DISSERTATION

MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 HIDE CONTAMINATION ROUTES - FEEDLOT TO HARVEST

Submitted by
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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KIRBY DAN CHILDS ENTITLED "MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 HIDE CONTAMINATION ROUTES - FEEDLOT TO HARVEST" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

MOLECULAR CHARACTERTIZATION OF *ESCHERICHIA COLI* O157:H7 HIDE CONTAMINATION ROUTES - FEEDLOT TO HARVEST

This study was conducted to identify the origin of *Escherichia coli* O157:H7 contamination on steer hides at the time of harvest by sampling the feedlot, transport trailers, packing plant holding pens, and colons of feedlot steers in conjunction with the hides. A total of 50 positive hide samples were collected in two geographical locations: the Midwest (n = 25 positive hides) and Southwest (n = 25 positive hides). At the feedlot, sample locations included: water troughs, pen floors, feed bunks, and loading chutes. Transport trailer samples were obtained from the inner walls and floors of trailers. At the packing plant, the following samples were collected: packing plant holding pen floors, side rails, drinking water, and restrainer boxes. Hide samples were screened and confirmed for presence of *E. coli* O157:H7. When at least one hide sample tested positive for *E. coli* O157:H7, all corresponding companion samples were screened and confirmed for presence of *E. coli* O157:H7. Identification of *E. coli* O157:H7 isolates was conducted by fingerprinting all positive samples using Pulsed Field Gel Electrophoresis after digestion with *Xanthomonas badrii* (XbaI). Furthermore, isolates were analyzed using multiplex Polymerase Chain Reaction for the known *E. coli* O157:H7 gene markers: stx_1, stx_2, *eaeA*, and *hlyA*. Feedlot water troughs, pen floors, feed bunks, loading chutes; truck trailer side walls, floors; packing plant holding pen floors, side rails, and packing plant cattle drinking water samples tested positive for presence of *E. coli* O157:H7. Data was analyzed by stratifying isolates by gene markers present and by packing plant using Pulsed Field Gel Electrophoresis. Isolates that had greater than 7 differences in banding patterns were not considered to be related. In this study hide samples were traced to other hide, colon, feedlot pen floor fecal, packing plant holding pen drinking water, and transport trailer side wall samples. Links were determined between packing plant side rails, feedlot loading chutes and feedlot pens; and between truck trailer, different feedlots, and colons of multiple cattle. The current study is the first to identify genotypic matches between transport side walls and cattle hide samples within the packing plant.

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CHAPTER I

INTRODUCTION

Escherichia coli O157:H7 has been one of the major factors affecting consumers' perception of the safety of beef since the early 1990's. This was due to the tragic death of 3 children in the Pacific Northwest who consumed undercooked ground beef contaminated with *E. coli* O157:H7. Among recorded foodborne outbreaks, up to 25% of infected individuals have been hospitalized, 6% have developed the potentially fatal, hemolytic uremic syndrome, and 1% has died from infection of *E. coli* O157:H7 (27). The U.S. Food Safety and Inspection Service in 1994 declared *E. coli* O157:H7 an adulterant in ground beef (4). Since 1996, the beef industry has operated under Hazard Analysis - Critical Control Point plans which emphasize the reduction or elimination of hazards including *E. coli* O157:H7 (18). During the Food Safety Summit in 2003, sponsored by the National Cattlemen's Beef Association, the Points of Focus for the Producer Sector addressed ways to reduce the prevalence of *E. coli* O157:H7 associated with feedlot cattle. At the summit, the following best management practices were suggested: (a) Good Management Practices (GMPs) of clean feed, clean water, clean pens, and clean cattle should be implemented and maintained, (b) evaluate adoption of interventions or GMPs that have been scientifically validated, and (c) packing houses, producers and USDA-FSIS should maintain open communication and share data within the beef industry regarding pre-harvest interventions and GMPs (36).

Chapman et al. (11), Elder et al. (18), and Sofos et al. (44) demonstrated that cattle may be reservoirs or transmission vectors for *E. coli* O157:H7 which can potentially occur from hide to meat during the harvesting/dressing phase. Hides of animals are a key source of cross-contamination on carcasses (4, 33). Furthermore, Ransom et al. (40) stated that the prevalence of *E. coli* O157:H7 on pre-evisceration carcasses was 7.1% if fecal prevalence at the feedlot was less than 20%; however, carcasses prior to evisceration exhibited a 12.5% prevalence of *E. coli* O157:H7 if pre-harvest fecal prevalence was more than 20%.

Several studies have examined the link between carcass contamination and the harvesting process with *E. coli* O157:H7 positive carcass samples (7, 22, 23, 24, 26). However, these studies did not directly link pre-harvest sources of *E. coli* O157 contamination with packing plant hide samples positive for *E. coli* O157:H7 or identify specific routes of carcass contamination (6). However, it has been shown that cattle hides may become contaminated with *E. coli* O157:H7 from feedlot pen floors, packing plant holding pens, or restrainer floors (14, 49).

Pulsed-field gel electrophoresis (PFGE) genotyping is a method that is used by the Centers for Disease Control and Prevention to trace genetic patterns of pathogens linked to food safety outbreaks. Barkocy-Gallagher et al. (6) and Tutenel et al. (49) were the first researchers to use PFGE genotyping to track *E. coli* O157 on carcasses throughout the harvesting process at various packing houses. Barkocy-Gallagher et al. (6) determined the prevalence of *E. coli* O157 on carcasses and in the slaughter environment and characterized the *E. coli* O157 strains by use of PFGE and shiga-toxin gene typing in order to detect contamination routes. In the Tutenel et al. (49) study, molecular characterization of *E. coli* O157 (PFGE profiles and *Stx* types) was used to determine that hide-to-hide contamination can occur after departure from the farm while being transported to the processing facility.

The purpose of this study was to investigate microbiological samples from feedlot pens, chutes, transport trailers, and harvest-plant holding pens, plus samples from hides and colons of cattle, to determine the origin of *E. coli* O157:H7 contamination on cattle hides at the time of harvest. There are multiple opportunities for cattle hides to become contaminated with *E. coli* O157:H7 at the feedlots, during transport to the packing plants, and while being held in holding pens at the packing plants. This study was designed to verify pre-harvest origins of *E. coli* O157:H7 on steer hides through microbiological sampling and molecular fingerprinting with PFGE and multiplex PCR analysis.

CHAPTER II

REVIEW OF LITERATURE

Escherichia coli O157:H7 is considered one of the major human pathogens throughout the world. In the United States alone, *E. coli* O157:H7 is estimated to be responsible for 74,000 foodborne infections per year (38). The first documented case of *E. coli* O157:H7 illness was reported in 1982 and was associated with consuming undercooked raw ground beef. A decade later in 1993, *E. coli* O157:H7 achieved national infamy with numerous cases of foodborne illness and the death of young children from consumption of undercooked beef product served by a fast food restaurant chain. In 1994, the United States Department of Agriculture, Food Safety Inspection Service (50) declared *E. coli* O157:H7 an adulterant in ground beef. *E. coli* O157:H7 was the first microorganism given this status by USDA-FSIS under the Federal Meat Inspection Act (6). Shiga toxin can be produced by *E. coli* O157:H7, and causes potentially fatal conditions of hemorrhagic colitis and hemolytic uremic syndrome (HUS). One of the most publicized sources of *E. coli* O157:H7 is the GI tract of healthy ruminants, particularly cattle. The *E. coli* O157:H7 may spread from the intestinal track of ruminants to the local environment, and subsequently to raw foods. Due to the seriousness of infection by *E. coli* O157:H7, the beef industry must continue to take progressive steps to reduce or eliminate this pathogen as early in the production chain as possible.

Numerous approaches have been used to characterize how *E. coli* O157:H7 contaminates food (17, 18). In order to combat *E. coli* O157:H7, two areas of research focus have developed technologies for implementation in recent years: pre-harvest and post-harvest interventions. The majority of microbial intervention studies have concentrated on harvest floor interventions (2, 10, 13, 40, 44). Research to identify pre-harvest interventions has only recently evolved, and studies in this area have observed supplementation of feedlot diets with competitive inhibitors of *E. coli* O157:H7 (52). Also, hide-on antimicrobial washes have been used to reduce bacterial populations before the hide is removed on the harvest floor (35). In the future, there is a possibility for approval of vaccines against *E. coli* O157:H7, which may also serve as a pre-harvest intervention strategy (29, 39). Other research, discussed below, focuses on the transfer of *E. coli* O157:H7 from the environment to the feedlot animal.

Pre-harvest *E. coli* O157:H7 Reservoirs

Typically, finishing cattle are in the feedlot between 90 to 160 days (16, 45). In the production of fed dairy cattle, time in the feedlot can easily reach up to 300 days (47). Cattle are on feed for various amounts of time which can potentially expose them to infection of *E. coli* O157:H7. According to Hancock et al. (28) and McGee et al. (34), cattle may not harbor *E. coli* O157:H7 continuously throughout the feeding phase, but are continually exposed and susceptible to re-infection. Furthermore, each feedlot facility can also vary greatly in type of material used for pen fencing, feedbunks, and water troughs, and in the frequency of sanitation of these areas. LeJeune et al. (32) discussed, at great length, variables associated with water trough design and water hygiene as it may correlate with the harborage of *E. coli* O157:H7. LeJeune et al. (32) reported no direct link with the last cleaning of the trough; however, the study only observed troughs that had been cleaned in the preceding six months before the study *versus* water troughs that had not been cleaned in over six months. LeJeune et al. (32) also noted that *E. coli* O157:H7 was isolated in water troughs that received less sanitation *versus* those being cleaned more frequently, and once *E. coli* O157:H7 is inoculated into a water trough, the bacteria can become a part of the water trough bacterial load or endogenous flora. Clean troughs would be less likely to support an aquatic microflora system. These observations highlight the importance of minimizing water trough sediment accumulation. Sediment in a trough increases the likelihood of bacterial colonization which can include *E. coli* O157:H7. McGee et al. (34) stated that *E. coli* O157:H7 was not detected in water with or without the presence of sediment; however, plastic containers with only a small opening for drinking were used, and the authors noted that the water trough was not conducive for sediment accumulation. Smith et al. (43) also detected *E. coli* O157:H7 in water troughs, but did not find a correlation with temperature, pH, or cleanliness of the water in the trough. Furthermore, Rice and Johnson (42) concluded that *E. coli* O157:H7 could survive in water troughs for up to two weeks, and LeJeune et al. (32) demonstrated that *E. coli* O157:H7 had survived in sediment for up to six months and was still colonize in the GI of cattle.

Sediments may be defined, according to LeJeune et al. (32), as bacterial contaminants including cud, fecal material, dust, feed, or bedding. Smith et al. (43) stated that excrement from rats, cats, or birds were also potential bacterial contaminants.

LeJeune et al. (32) also reported more sediment accumulation when the feedbunks were closer to the water trough.

A strategy suggested by Zhao et al. (53) to reduce bacterial growth was to chlorinate the water. However, when LeJune et al. (31) added chlorine to water at a level of 1 ppm of free chlorine, they found no difference in prevalence of *E. coli* O157:H7 between water troughs treated or not treated with chlorine. LeJeune et al. (31) stated that large amount of sediments found in the water troughs (mainly consisting of feed material) may inactivate the bactericidal activity of the chlorine. Cattle continually "stirred" the water, which allowed the sediment to float freely throughout the water, which may have contributed to the ineffectiveness of chlorine to reduce pathogen prevalence. LeJeune et al. (31) concluded that chlorine would not likely control *E. coli* O157:H7 populations in water troughs unless water troughs were redesigned to eliminate accumulation of organic sediment.

Direct exposure of the water trough to sunlight is another factor that may influence the *E. coli* O157:H7 population. Two studies (31, 32) have concluded that levels of detectable *E. coli* O157:H7 populations are lower in troughs exposed to direct sunlight (ultraviolet radiation). Exposure to direct sunlight may influence water temperature, which in turn may affect bacterial growth. McGee et al. (34) and LeJeune et al. (32) both identified that 15° C is the key temperature for *E. coli* O157:H7 proliferation. Several factors influence *E. coli* O157:H7 population in water troughs; however, the two elements that appear to be key in harboring *E. coli* O157:H7 populations in feedlot water are the amount of sediment in the water and water temperature. Further research is needed to determine the effectiveness of designing water troughs that minimize the accumulation of sediment and that control water temperature (below 15° C) in order to reduce the potential for *E. coli* O157:H7 proliferation.

Conditions of the pen floor, transport trailers, and production systems (grass fed *versus* grain fed in a feedlot) are other pre-harvest environmental factors that have been examined to ascertain their relationship with *E. coli* O157:H7 harborage. Smith et al. (43) observed that condition of the pen floor was associated with incidence of cattle shedding of *E. coli* O157:H7; if the pen was designated as "muddy", a significant percentage of cattle were shedding *E. coli* O157:H7. The pen floor was designated as muddy if at least 50 % of the pen surface was muddy to a depth of 8.0 cm and 20 % of the pen was muddy to a depth of 30.0 cm. Smith et al. (43) attributed association of muddy pen conditions with increased cattle shedding of *E. coli* O157:H7 to an increase in prevalence of *E. coli* O157:H7 in the environment. If *E. coli* O157:H7 was able to maintain or increase prevalence in the mud, there would be opportunity for new or repeated infections. In a practical sense, if cattle are wading through mud, *E. coli* O157:H7 could be easily re-introduced to the hide surface of the animal. This was also confirmed by Kudva et al. (30) who showed that cattle and sheep manure may harbor *E. coli* O157:H7 for several months under wet environmental conditions. Dry manure was less likely to serve as a harborage point than wet manure.

There is limited, conflicting data that does not clearly illustrate if cattle transport trailers harbor *E. coli* O157:H7. Bach et al. (1) stated that *E. coli* O157:H7 was not detected in fecal samples before cattle were shipped to the feedlot, but on arrival at the feedlot, six of 43 calves tested positive for *E. coli* O157:H7. However, Barham et al. (3) found that hide samples testing positive for *E. coli* O157:H7 went from 18.0% before shipment from the feedlot to 4.5% at the packing plant. Positive fecal samples for *E. coli* O157:H7 went from 9.5% before shipment to 5.5% at the processing facility. Further research is needed to clarify the significance of transport trailers as a harborage point for *E. coli* O157:H7.

Different production systems (high roughage or grass-fed *versus* a high concentrate grain diet) do not appear to be a contributing factor to the prevalence of *E. coli* O157:H7 in feces of cattle. Some researchers believe that high roughage diets may decrease the shedding of *E. coli* O157:H7 (15). However, other studies (9, 20, 48) have shown no difference in shedding of *E. coli* O157:H7 between cattle fattened on grass or a high concentrate grain diet.

Molecular Characterization Techniques

Two techniques have allowed researchers the opportunity to molecularly characterize and trace bacterial strains to the host: Pulsed Field Gel Electrophoresis (PFGE) and multiplex Polymerase Chain Reaction (mPCR). Barkocy-Gallagher et al. (6) was one of the first groups to use PFGE analysis to investigate the source of *E. coli* O157:H7 contamination on the carcass following harvest. Analysis of pathogenic strains of bacteria using PFGE techniques had previously been used to locate sources of *Listeria* contamination (25) and to assist in investigating sources of *E. coli* O157:H7 foodborne outbreaks by the Centers for Disease Control and Prevention (5). In a land mark study, Elder et al. (18) reported that 43% of hides were positive for *E. coli* O157:H7 before evisceration, 18% post-evisceration, and 2% post-harvest floor. Barkocy-Gallagher et al. (6) further investigated *E. coli* O157:H7 positive samples collected by Elder et al. (18) by fingerprinting the samples to examine the potential source of cross-contamination. Of particular interest was the correlation between isolates entering the plant and isolates that appeared on carcasses within the same lot; and the relationship between carcasses that had been detected with *E. coli* O157:H7 isolates in the cooler and those carcasses that had been detected with *E. coli* O157:H7 positive isolates during processing. Barkocy-Gallagher et al. (6) concluded, after PFGE genotype fingerprinting, that *E. coli* O157:H7 found on carcasses appears to be the result of transfer of the bacteria from cattle within a lot rather than cross-contamination from other cattle of other lots. Barkocy-Gallagher et al. (6) did allow for one exception, which was observed by Byrne et al. (8), *E. coli* O157:H7 may be spread by processing plant employee hands and knives. However, if proper sanitation of knives and hands of processing plant employees is maintained, the likelihood of cross-contamination is minimal. Evidence also indicates that the majority of *E. coli* O157:H7 found on carcasses after processing may have been introduced before evisceration. Furthermore, Barkochy-Gallagher et al. (5) stated that there is a "substantial" level of genomic variation among the *E. coli* O157:H7 isolates; "substantial" variation was explained by the potential incorporation of new genes by *E. coli* O157:H7, which is the result of horizontal DNA transfer from different species of bacteria, as well as different strains of *E. coli* (possibly from wild animals, i.e. deer, rats and cats).

Using PFGE analysis, Tutenel et al. (49) identified harborage points from the production chain to the harvest floor that have potential to contaminate carcasses with *E. coli* O157:H7. After three days of sampling, Tutenal et al. (49) found low levels of *E. coli* O157:H7 samples in feces and in the environment, but employee aprons and holding pens of the processing facility were found by Tutenel et al. (49) to also be positive for *E. coli* O157:H7. Correlations were noted between hide sample *E. coli* O157:H7 and environmental sample positives. Tutenel et al. (49) stated that carcass contamination most likely did not occur at the processing facility, but most likely prior to the arrival of the animals to the processing facility. This observation was noted due to the fact that *E. coli* O157:H7 positives on carcasses did not come from consecutive carcasses but occurred randomly throughout sampling. Tutenel et al. (49) concluded that direct hide-to-hide contamination prior to arrival to the processing facility was the most probable source of pathogen transmission to carcasses. Analysis using PFGE of samples showed conclusively that *E. coli* O157:H7 positive samples from carcasses had similar PFGE profiles as the *E. coli* O157:H7 positive samples from the environment (loading chute, restrainer, and aprons).

CHAPTER III

MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 OF HIDE CONTAMINATION ROUTES - FEEDLOT TO HARVEST

ABSTRACT

This study was conducted to identify the origin of *Escherichia coli* O157:H7 contamination on steer hides at the time of harvest by sampling the feedlot, transport trailers, packing plant holding pens, and colons of feedlot steers in conjunction with the hides. A total of 50 positive hide samples were collected in two geographical locations: the Midwest (n = 25 positive hides) and Southwest (n = 25 positive hides). At the feedlot, sample locations included: water troughs, pen floors, feed bunks, and loading chutes. Transport trailer samples were obtained from the inner walls and floors of trailers. At the packing plant, the following samples were collected: packing plant holding pen floors, side rails, drinking water, and restrainer boxes. Hide samples were screened and confirmed for presence of *E. coli* O157:H7. When at least one hide sample tested positive for *E. coli* O157:H7, all corresponding companion samples were screened and confirmed for presence of *E. coli* O157:H7. Identification of *E. coli* O157:H7 isolates was conducted by fingerprinting all positive samples using Pulsed Field Gel Electrophoresis after digestion with *Xanthomonas badrii* (XbaI). Furthermore, isolates were analyzed using multiplex Polymerase Chain Reaction for the known *E. coli* O157:H7 gene markers: *stx*$_1$, *stx*$_2$, *eaeA*, and *hlyA*. Feedlot water troughs, pen floors, feed bunks, loading chutes; truck trailer side walls, floors; packing plant holding pen floors, side rails, and packing plant cattle drinking water samples tested positive for presence of *E. coli* O157:H7. Data was analyzed by stratifying isolates by gene markers present and by packing plant using Pulsed Field Gel Electrophoresis. Isolates that had greater than 7 differences in banding patterns were not considered to be related. In this study hide samples were traced to other hide, colon, feedlot pen floor fecal, packing plant holding pen drinking water, and transport trailer side wall samples. Links were determined between packing plant side rails, feedlot loading chutes and feedlot pens; and between truck trailer, different feedlots, and colons of multiple cattle. The current study is the first to identify genotypic matches between transport side walls and cattle hide samples within the packing plant.

INTRODUCTION

Media attention to *Escherichia coli* O157:H7 related outbreaks have influenced consumers' perception of the safety of beef since the early 1990's, due primarily to the tragic human death that can occur from an infection with *E. coli* O157:H7 in the young and elderly. This was due to the tragic deaths of three children in the Pacific Northwest who consumed undercooked ground beef contaminated with *E. coli* O157:H7. Among recorded foodborne outbreaks, up to 25% of infected individuals have been hospitalized, 6% have developed the potentially fatal hemolytic uremic syndrome, and 1% has died from infection of *E. coli* O157:H7 (27). The U.S. Food Safety and Inspection Service in 1994 declared *E. coli* O157:H7 an adulterant in ground beef (4). Since 1996, the beef industry has operated under Hazard Analysis Critical Control Point plans which emphasize reduction or elimination of hazards including *E. coli* O157:H7 (18). During the Food Safety Summit in 2003, sponsored by the National Cattlemen's Beef Association, the Points of Focus for the Producer Sector addressed ways to reduce prevalence of *E. coli* O157:H7 associated with feedlot cattle; the following best management practices were suggested: (a) Good Management Practices (GMPs) of clean feed, clean water, clean pens, and clean cattle should be implemented and maintained, (b) evaluate adoption of interventions or GMPs that have been scientifically validated, and (c) packing houses, producers and USDA-FSIS should maintain open communication and share data within the beef industry regarding pre-harvest interventions and GMPs (36).

Chapman et al. (11), Sofos et al. (44), and Elder et al. (18) demonstrated that cattle may be reservoirs or transmission vectors for *E. coli* O157:H7 which can potentially be transferred from hide to meat during the harvesting/dressing process. Hides of animals are a key source of cross-contamination of carcasses (4, 33). Furthermore, Ransom et al. (40) stated that prevalence of *E. coli* O157:H7 on pre-evisceration carcasses was 7.1% if fecal prevalence at the feedlot was less than 20%; however, carcasses sampled before evisceration exhibited a 12.5% prevalence of *E. coli* O157:H7 if pre-harvest fecal prevalence was more than 20%.

Several studies have examined links between carcass contamination with *E. coli* O157:H7 throughout the harvesting process (7, 22, 23, 24, 26). However, these studies did not directly link pre-harvest sources of *E. coli* O157 contamination with packing plant hide samples positive for *E. coli* O157:H7 or identify specific routes of carcass contamination (6). However, cattle hides may become contaminated with *E. coli* O157:H7 from feedlot pen floors, packing plant holding pens, or restrainer floors (14, 49).

Pulsed field gel electrophoresis (PFGE) genotyping is a method that is used by the Centers for Disease Control and Prevention to trace genetic patterns of pathogens linked to food safety outbreaks. Barkocy-Gallagher et al. (6) and Tutenel et al. (49) were the first researchers to use PFGE genotyping to track *E. coli* O157 on carcasses throughout the harvesting process in various packing plants. Barkocy-Gallagher et al. (6) determined prevalence of *E. coli* O157 on carcasses and in the slaughter environment and characterized the *E. coli* O157 strains using PFGE and shiga-toxin gene typing in order to detect contamination routes. In the Tutenel et al. (49) study, molecular characterization of *E. coli* O157 (PFGE profiles and *Stx* types) was used to determine that hide-to-hide contamination can occur after departure from the farm, while being transported to the packing plant.

The purpose of this study was to investigate microbiological samples from feedlot pens, chutes, transport trailers, and harvest-plant holding pens, plus samples from hides and colons of cattle, to determine the origin of *E. coli* O157:H7 contamination on cattle hides at the time of harvest. There are multiple opportunities for cattle hides to become contaminated with *E. coli* O157:H7 in feedlots, during transport to the packing plants, and while being held in holding pens at packing plants. This study was designed to verify pre-harvest origins of *E. coli* O157:H7 on steer hides via characterization of microbiological samples using molecular PFGE fingerprinting and multiplex Polymerase Chain Reaction analysis.

MATERIALS AND METHODS

Cattle, Feedlots and Packing Plant. Cattle originating from six feedlots in the Midwest (n = 3) and Southwest (n = 3) were sampled in this study. Samples were collected from eight feedlot shipments of cattle during the period between July 2004 thru February 2005. Cattle were transported distances of 2411, 1685, or 55 kilometers from the three Midwestern commercial feedlots to two Upper Midwest beef packing plants. In the Southwestern region, cattle were transported distances of 81, 86, or 561 kilometers to a single packing plant. For each feedlot shipment, samples were collected from eight pre-harvest environmental sample locations and two packing plant harvesting-processes, including (1) feedlot pen floor sample composites, (2) feedlot pen water sample composites, (3) feed bunk and feed composite sample composites, (4) loading chute sample composites, (5) transport truck trailer inner wall and floor composites, (6) packing plant holding pen sample composites, (7) plant pen water sample composites, (8) restrainer wall samples, (9) cattle hides during harvesting, and (10) corresponding colons on the harvest floor. Sample sets were collected until at least 25 hide samples were confirmed positive for *E. coli* O157:H7 by biochemical analysis from each region (Midwest and Southwest).

Feedlot Pen Sample Collection. On the day of or the day before cattle shipment to the packing plant, researchers traveled to selected feedlots and collected samples for microbiological characterization. Each feed bunk was aseptically sampled by swabbing a large area (~1000 cm^2) of the feed bunk with a hydrated sterile sponge (HydraSponge, Biotrace International, Bothwell, WA), and random feed samples were collected to select the entire length of the feed bunk in sterile cups. Water tanks in each pen were sampled by swabbing the exterior of the trough and the tank ball if applicable (trough base was not sampled) with a hydrated sterile sponge (HydraSponge, Biotrace International, Bothwell, WA). Water samples also were collected by submersion of a hydrated sterile sponge into the water tank. All sponges were placed back into individual sterile bags, excess air was removed, and the samples were placed in a foam cooler with icepacks until shipment to a commercial laboratory (Food Safety Net Services, Inc., San Antonia, TX) for analysis. Pen floor samples were collected aseptically by a researcher using a sterile tongue depressor and sterile cup. Each fecal and dirt composite sample represented approximately 10 square yards of the pen floor.

Loading Chute and Truck Trailer Sample Collection. Cattle loading chutes at the feedlots, and the associated alley, were sampled immediately before loading cattle onto the first truck trailer during shipment. If more than one loading chute was used, both were sampled and samples were combined. Hydrated sterile sponges (HydraSponge, Biotrace International, Bothwell, WA) were used to take a 500 cm^2 representative sample of the loading chute walls and railing. Sterile tongue depressors and sterile cups were used to aseptically collect composite dirt and fecal samples from the floors of the loading chutes.

Before cattle were loaded, the upper deck, lower deck, and the back of trailers at the point of entry were sampled. Trailer walls were sampled by swabbing a 500 cm^2 with a single hydrated sterile sponge (HydraSponge, Biotrace International, Bothwell, WA) at each location within the trailer (upper deck, lower deck, back), comprising a representative wall sample (500 cm^2) for each location. If visible floor contamination was present, trailer floor samples were collected, using a sterile tongue depressor and sterile cup from the top deck, bottom deck, and back of each trailer.

Plant Holding Pen Sample Collection. Before cattle were unloaded from truck trailers at the packing plant, empty pens that would house the research cattle were sampled with a hydrated sterile sponge (HydraSponge, Biotrace International, Bothwell, WA). Objects inside the pens that were sampled included side railings, perimeter walls, gates, and pillars. If present, representative fecal material and associated pen bedding were collected into sterile cups from each holding pen floor. Hydrated sterile sponges (HydraSponge, Biotrace International, Bothwell, WA) were used to swab water troughs in each holding pen, along with any fecal contamination attached to the trough. Hydrated sterile sponges also were used to collect water, by submersion of the entire sponge in the water tank for ~10 sec. All sponges were placed back into sterile bags after sample collection; excess air was removed, the bag was labeled, and bags were placed, along with labeled fecal cups, in a foam shipper with ice packs until shipment to the commercial laboratory for analysis.

Harvest Floor Sample Collection. Hide samples were collected from randomly selected cattle of each shipped lot of live cattle in each plant directly before hide removal. Hide samples were collected using an individual, sterile sponge from a 500 cm^2 area of hide at the dorsal midline by swabbing with 20 strokes (up and down comprising one stroke) up and down and side to side, using enough pressure to remove any dried blood that may be present. Each sampled carcass and corresponding colon was tagged to maintain identity. All colons were collected from animals that had the hide swabbed by excising the digestive tract at the rectoanalmucosal (RAM) junction (41) and placed, along with the carcass tag, in a labeled WhirlPak bag. Excess air was removed, and the bag was closed.

Microbial Analysis. Before shipping, all samples were cooled to 4° C and packed with frozen ice-packs into insulated shipping boxes. All samples, excluding colons which were cooled overnight before shipping, were shipped overnight for microbiological analysis at Food Safety Net Services, San Antonio, TX. Upon arrival at the laboratory, the temperature of each shipping container was measured and documented. Samples exceeding 4° C upon arrival were discarded. Intact colons were dissected, and the mucosal membrane of the colon was vigorously swabbed with a hydrated sterile sponge (HydraSponge, Biotrace International, Bothwell, WA) which was then incubated in Tryptic Soy Broth (TSB; Biotrace International, Bothwell, WA) at 37° C for 24 h. Detection of *E. coli* O157:H7 in all samples was conducted following the USDA-MARC method described by Barkocy-Gallagher et al. (5).

A 10 ml aliquot of fluid was taken from each sample bag and suspended in 90 ml of TSB and incubated for 2 h at 25° C, followed by 6 h at 42° C, followed by an overnight incubation at 4° C. After incubation, 20 µl of anti-*E. coli* O157:H7 Dynabeads (Dyna Laboratories, Lake Success, NY) and 100 µl 0.05% protamine (Sigma, St. Louis, MO) was added to 1 ml aliquots and incubated again for 30 min on a rocker at room temperature (24 ± 2° C). Tubes were placed in a magnetic separation rack to bind beads and incubated for an additional 5 min at room temperature (24 ± 2° C) on a rocker. A 1 ml portion of supernatant was removed from each tube, and beads were washed 3 times with 1 ml of a 7.0 pH phosphate buffered saline (PBS) and 0.05% of Tween 20 solution, (Fisher Scientific, Fair Lawn, NJ) and then re-suspended in 100 µl of PBS and 0.05%

Tween 20 solution. A 50 µl portion of the suspended bead solution was spread onto Sorbitol MacConkey agar supplemented with cefeximine (0.05 mg/l) and 2.5 mg/l potassium tellurite (ctSMAC; Sigma-Aldrich, St. Louis, MO), and another 50 µl was spread on Rainbow-plus agar (Rainbow-Agar O157, Bilog Inc., Hayward, CA) containing 0.8 g/ml potassium tellurite (Sigma-Aldrich, St. Louis, MO) and 20 g/ml novobiocin (Sigma-Aldrich, St. Louis, MO). Each plate was incubated for 18 h at 37° C. Following incubation, typical *E. coli* O157:H7 colonies identified on ctSMAC plates (colorless with or without a dark center) or Rainbow-agar (dark slightly blue colonies) were removed and screened with the latex agglutination assay, Oxoid *E. coli* O157:H7 Test Kit (Oxoid; Ogdensburg, NY). If agglutination occurred, the colony was tested with a control latex reagent to ensure the isolate was not an auto-agglutinating strain.

Initial biochemical analysis of presumptive isolates included cellubiose, triple sugar iron (TSI) slants, and motility tests. Following initial biochemical analysis, presumptive isolates were then screened with an O and H antigen agglutination test (RIM *E. coli* O157:H7 Latex test, Remel, Lenexa, KS) and each primary *E. coli* O157:H7 isolate from each sample also was analyzed with additional biochemical testing, including VITEK analysis (bioMerieux, Durham, NC). Once confirmation testing was complete, isolates were stored in a 20% glycerol-TSB solution and frozen until molecular characterization analysis could be conducted.

Molecular Characterization of Recovered *E. coli* O157:H7 Isolates. Multiplex Polymerase Chain Reaction (PCR) of *Escherichia coli* O157:H7 isolates was conducted using genetic markers (21, 51), and Pulsed Field Gel Electrophoresis (PFGE) of restricted genomic DNA was used to trace and molecularly characterize genetic fingerprints between hide, colon, and environmental companion samples.

Multiplex PCR. Each isolate was subjected to each of three multiplex PCR procedures, a combination of different primers for amplification was used as follows: (1) for Shiga toxin genes 1 and 2 (stx_1 and stx_2) per Wang et al. (51); (2) for enterohemorrhagic *E. coli* (EHEC) enterohemolysin (*hlyA*), *E. coli* somatic antigen O157 ($rfbE_{O157}$), and *E. coli* structural flagella antigen H7 ($fliC_{H7}$) per Wang et al. (51); and 3) for effacing and attachment of *E. coli* O157:H7 ($eaeA_{O157}$) per Gannon et al. (21). All reactions included a primer set for the 16S rRNA gene for *E.coli* to serve as an internal control as described by Wang et al. (51).

DNA was prepared from each *E. coli* O157:H7 isolate evaluated using the Instagene System (Bio-Rad, Hercules, CA). All plastic ware and pipettes used for the preparation of the PCR reaction Master Mixes were placed under UV light for 20 min before mixing under a laminar flow hood to dimerize any residual DNA fragments. According to the procedure described by Wang et al. (51), the therma-cycler (iCycler, Bio-Rad, Hercules, CA) settings were as follows: cycle 1 (1 rep) was 95° C for 8 min; cycle 2 (30 rep) was 95° C for 30 sec, 58° C for 30 sec and 72° C for 7 min; cycle 3 consisted of (1 rep) 72° C for 7 min; and cycle 4 (1 rep) was 4° C for 18 min. Gannon et al. (21) described the following settings for the therma-cycler (iCycler, Bio-Rad, Hercules, CA): cycle 1 (35 reps) was 94° C for 15 sec, 65° C for 15 sec and 72° C for 90 sec; cycle 2 (1 rep) was 72° C for 5 min; and cycle 3 (1 rep) was 25° C for 18 min. After the therma-cycler programs were complete and samples had cooled, 2µl of sample were mixed with 1 µl 10 x Bluejuice (Invitrogen, Carlsbad, CA) and added to a 100 ml 1 x

TBE buffer. The sample was then loaded in a 1g agarose gel (Certified PCR Agarose, Bio-Rad, Hercules, CA) covered by 80 ml 1 x TBE (Tris, EDTA, and boric acid) buffer. A 20 well comb was used to cast each PCR gel; therefore, 15 samples, 3 *lamda* ladder wells, one positive control, and one negative control were run on each PCR gel for 1 h at 80V. After electrophoresis, gels were removed from buffer and stained for 30 min with ethidium bromide and de-stained for 20 min. The gel was then removed from the de-staining solution and placed in Gel Doc EQ (Bio-Rad, Hercules, CA) for UV photo imaging. Exported gel images were analyzed using the QuantityOne software program (Bio-Rad, Hercules, CA).

Pulsed Field Gel Electrophoresis. A 3.0 McFraland (Remel Colormeter Standards kit, Lenexa, KS) turbidity solution of isolate cells was suspended in Cell Suspension Buffer (CSB: 1.0 M NaCl, 10 ml 1.0 M Tris [Sigma, Desienhofen, Germany], pH 7.6), and then added in a 1:1 ratio to a 50° C 1.6% Low Melt Agarose (Bio-Rad, Hercules, CA) and CSB solution. After gentle pipette mixing, sample solutions were added to disposable 10 mm x 5 mm x 1.5 mm plug molds (Bio-Rad, Hercules, CA) and chilled at 4° C for 20 min. After chilling, plugs were removed from the mold and placed in 4 ml cell lysis buffer (0.5 M EDTA [Bio-Rad, Hercules, CA], 1.0% Sodium Lauroyl Sarcosine[Sigma, Desienhofen, Germany], 0.5 mg Proteinase K [Sigma, Desienhofen, Germany]) and incubated at 50° C in a shaking water bath overnight. After 24 h incubation, expired cell lysis buffer was removed and replaced with an additional 4 ml cell lysis buffer and incubation was allowed to continue in a shaking water bath overnight at 50° C. After incubation, cell lysis buffer was removed and replaced with 5 ml TE buffer (10 mM Tris[Bio-Rad, Hercules, CA], 0.1mM EDTA [Bio-Rad, Hercules, CA]) and incubated in a shaking water bath at 37° C for 30 min. After incubation, the sample was washed with TE buffer 4 times.

Plugs were rinsed with 400 µl sterile H_2O, added to 40 µl of 10 x reaction buffer solution, and then incubated at room temperature (24 ± 2° C) for an additional 4 h. After incubation, 6 µl of *Xba*I (Roche Molecular Biochemicals, Indianapolis, IN) restriction enzyme was added to each centrifuge tube and incubated in a dry incubator at 37° C overnight. The reaction was terminated by removing the reaction buffer and replacing with TE buffer; the samples were then incubated in TE buffer for 1 h at 37° C.

After incubation, plugs were added to a solidified (30 min solidification interval) 1% Pulsed Field Certified Agarose (Bio-Rad, Hercules, CA) and 0.5 x TBE buffer (Tris-Borate EDTA Buffer [Sigma, St. Louis, MO]) gel by insertion of individual plugs into formed wells. The gel was then placed into 2 L of 0.5 x TBE buffer at 14° C in the electrophoresis chamber of the CHEF Mapper PFGE System (Bio-Rad, Hercules, CA). Each electrophoresis was performed according to the following parameters: (1) gradient 6.6 V/cm, (2) 120° Angle, (3) initial switch time of 2.16 sec, (4) final switch time of 54.17 sec, (5) linear ramp factor, and (6) run time of 20 h. After electrophoresis was complete, gels were stained for 20 min in 1 L of sterile distilled water containing 100 µl of ethidium bromide (10mg/ml) and destained for 40 min in sterile distilled water. The gel was then removed from the destaining solution and placed in Gel Doc EQ (Bio-Rad, Hercules, CA) for UV photo imaging. Exported gel images and band patterns were analyzed using the FingerPrinting II software program (Bio-Rad, Hercules, CA).

Data analysis was conducted using the method described by Tenover et al. (46), which is very stringent and is used during epidemiologic studies for foodborne outbreaks.

The guidelines are based on the use of a single restriction endonuclease. The current study used one restriction endonuclease, and samples were collected in less than one year. For the current study, only isolates with band differences of ≤7 were utilized to determine relationships between samples. Tenover et al. (46) suggested the following relationships: 0-1 band difference is indistinguishable and is considered a food borne outbreak, 2-3 band differences are closely related and is probably part of an outbreak, and 4-6 band differences is possibly related and is possibly part of an outbreak.

RESULTS

Distributions of *E. coli* O157:H7 positive samples are illustrated in Table 1. *E. coli* O157:H7 was detected at feedlot water troughs, pen floors, feed bunks, loading chutes; truck trailer side walls and floors; and packing plant packing plant holding pen floors, side rails, drinking water samples tested positive for the presence of *E. coli* O157:H7. The only sample locations that did not test positive for *E. coli* O157:H7 were feed and restrainer sidewalls. The study was not designed to diagnose *E. coli* O157:H7 prevalence for environmental samples or carcasses, but to identify potential cross-contamination points at the feedlot, during transportation, and holding pens at the packing plant. In both the Midwest and Southwest, all feedlots tested positive for *E. coli* O157:H7 at both the pen floor and loading chute side panel sample locations. However, *E. coli* O157:H7 was detected in feedbunk samples and fecal samples collected at the loading chute in Midwest feedlots, but not detected in loading chute fecal samples from the feedlots located in the Southwest (Table 1).

E. coli O157:H7 was detected in both Midwest and Southwest truck trailer side walls. Only fecal samples from truck trailer floors from the Southwest tested positive for *E. coli* O157:H7 (Table 1).

At the packing plants, *E. coli* O157:H7 was detected on the receiving pen side rails, as well in water samples in the receiving pens. The receiving pen floor samples were positive for *E. coli* O157:H7 in the Midwest, but not in the Southwest. Colon samples also tested positive for *E. coli* O157:H7 in both the Midwest and Southwest.

Multiplex PCR analysis. The distribution of *E. coli* O157:H7 virulence genes are separated by sample location in Table 2. Overall, Stx_1, *hlyA*, $rfbE_{O157}$, $fliC_{H7}$, and $eaeA_{O157}$ genes were detected in at least one sample at each location. The only locations that were positive for *E. coli* O157:H7 but that did not have Stx_2 present were the water sample at the feedlot and the receiving pen floor at the packing plant. In over 50.0% of samples from feedlot chute side panels, truck trailer side walls, packing plant receiving pen side rails, and packing plant water samples, both Stx_1 and Stx_2 genes were detected (Table 2). The number of hide samples positive for Stx_1 and Stx_2 genes was greater than the other companion samples; this category also had the most isolates, 176. There were 48 colon isolates, but only 3 expressed both Stx_1 and Stx_2 genes compared to the 45 pen floor isolates where 11 samples had Stx_1 and Stx_2 genes present. In samples from the carcass (hide and colon), there were more samples that did not have either Stx_1 or Stx_2 genes expressed than isolates that did express both Stx_1 and Stx_2 genes. In environmental samples, and with the exception of the feedlot chute floor, more isolates expressed both Stx_1 and Stx_2 genes than isolates that did not express either Stx_1 and Stx_2 genes (Table 3).

Water and feed bunk samples at the feedlot did not express all genes. Also, at the processing facility, the receiving pen floor samples did not express all virulence genes. Of particular interest, over 50.0% of the isolates collected from the feedlot loading chute side-panels, truck trailer side panels, receiving pen side rails and receiving pen water samples expressed all virulence genes (Table 4).

PFGE analysis. For PFGE analyses, data were first stratified into groups of isolates that had the same virulence gene expression, and then further stratified by packing plant of origin. Plant 1 and Plant 2 are located in the Upper Midwest and Plant 3 is located in the Southwestern United States. As illustrated in Tables 4 through 6, three samples were indistinguishable; a feedlot pen floor fecal sample and packing plant pen floor sample (2348 kilometers apart), and two sets of colons. Eleven samples were determined to be closely related (2-3 band differences). Comparisons of note were feedlot loading chutes with plant pen side rails, hides on consecutive carcasses, feedlot pens in different locations of the feedlot, feedlot pen fecal samples with hide and colon samples, and truck trailer side rails with colon samples. Also of interest, a feedlot pen floor fecal sample was closely related with a processing plant pen floor, even though the sampling date was 4 months apart (10/4/2004 and 2/7/2005, respectively).

Multiplex PCR and PFGE analysis. Davis et al. (12) suggested that the classification system described by Tenover et al. (46) using one restriction enzyme digest has the potential to misclassify unrelated items as closely related or may not show a close relationship when in fact two PFGE patterns are related. However, Davis et al. (12) did state that when there is a high probability that isolates are epidemiologically linked, that PFGE analysis with one restriction enzyme is appropriate. In order to demonstrate that samples in the current study did have an epidemiological link, PFGE patterns were only compared when isolates had the same molecular characteristics (i.e., virulence gene expression as determined by multiplex PCR analysis) (Table 4 through 6). Samples from different packing plants were not analyzed together; only samples from the packing plant and corresponding feedlot were analyzed for similar PFGE patterns.

For Plant 1 located in the Midwest (Table 4), there was an indistinguishable relationship between a feedlot pen floor fecal isolate and a packing plant pen floor fecal isolate. A feedlot loading chute side panel isolate, a packing plant holding pen side rail, and a hide sample with a truck trailer side rail were related. Specifically, packing plant pen 1 sample 20 matched loading chute side rail sample 6. Packing plant pen 1 sample 20 also matched feedlot pen sample 23, and in turn feedlot pen sample 23 matched a fecal sample from feedlot pen 2. For the Midwest, plant 1 companion samples showed relationships that linked the *E. coli* O157:H7 found at the packing plant holding pen side rail with the feedlot loading chute and feedlot pen floor.

Plant 2 samples from the Midwest (Table 5) generated a relationship that was indistinguishable between two colons that were collected from two consecutive carcasses. Also showing a relationship was a feedlot pen floor fecal sample with colon samples. Colon sample 23 matched colon sample 24; and colon sample 24 matched feedlot pen floor sample 3. This suggests that multiple cattle within a pen may be shedding a genetically similar *E. coli* O157:H7 strain, or animals within a pen may become infected with *E. coli* O157:H7 from other animals within the pen or during transport.

Plant 3 samples from the Southwest, (Table 6) generated numerous relationships that were closely linked. Sample 23, a fecal sample from the pen floor of feedlot 2 in the

Southwest, matched a hide sample from feedlot 1. Also, a feedlot pen floor fecal sample 23 also matched colon samples 37 and 38. Colon sample 38 matched colon sample 47 and truck trailer sample 5. Upon further investigation, the same truck trailers used to transport cattle from feedlot 1 of the Southwest also transported cattle from feedlot 2. In this case, the truck trailer was a potential transfer point between two feedlots and colon samples of multiple cattle.

DISCUSSION

Results of this study corresponded similarly with those described by Tutenal et al. (49). *E. coli* O157:H7 is detectable in packing plant holding pens; and stx_1 and stx_2 are often, but not always expressed. Although Tutenal et al. (49) did not sample any locations except at the packing plant, they concluded that direct hide-to hide contamination between the departure from the cattle's origin to the processing facility was the most likely transmission route. The current study illustrated that potential cross-contamination points may serve as vectors of transmission from the companion samples (feedlot pen fecal samples, feedlot loading chute, packing plant holding pen side rails, truck trailer side rails, and colons) to hides. The current study is the first to identify genotypic matches between transport trailer side walls with packing plant cattle hide samples. Also of interest were the genotypic matches for *E. coli* O157:H7 samples between: 1) truck trailer side walls, feedlot feed bunk samples, and loading chutes; and 2) packing plant receiving pens floors, side rails, and colon samples. Other studies have demonstrated that water troughs, feed (although not detected in the current study), feces from feedlot pen floors and anus (during the removal process on the harvest floor) are potential cross-contamination points (19, 32, 34, 43).

Although different regional locations were sampled than in the present study, Dewell et al. (14) found regional differences between cattle from eastern Colorado *versus* central Nebraska. The current study although not designed to determine prevalence, did result in companion samples that were positive for *E. coli* O157:H7 in the Midwest that were not detected in the Southwest (Table 1). For feedlots, *E. coli* O157:H7 was not found in the Midwest trailer floor fecal samples, but *E. coli* O157:H7 was found in trailers in the Southwest. For the Southwest feedlots, *E. coli* O157:H7 was detected in samples of water or feed bunks, but were in Midwest feedlots. For packing plants, *E. coli* O157:H7 was detected in Midwestern water samples, but not in those from the Southwest. Southwestern packing plant generated positive samples from the holding pen floor, but no positive samples were identified in the Upper Midwest. Regions vary greatly in humidity, sea level, average temperature, wind, and concentration of cattle in the feedlots and surrounding feedlots. This study did not account for all environmental factors that could influence prevalence of *E. coli* O157:H7 or differences in virulence gene expression rates.

Findings agreed with those of Naylor et al. (37) in that *E. coli* O157:H7 is found in the rectoanal-juction of animals. The current study suggested that *E. coli* O157:H7 samples from the colon matched numerous environmental sample locations as confirmed by PFGE analysis (Tables 4 through 6) and multiplex PCR. This study did not investigate the mode of transmission between the *E. coli* O157:H7 found in the colon and the *E. coli* O157:H7 found in environmental samples. These data suggested that colon shedding plays a critical role in distributing *E. coli* O157:H7 throughout feedlot pen floors and truck trailer side panels. In addition to the environmental samples, genotypic matches for *E. coli* O157:H7 were found between colons, as well as between hides from different animals. This study demonstrated the need for the beef industry to implement standard sanitation practices and/or decontamination interventions that may reduce or eliminate *E. coli* O157:H7 from colons.

This study demonstrated that pre-harvest environmental samples genetically matched hide samples. Therefore, *E. coli* O157:H7 hide contamination can be traced to feedlot, truck trailer, and packing plant holding pens. Further research is needed to determine modes of transmission and effective methods for reduction of *E. coli* O157:H7 at feedlot loading chutes, truck trailer side panels, and water troughs, both in feedlots and beef packing plants, as well as in feed bunks in feedlots.

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TABLES

TABLE 1. *Number of* E. coli *O157:H7 positives recovered at the feedlot, truck trailers and packing house.*

Collection Site	Total % Positive (Isolates)	[a]Midwest	[a]Southwest
Feedlot			
Water	2/64 (3)	2	0
Pen floor	19/96 (45)	8	11
Feed	0/48 (0)	0	0
Feedbunk	1/32 (2)	1	0
Chute (Swab of side-panels)	2/20 (9)	1	1
Chute (Fecal from chute floor)	2/30 (5)	2	0
Transport			
Trailers (Swab of side-panels)	5/75 (14)	3	2
Trailers (Fecal from trailer floor)	3/75 (5)	0	3
Processing facility			
Receiving pen floor	2/100 (3)	0	2
Receiving pen side rails	2/50 (8)	1	1
Water	1/50 (5)	1	0
Restrainer sidewalls	0/8 (0)	0	0
Carcasses			
Hide	75/442 (176)	25	50
Colon	16/75 (48)	8	8

[a] Represents the number of *E. coli* O157:H7 positives by region.

TABLE 2. *Distribution of* E. coli *O157:H7 virulence genes among carcass and companion samples.*

Feedlot	**# of Positive Samples**	**# of Positive Isolates**	***EAE 157***	***Stx1***	***Stx2***	***HlyA***	***RfbE***	***Flic***	**All genes**
Water	2	3	(2/3)	(2/3)	(0/3)	(3/3)	(3/3)	(3/3)	0
Pen floor	19	45	(43/46)	(30/46)	(24/46)	(44/46)	(42/46)	(43/46)	9
Feed	nd								0
Feedbunk	1	2	(1/2)	(1/2)	(1/2)	(1/2)	(1/2)	(1/2)	0
Chute (Swab of side-panels)	2	9	(9/9)	(5/9)	(8/9)	(9/9)	(9/9)	(9/9)	5
Chute (Fecal from chute floor)	2	5	(4/5)	(2/5)	(1/5)	(4/5)	(4/5)	(4/5)	1
Transport									
Trailers (Swab of side-panels)	5	14	(13/14)	(8/14)	(12/14)	(13/14)	(13/14)	(13/14)	7
Trailers (Fecal from trailer floor)	3	5	(3/5)	(2/5)	(3/5)	(2/5)	(3/5)	(2/5)	1
Processing facility									
Receiving pen floor	2	3	(1/3)	(1/3)	(0/3)	(1/3)	(3/3)	(1/3)	0
Receiving pen side rails	2	8	(7/8)	(6/8)	(6/8)	(6/8)	(8/8)	(6/8)	6
Water	1	5	(5/5)	(4/5)	(4/5)	(5/5)	(5/5)	(5/5)	4
Restrainer sidewalls	nd								0
Carcasses									
Hide	75	176	(101/173)	(59/173)	(67/173)	(88/173)	(136/173)	(92/173)	24
Colon	16	48	(45/48)	(23/48)	(17/48)	(40/48)	(42/48)	(40/48)	3
Totals	130	322							60

TABLE 3. *Number of* E. coli *O157:H7 positives recovered at the feedlot, truck trailers and packing house.*

Collection Site	Total # Positive Isolates	$^{a}Stx_1$ and Stx_2 Not Present
Feedlot		
Water	3	1
Pen floor	45	3
Feed	0	0
Feedbunk	2	0
Chute (Swab of side-panels)	9	1
Chute (Fecal from chute floor)	5	3
Transport		
Trailers (Swab of side-panels)	14	2
Trailers (Fecal from trailer floor)	5	1
Processing facility		
Receiving pen floor	3	2
Receiving pen side rails	8	2
Water	5	1
Restrainer sidewalls	0	0
Carcasses		
Hide	176	88
Colon	48	11

TABLE 4. *Comparison of sample relationship by comparing the similarities of band presence or absence in PFGE analysis based on the procedures of Tenover et al. (49) for Plant 1 (Midwest). Comparisons were made only with isolates expressing similar genotypes (Multiplex PCR analysis).*

Sample Types	Genes Present	No. of fragment differences	Epidemiologic Interpretation
Feedlot pen floor fecal/Plant pen floor fecal	$eaeA_{O157}$, stx_1, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	1	Indistinguishable
Loading chute side panel/Plant pen side rail	$eaeA_{O157}$, stx_1, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	2	Closely related
Loading chute side panel/Plant pen side rail	$eaeA_{O157}$, stx_1, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Feedlot Pen 1/Feedlot Pen 2 (Same feedlot)	$eaeA_{O157}$, stx_1, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	6	Possibly related
Hide/Truck trailer side wall	$eaeA_{O157}$, stx_1, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	5	Possibly related
Feedlot pen floor fecal/Plant pen side rail	$eaeA_{O157}$, stx_1, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	6	Possibly related
Truck trailer side wall/Feedbunk swab	$eaeA_{O157}$, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	5	Possibly related
Hide/Truck trailer side wall	$eaeA_{O157}$, stx_2, *hlyA*, $rfbE_{O157}$, *fliC*	6	Possibly related
Colon/Colon	$eaeA_{O157}$, stx_1, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Hide/Feedlot pen floor fecal	$eaeA_{O157}$, stx_1, *hlyA*, $rfbE_{O157}$, *fliC*	2	Closely related
Hide/Feedlot pen floor fecal	$eaeA_{O157}$, stx_1, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Colon/Colon	$eaeA_{O157}$, stx_1, *hlyA*, $rfbE_{O157}$, *fliC*	6	Possibly related
Hide/Hide	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Hide/Hide (Consecutive carcasses)	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	3	Closely related
Hide/Hide	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Truck trailer side wall/Plant pen water	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Hide/Plant pen water	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	5	Possibly related
Hide/colon	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	5	Possibly related
Hide/Hide	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Loading chute floor fecal/Truck trailer side wall	$eaeA_{O157}$, *hlyA*, $rfbE_{O157}$, *fliC*	5	Possibly related
Hide/Hide	*hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Hide/Hide	*hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Hide/Hide	*hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related
Hide/Pen water	*hlyA*, $rfbE_{O157}$, *fliC*	4	Possibly related

TABLE 5. *Comparison of sample relationship by comparing the similarities of band presence or absence in PFGE analysis based on the procedures of Tenover et al. (49) for Plant 2 (Midwest). Comparisons were made only with isolates expressing similar genotypes (Multiplex PCR analysis).*

Sample Types	Genes Present	No. of fragment differences	Epidemiologic Interpretation
Colon/Colon (consecutive carcasses)	$eaeA_{\mathrm{O157}}$, stx_1, *hlyA*, $rfbE_{\mathrm{O157}}$, *fliC*	1	Indistinguishable
Feedlot pen floor fecal/Colon	$eaeA_{\mathrm{O157}}$, stx_1, *hlyA*, $rfbE_{\mathrm{O157}}$, *fliC*	5	Possibly Related
Feedlot pen 1 floor fecal/Feedlot pen 2 floor fecal	$eaeA_{\mathrm{O157}}$, stx_1, *hlyA*, $rfbE_{\mathrm{O157}}$, *fliC*	2	Closely Related
Feedlot pen 1 floor fecal/Feedlot pen 2 floor fecal	$eaeA_{\mathrm{O157}}$, stx_1, *hlyA*, $rfbE_{\mathrm{O157}}$, *fliC*	5	Possibly Related
Colon/Feedlot pen floor fecal	$eaeA_{\mathrm{O157}}$, stx_1, *hlyA*, $rfbE_{\mathrm{O157}}$, *fliC*	5	Possibly Related

TABLE 6. *Comparison of sample relationship by comparing the similarities of band presence or absence in PFGE analysis based on the procedures of Tenover et al. (49) for Plant 3 (Southwest). Comparisons were made only with isolates expressing similar genotypes (Multiplex PCR analysis).*

Sample Types	Genes Present	No. of fragment differences	Epidemiologic Interpretation
Truck trailer side rail/Colon	*eaeA*$_{O157}$, *stx*$_1$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	3	Closely related
Feedlot 5 pen floor fecal/Hide	*eaeA*$_{O157}$, *stx*$_1$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	2	Closely related
Feedlot 4 pen floor fecal/Hide	*eaeA*$_{O157}$, *stx*$_1$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	4	Possibly related
Hide/Hide	*eaeA*$_{O157}$, *stx*$_1$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	3	Closely related
Feedlot 4 pen floor fecal/Hide (from Feedlot 5)	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	4	Possibly related
Feedlot 4 pen floor fecal/Colon	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	3	Closely related
Feedlot 4 pen floor fecal/Colon	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	2	Closely related
Feedlot 4 pen floor fecal/Colon	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	5	Possibly related
Feedlot 4 pen floor fecal/Colon	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	4	Possibly related
Colon/Colon	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	1	Indistinguishable
Truck trailer side rail/Colon	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	2	Closely related
Truck trailer side rail/Feedlot 4 pen floor fecal	*eaeA*$_{O157}$, *stx*$_2$, *hlyA*, *rfbE*$_{O157}$, *fliC*	5	Possibly related
Feedlot pen floor fecal/Plant pen floor [a]	*eaeA*$_{O157}$, *stx*$_1$, *hlyA*, *rfbE*$_{O157}$, *fliC*	3	Closely related
Truck trailer fecal/Colon [b]	*eaeA*$_{O157}$, *stx*$_1$, *hlyA*, *rfbE*$_{O157}$, *fliC*	6	Possibly related
Feedlot pen floor fecal/colon [a]	*eaeA*$_{O157}$, *stx*$_1$, *hlyA*, *rfbE*$_{O157}$, *fliC*	6	Possibly related
Hide (Feedlot 4)/Colon (Feedlot 5)	*eaeA*$_{O157}$, *hlyA*, *rfbE*$_{O157}$, *fliC*	6	Possibly related

[a] Sample dates were 10/4/2004 and 2/7/2005, respectively

[b] Sample dates were 10/13/2004 and 2/7/2005, respectively