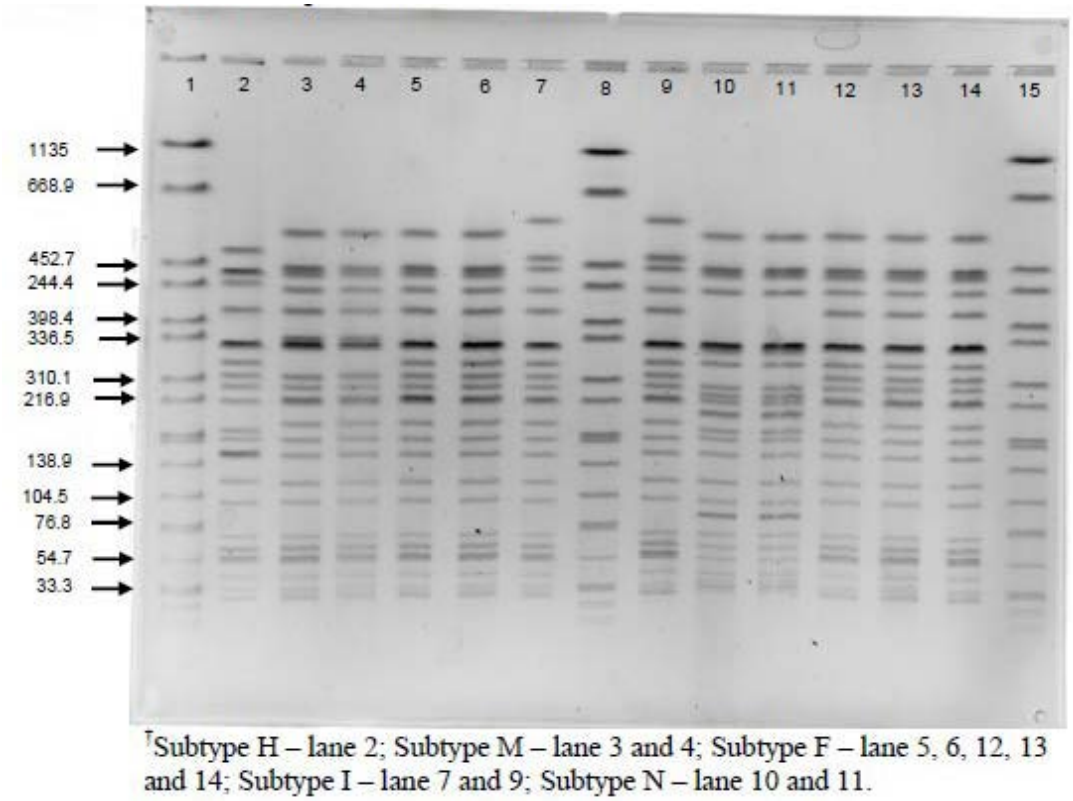


Figure 2. Composite multiplex PCR gel displaying isolates with differing genotypes.



[Subtype H – lane 2; Subtype M – lane 3 and 4; Subtype F – lane 5, 6, 12, 13 and 14; Subtype I – lane 7 and 9; Subtype N – lane 10 and 11.

Figure 3. PFGE image showing *E. coli* O157:H7 isolates (lanes 2-7 and 9-14) and the standard S. Braenerup H9812: lanes, 1, 8, and 15) restricted with XbaI and approcimate kilobase values for band positions of the standard.

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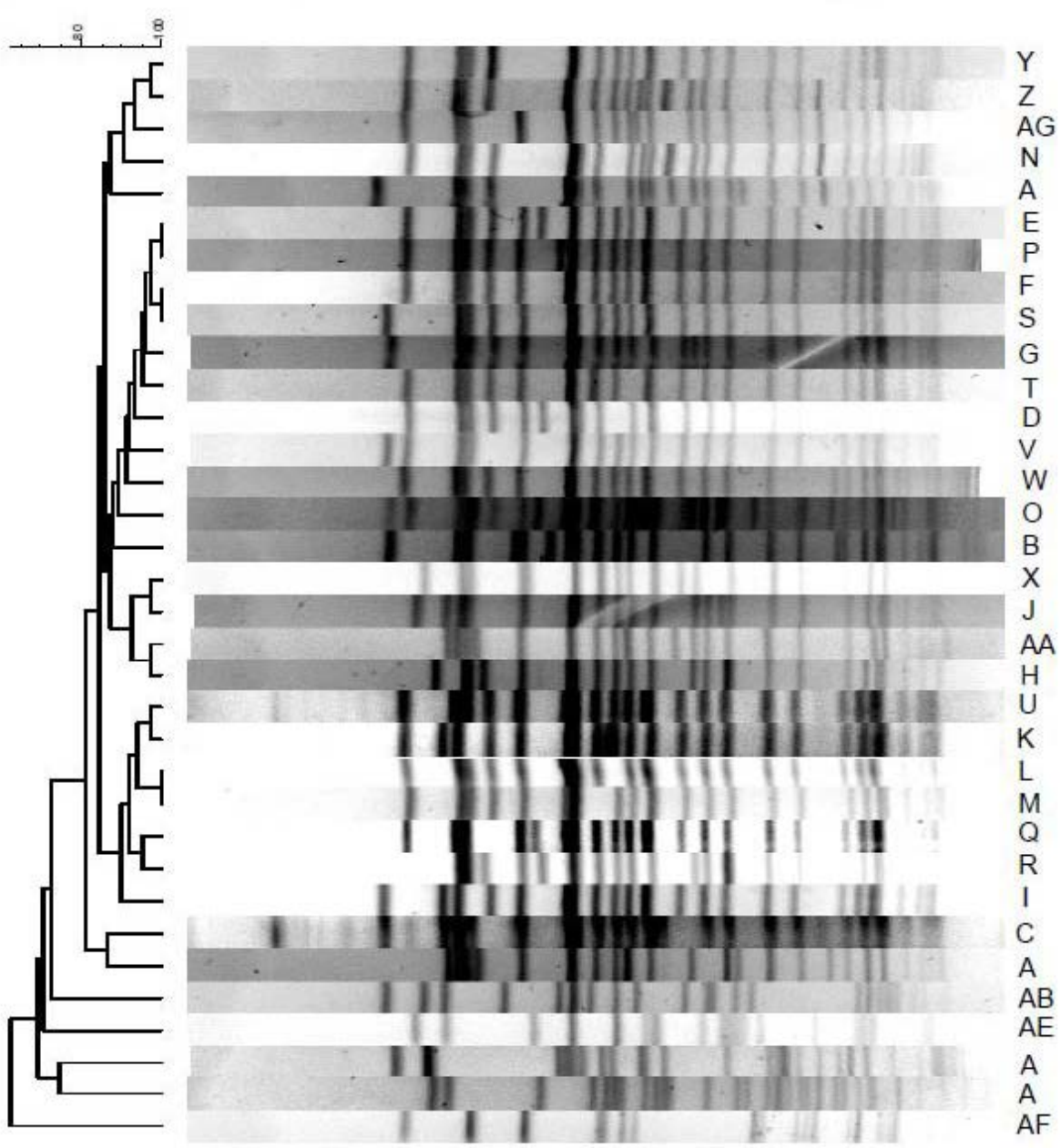

†Partial dendogram only containing one isolate for each distinct PFGE pattern.

Figure 4. Dendogram showing band differences between 34 distinct PFGE patterns.

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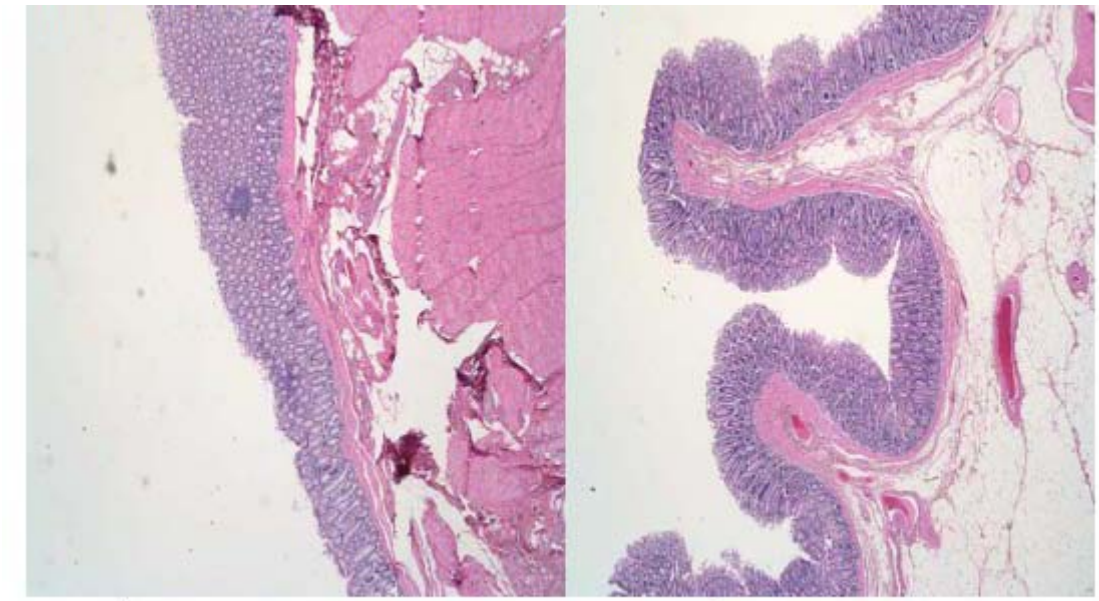
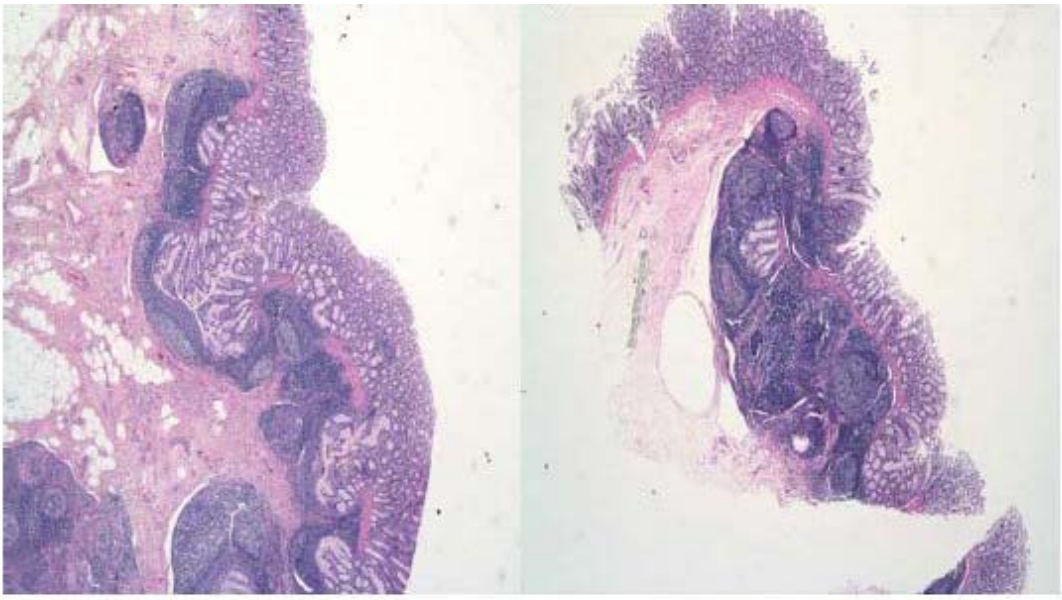


[1]NS colon sample [2]PS colon sample

Figure 5. Histopathology images of colon epithelial linings from NS[1] and PS[2] stained with hematoxylin and eosin and magnified 20 times.



[1]NS ileum sample [2]PS ileum sample

Figure 6. Histopathology images of ileum linings from NS[1] and PS[2] stained with hematoxylin and easin and magnified 20 times.

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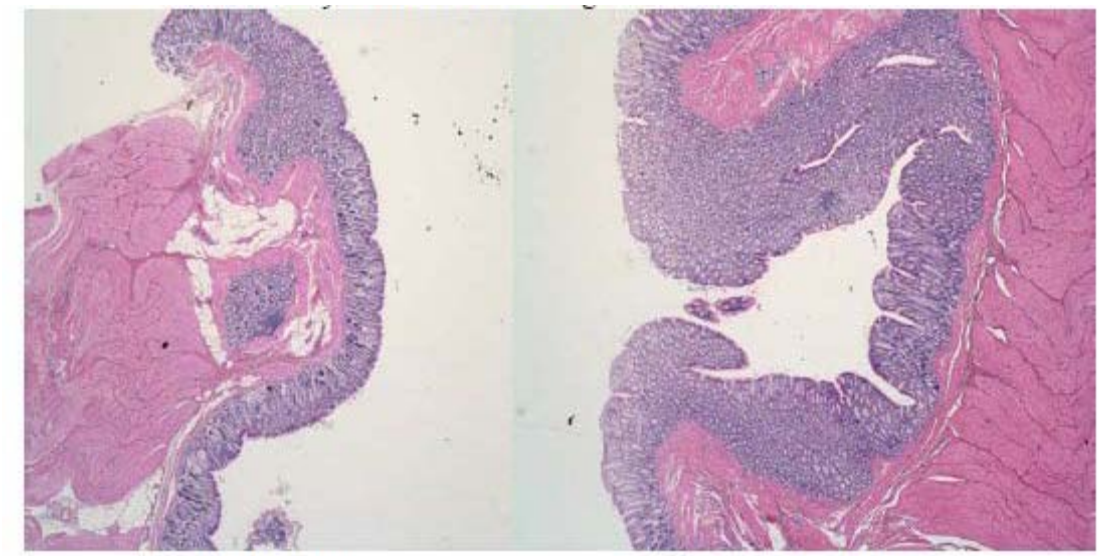

Figure 7. Histopathology images of cecum epithelial linings from NS[1] and PS[2] stained with hemotoxylin and eosin and magnified 20 times.

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