

Translocation of *E. coli* in Whole Muscle Non-Intact Beef

by

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Abstract

Beef striploins were inoculated on the lean side with 8.2 to 10.1 log CFU/mL ranging from 6.4 to 7.2 mL of a nalidixic resistant *Escherichia coli*. Total CFU inoculated on striploins ranged from 1.12×10^9 to 9.10×10^{10} . Striploins were passed once, lean side up, anterior end first, through a mechanical blade tenderizer. Subsequent uninoculated beef striploins ($n = 5$) were passed once, lean side up, anterior end first, through the same mechanical tenderizer following inoculated striploins. Six core samples were taken from each striploin starting with the anterior end and each core was cut into six segments. Segments 1 through 4 represented the top 4 cm and segments 5 and 6 represent the remaining portion split in half. Following tenderization, pathogen levels were highest ($P < 0.05$) in loin 1. Loin 2 had higher ($P < 0.05$) levels than loins 4, 5, and 6. There were no differences ($P > 0.05$) between loins 3, 4, 5, and 6 which were below detectable limits. Levels of *E. coli* from segments 1 were higher than all other segments. Segment 2 had higher ($P < 0.05$) levels than 3, 4, 5, and 6. Pathogen recovery from segment 6 was higher ($P < 0.05$) than 3, 4, and 5. There were no differences ($P > 0.05$) between segments 3, 4, and 5. Data indicates that even with high initial inoculum numbers, contamination from one loin to another is quickly reduced to non-detectable levels.

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Review of Literature

Introduction

Consumers are becoming increasingly concerned about where their food comes from and how it is handled. This is especially true for today's modern meat packers as they face more public scrutiny than ever before. The biggest concern to the meat supply is the, "Adulteration," of meat with *E. coli* O157:H7, which is a virulent pathogen. Today, the United States Department of Agriculture (USDA) and Food Safety Inspection Service (FSIS) has implemented a testing protocol for tenderized beef because of the possibility that *E. coli* O157:H7 could be transferred from the surface of whole intact muscle or trim to the inner portions. Any lot of product testing positive must be used in a fully processed product or disposed of under the Federal Meat Inspection Act. According to USDA recommendations ground beef should be cooked to an internal temperature of 70°C to insure destruction of pathogenic bacteria that may exist internally in the product. The same thought process about ground beef is now being applied to whole muscle non-intact beef. The major food safety concerns are with blade tenderized or mechanically enhanced steaks. The concept is that people who enjoy their steaks cooked to a rare or medium degree of doneness may not reach internal cooking temperatures high enough to destroy potential pathogens. Questions to be asked and evaluated by both the government and industries are: (1) Does *E. coli* O157:H7 exist on primals destined for tenderization processes and if so, at what concentration? (2) Are these known concentrations being translocated to the inner portion of steaks and roasts? (3) If so, are

conventional cooking methods adequate to destroy the pathogens? (4) How effective are current intervention strategies on reducing pathogen numbers on primals destined for tenderization processes? *Escherchia coli* O157:H7 is the major health risk in non-intact beef but there are many other concerns involving the food safety of the product. Several pathogenic organisms can exist in beef products, and there are several processes that can be used to control pathogen's growth and contamination of raw products.

Campylobacter

The Campylobacter genus consists of 14 species, of which *C. jejuni* is of most importance to the food industry. This particular pathogen is a slender, spirally shaped rod that possesses a single polar flagellum at one or both ends. It is oxidase and catalase positive and it will not grow in the presence of 3.5% NaCl nor at a temperature of 25°C (Jay, 2000). Some strains of *C. jejuni* produce a heat-labile enterotoxin. This organism is also microaerophilic, only requiring 3-6% oxygen for growth. *Campylobacter jejuni* is also heat sensitive. When testing the heat sensitivity of *C. jejuni* in ground beef at 70°C, 10^7 cells/g inoculated into the beef could not be detected after the beef was heated for about 10 minutes (Stern and Kotula, 1982). Freezing also appears to significantly reduce cell numbers. Chicken with 10^5 *C. jejuni* per carcass frozen at -18°C showed substantial reduction or elimination of *C. jejuni* (Gill and Harris, 1984). In another study, the prevalence of *C. jejuni* and *C. coli* in 396 frozen and 405 fresh meats was examined. It was observed that about 12% of fresh meat tested positive while only 2.3% of the frozen meat tested positive, suggesting the lethal effects of freezing on the organisms (Stern et al., 1984). Campylobacter species also seem to be sensitive to drying. It has been demonstrated that the forced air ventilation used in the abattoir cooling rooms limits growth (Hui, 2001).

Unlike many other pathogens, *C. jejuni* is not found in the environment and is solely associated with warm-blooded animals. Initially Campylobacter spp. were known

primarily as organisms that cause spontaneous abortions in cattle and sheep (Jay, 2000). After more than 90 years of being identified as only an animal pathogen it was isolated from human diarrhea stools in 1971. Since then *C. jejuni* has been recognized as one of the leading causes of gastroenteritis. Today, it is known that a large percentage of all major meat animals harbor these organisms in the feces, with poultry contamination being prominent. Its prevalence in fecal samples can range from 30% to 100% (Jay, 2000). A study was conducted in 1985 which evaluated 2000 samples of a variety of retail store meats. Of these samples, 29.7% of chicken samples, 4.2% of pork sausage, 3.6% of ground beef, and about 5.1% of 1,800 red meats tested positive for *C. jejuni* or *C. coli* (Stern et al., 1985). Although a high percentage of cases indicate poultry as the principal carrier of the bacterium, raw dairy products or contaminated water are more often the vehicle of transmission (Hui, 2001).

The enteritis syndrome can be highly variable. Onset of the disease is anywhere from 48-82 h but may be as long as 7-10 d or more. The most common symptoms with campylobacter infection are diarrhea, malaise, fever, and abdominal pain (Hui, 2001). A reoccurrence of symptoms is experienced by 25% of patients (Mandel et al., 1984). The organisms can also be shed in the feces for more than two months after symptoms subside (Jay, 2000). Although campylobacter is one of the leading causes of gastroenteritis, it is not as virulent of an organism as *E. coli* O157:H7 and is mostly associated with poultry products which make it a minor concern for non-intact beef.

Clostridium

Clostridium botulinum is a Gram positive, anaerobic, rod shaped, spore forming bacteria. There are seven types that are recognized: A, B, C, D, E, F, and G. Of these types only A, B, E, F, and G cause illness to humans (Jay, 2000). They are also classified into two categories: proteolytic and nonproteolytic. One process of major concern is home canning because clostridia endospores can be fairly heat tolerant. This

is especially important when canning vegetables because the organism is most prevalent in soils and water. In the United States, type A occurs more frequently in the western states and type B is found more frequently in the eastern states as well as in Europe (Jay, 2000). Type E spores tend to be more confined to marine waters which makes them a major concern in seafood.

USDA classifications for shelf-stable food exist based on the growth and toxin production limits of *C. botulinum* strains. In order to be considered shelf-stable, foods must utilize at least one method of microbial inhibition. Such microbial hurdles exist in the form of low water activity, low pH, and addition of non-meat ingredients. It is generally recognized that growth does not occur at or below pH 4.5 (Jay, 2000). This is also the USDA limit for shelf stability when pH is the limiting factor. The lowest a_w that will permit growth and toxin production of any strain of Clostridia is 0.94 (Jay, 2000). There are many ingredients that are added to foods as a function of lowering a_w . Salt and sugar, at a level of about 10% and 50% respectively, will inhibit the growth of types A and B, and 3-5% salt has been found to inhibit toxin production in smoked fish chubs (Christiansen, 1968). In regards to heat resistance, proteolytic strains produce spores of high heat resistance, and have a minimum growth temperature of 10°C. In contrast, nonproteolytic strains produces spores with low heat resistance, and are capable of growing at refrigeration temperatures (Hui et al., 2001). Of the different types of strains of clostridia, type A strains have the highest thermal death time (D) for endospores with $D_{110} = 2.72 - 2.89$ (Jay, 2000). The radiation D values for endospores follow much of the same pattern as heat resistance D values. As with heat resistance, type A is the most resistant to radiation with a D value = 1.2 - 1.5 kGy (Jay, 2000). One reason why the growth of *C. botulinum* is more apparent in canned goods that have been either heat treated or irradiated is that clostridia cannot grow and compete with large numbers of other microorganisms (Jay, 2000). Foods that have been improperly heat treated may

have likely destroyed most vegetative cells but some spores may have survived. Spores can then germinate with little or no competition.

Clostridia produce a potent neurotoxin which is the most toxic substance known today. The lethal dose for humans is estimated to be in the range of 0.1 - 1 ng/kg (Montecucco et al., 1996). The toxin from type A strains has been reported to be more lethal than the toxins from types B or E (Jay, 2000). Although the toxin is the most potent substance known to man, it is quite heat sensitive, unlike the heat-stable toxins of some other pathogens. The botulinal neurotoxin can be destroyed at 80°C for 10 min, or at boiling temperatures (Jay, 2000). Symptoms of botulism can develop anywhere from 12 to 72 h after ingestion of the toxin-containing food. Symptoms consist of nausea, fatigue, dizziness, paralysis of the muscles, double vision, and respiratory failure. The mortality rate varies between 30% and 65%, with the rate generally being lower in European countries than in the United States (Jay, 2000). There is also another condition known as infant botulism that was first recognized in California in 1976. The condition typically affects infants less than a year old who have not yet established a normal intestinal biota. In infant botulism, viable spores are ingested, and upon germination in the intestinal tract, the toxin is synthesized (Jay, 2000). Infants can get viable spores from baby foods and possibly the environment. In a test conducted in 1982, 910 infant foods from ten product classes were analyzed. Of these classes, only two tested positive for spores: honey and corn syrup (Kautter et al., 1982). Of the 100 honey samples tested 2 samples contained type A, while 8 of the 40 corn syrup samples yielded type B.

It is obvious that pathogenic clostridia cells are a major food safety concern. However, they are more of a concern in canned goods in situations where canned goods were undertreated and only the heat tolerant endospores survived sterilization. Clostridia thrive in these conditions because they have no other microorganisms to compete with.

In a whole muscle non-intact beef product many other organisms exist naturally in the surface microflora making clostridia a minor health concern.

Listeria

Listeriae are Gram positive, non-spore forming, and non-acid fast rods. There are six species of *Listeria* that are recognized. Of the listerial species, *L. monocytogenes* is the pathogen of most concern to humans (Jay, 2000). Within this primary pathogen species there are 13 serovars. *Listeriae* is a very hardy pathogen which is one reason for its major food safety concern. Many studies have been conducted on the minimum pH range for growth of *L. monocytogenes*. In one such study, four strains of *L. monocytogenes* grew at pH 4.5 after 30 d in a culture media incubated at 30°C, but no growth occurred at pH 4.0 or lower (Parish and Higgins, 1989). Salt has also been found to inhibit growth but is not a method of prevention. At pH 4.66, time to visible growth was 5 d at 30°C with no NaCl, 8 d at 4.0% NaCl, and 13 d at 6.0% NaCl (Cole et al., 1990). *Listeriae* have also been shown to be psychotropic. The mean minimum growth temperature range on Trypticase Soy Agar of 78 strains of *L. monocytogenes* was found to be $1.1^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ (Junttila et al., 1988). Temperature is particularly important in food processes where temperature is a control point for pathogen reduction. *Listeria monocytogenes* has also been found to grow at low a_w values <0.93 , second only to *Staphylococci spp.* (Jay, 2000).

The *Listeriae* are widely distributed in nature. They can be found on decaying vegetation and in soils, animal feces, sewage, silage, and water (Jay, 2000). Within the food supply, organisms can be found in raw milk, soft cheeses, fresh and frozen meat, poultry, seafood products, and on fruits and vegetable products. In milk products, standard pasteurization protocols are adequate for destroying *L. monocytogenes* at levels of 10^5 - 10^6 /mL (Jay, 2000). In meat products, cooking to an internal temperature of

70°C for 2 min will destroy *L. monocytogenes* (Mackey and Bratchell, 1989). However, the addition of cure ingredients can have an effect on D-values. In a sausage type meat, the D value at 62°C was 61 sec, but when cure ingredients were added, the D value increased to 7.1 min, indicating some heat protective effects of the cure compounds, which consisted of nitrite, dextrose, lactose, corn syrup, and 3% NaCl (Farber, 1989).

Listeriosis in humans is not characterized by a unique set of symptoms because the disease depends on the condition of the host. Immuno-compromised hosts are the most susceptible. When susceptible adults contract the disease, meningitis and sepsis are the most common symptoms. Of 641 human cases, 73% of victims had meningitis, meningoencephalitis, or encephalitis (Jay, 2000).

Salmonella

The Salmonella bacteria are small, Gram negative, non-spore forming rods. Their optimum temperature range for growth is 35-37°C, but they can grow over a much wider range with growth occurring at temperatures as low as 4°C (Kelterborn, 1967). The Salmonella genus has 2,324 serovars and all have been placed into one of two species, *S. enterica* and *S. bongori*. There are 2,000 or so serovars that have been divided into five subspecies, most of which are classified under *S. enterica* which is the type species (Le Minor and Popoff, 1987). For epidemiological purposes, the salmonella can be placed into three different groups: 1) those that infect humans only, 2) those that are host-adapted serovars, 3) those that are un-adapted serovars with no host preference. Those that infect humans only include the agents that cause typhoid fever and the paratyphoid fevers, which are the most severe of all diseases caused by *Salmonellae* (Jay, 2000). The host-adapted serovars include those that can cause human illness and may be contracted from foods (Jay, 2000). Lastly, the un-adapted serovars are those that are pathogenic for humans and other animals, and they include most foodborne serovars (Jay, 2000). These organisms are widely distributed in nature. Humans and

animals are the primary carriers of the organism. Rodents, pests, reptiles and insects may also act as vectors for the transmission of salmonella (Hui et al., 2001). Salmonella food poisoning occurs from the ingestion of specific strains in significant numbers. The number of cells necessary to cause salmonellosis is 10^7 - 10^9 /g (Jay, 2000). From the time of ingestion symptoms usually develop in 12-14 h (Jay, 2000). Symptoms include muscle weakness, moderate fever, restlessness, drowsiness and persist for 2-3 d (Jay, 2000). Although these organisms generally disappear rapidly from the intestinal tract, up to 5% of patients may become carriers of the organism upon recovery from this disease (Jay, 2000).

Salmonellosis was first observed in cattle as early as 1902, but infection of cattle by salmonella had been considered rare as early as the 1960's. At the time a 5-y study at Cornell University of 731 *Salmonella typhimurium* cultures isolated from animal sources revealed that only 2 isolates originated in cattle (Jensen and Mackey, 1971). In poultry there are two public health issues related to asymptomatic carriage: contaminated broiler carcasses and salmonella-contaminated shell eggs (Hui et al., 2001). There is also a very high incidence of salmonella in swine. The USDA, Animal Plant Health Inspection Service analyzed 6,655 samples from 152 grower/finisher farms in a 1995 study. The sample and herd prevalence rates were 6.2% and 38.2%, respectively (Bush, 1995). It has been estimated that nationwide swine salmonellosis cost producers \$100 million yearly (Schwartz, 1990). Salmonella also poses a potential public health problem when meat from infected animals is sold; therefore disease prevention at the farm level is important. Animal feed can also be a vehicle for the spread of salmonella to animal herds. In a study of breeder/multiplier and broiler houses, 60% of meat and bone meal contained *salmonellae*, and feed was considered to be the ultimate source of *salmonellae* to breeder/multiplier houses (Jones et al., 1991).

Although *salmonellae* can cause extreme gastroenteritis and is widely distributed in animal feces and nature they are a minor concern in whole muscle non-intact beef. Since 10^7 - 10^9 cells/g are required to cause illness it is highly unlikely that this level of contamination would be translocated to the inner portions of steaks and roasts. The translocation rate of bacteria from the surface to the inner portions of non-intact beef has been reported to be less than 7% of the bacteria numbers on the surface of beef (Luchansky et al., 2008). Salmonella numbers that might exist at high levels on the surface of beef to cause infection in humans would be destroyed by conventional cooking methods.

Escherchia coli

Escherichia coli spp. is now a pathogen of major concern in today's modern meat industry. It is a Gram negative, facultative anaerobe that ferments lactose to gas but does not produce endospores. *Escherichia coli spp.* can be broken down into four virulence groups: enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC). There is a fifth group called enteroaggregative (EAEC) but this group lacks properties typical of the first four groups and possesses a particular adherence phenotype. All four virulence groups are widely distributed in the intestinal flora of humans and warm-blooded animals (Hui, 2001).

Enterohemorrhagic *E. coli* only affects the large intestine and produces large quantities of Shigella-like toxins (Jay, 2000). These toxins are now referred to as Stx1 and Stx2 toxins. The effects of temperature on Stx production varies. In one study, Stx production occurred at all temperatures that supported growth, however less toxin was found when cells were grown at 21°C than at 37°C even though cell numbers were similar (Abdul-Raouf et al., 1995). Of this group of *E. coli* the one strain of major concern to the meat industry is O157:H7. The prevalence of O157:H7 in foods is highly variable. According to a study on its prevalence in meats 3.7% of 164 beef, 1.5% of 264 pork,

1.5% of 263 poultry, and 2.0% of 205 lamb samples tested positive (Doyle and Schoeni, 1987). However progress has been made. In 1991-1992, a total of 1,400 samples of ground beef from retail stores in Seattle were examined for *E. coli* O157:H7 and all were negative (Tarr et al., 1999). Because of recent foodborne outbreaks of EHEC in ground beef it is widely considered that dairy herds are the primary carriers of this organism. A study on dairy herd fecal samples from 14 states in 1993 found that 31 of 965 (3.2%) tested positive for *E. coli* O157:H7 (Zhao et al., 1993). This suggests that there are other avenues for *E. coli* to reach the consumer and cause illness. In a risk assessment study on hamburger for O157:H7, the three highest ranked predictive factors relative to the probability of illness by this organism from hamburgers were concentration of the organism in animal feces, host susceptibility, and carcass contamination (Cassin et al., 1998). A number of studies have addressed the concentration of this organism in feces. In one study, cattle were fed a high-roughage diet for 4 d which significantly lowered *E. coli* O157:H7/g of feces but after 48 h of fasting they had significantly higher numbers (Jordan and McEwen, 1998). In another study on cattle fed mostly grain, cattle had a lower colonic pH and more acid-resistant *E. coli* than those fed hay (Diez-Gonzalez et al., 1998).

Enteroinvasive *E. coli* causes illness mostly in the very young and very old. Members of this group of *E. coli* prefer the colon and produce bloody or non-bloody, voluminous diarrhea. These cells generally do not produce enterotoxins, but they enter and multiply in colonic epithelial cells and then spread to adjacent cells in a manner similar to the *Shigellae* (Cheasty and Rowe, 1983).

Enteropathogenic *E. coli* generally do not produce enterotoxins but they can cause diarrhea. They possess adherence factor plasmids that enable adherence to the intestinal mucosa. After colonizing the intestinal mucosa, attachment effacement lesions

are produced (Jay, 2000). Enteropathogenic *E. coli* strains generally cause diarrhea in children under a year of age.

Enterotoxigenic *E. coli* affect both children and adults. It has been estimated that 10^8 - 10^{10} CFU are necessary to cause diarrhea by an ETEC strain in adult humans (Hui, 2001). There are two types of enterotoxins that are produced. One is a heat-labile (LT) and the other is heat-stable (ST). The LT toxin is destroyed at 60°C for 30 min, whereas the ST toxin can withstand 100°C for 15 min (Jay, 2000). Gastroenteritis by this group is characterized by non-bloody diarrhea without inflammatory exudates in stools. The diarrhea is watery and is similar to that caused by *V. cholera* (Jay, 2000). The first well-documented outbreak was a waterborne outbreak that occurred in a national park in Oregon in 1975. There were about 2,200 victims who drank improperly chlorinated water (Jay, 2000). However, there are many hurdles put in place today to help minimize or eliminate microbial growth.

Organic Acid Intervention

Carcass contamination is a major concern even for today's highly regulated industry practices. Even in the wake of the Hazard Analysis Critical Control Points (HACCP) programs and good management practices it is still possible that a carcass can become adulterated. Today, there are a few post-mortem intervention steps to decrease the surface microbe numbers. It is common practice to wash and trim the carcass of all visible contamination before final inspection is made.

One such practice is a hot water wash. A study conducted in 2002 evaluated the effectiveness of water temperatures ranging from 25 to 80°C at reducing *Enterobacteriaceae* populations on pork carcasses. It was found that temperatures of 55, 65, and 80°C reduced populations anywhere from 1 to 1.5 log/cm² on inoculated carcasses that were scalded (Eggenberger-Solorzano et al., 2002). An additional wash with an organic acid further lowered the *Enterobacteriaceae* count on the carcasses.

There are a few organic acid washes that are commonly used. The most commonly used are varying concentrations of lactic acid, acetic acid, and trisodium phosphate. In one study an industrial spray cabinet was used to deliver 1.5% and 3.5% lactic or acetic acid, or 12% trisodium phosphate wash. Initial aerobic plate count (APC) levels of approximately 5.6 log CFU/cm² were reduced by 1.3 to 2.0 log CFU/cm² by all five treatments (Dorsa et al., 1997). In a study to suppress the growth of bacteria in meat stored at 4°C for 21 d, organic acid intervention was evaluated. Beef carcass neck meat was collected immediately after harvest and inoculated with a bovine fecal cocktail. The meat was treated with either 2% lactic, 2% acetic acid, 12% trisodium phosphate, washed with 72°C or 32°C water, ground and stored up to 21 d at 4°C. Growth of bacteria was suppressed or not observed in the ground beef treated with lactic, acetic, or trisodium phosphate (Dorsa et al., 1998). Both water treatments offered little suppression of growth of pathogens.

Acid resistance of *E. coli* can be a concern when organic acid washes are applied as an intervention step. Exposure to low pH and organic acids in the bovine gastrointestinal tract may result in induced acid resistance. In one study, cattle fed mostly grain had a lower colonic pH and a 10⁶-fold higher level of acid resistant *E. coli* than those fed hay only (Diez-Gonzalez et al., 1998). This introduces the question of whether or not organic acid washes are effective against acid-resistant *E. coli*. One study tested the effectiveness of 2% acetic acid on three different strains of *E. coli*. The three strains were categorized as acid resistant, acid sensitive and inducible acid tolerance. It was observed that larger populations of acid-adapted cells than un-adapted cells remained after 2% acetic acid treatment and these differences remained after 14 d storage at 4°C storage (Berry and Cutter, 2000). This might suggest that the ante-mortem colonic pH of ruminants may be just as important as the post-mortem intervention steps used to keep residual pathogen levels down.

Many studies have been conducted on the effectiveness of organic washes. In 2007, Algino et al., did a study over a 9 month period, 265 head of beef were sampled from 22 very small beef slaughter facilities before and after their intervention treatments. Treatments applied were dry-aging, low pressure hot water spray, high pressure hot water spray, 2.5% acetic acid, and Fresh Bloom (Excaliber, Pekin, IL) spray. There were no significant differences among treatments and all treatments caused significant reductions in indicator organisms (Algino et al., 2007).

Chilling

A major intervention step in preventing the growth of pathogens on carcasses is carcass chilling. Modern facilities use forced air chilling rooms with subzero temperatures to quickly lower carcass temperatures. More importantly in terms of microbial control, the surface of these carcasses can be rapidly cooled so that the enzymatic activities of microbes that exist on the surface are slowed drastically. For most biological systems, every 10°C decrease in temperature results in a 1.5-2.5 decrease in the rate of reaction (Jay, 2000). However, microbes differ in their optimum growing temperatures. Organisms that grow well between 20°C and 45°C are referred to as mesophiles. In comparison, organisms that grow at refrigeration temperature are referred to as psychrophiles which would be more tolerant to cooling temperatures (Jay, 2000).

The effectiveness of chilling on *Salmonella* reduction on pork carcasses was evaluated at 10 different Wisconsin abattoirs. Within the study, 181 pork carcasses were sponge sampled at 24 and 48 h after chilling. *Salmonella* prevalence on chilled carcasses was 19.5% for 1 d and 14.7% for 2 d (Algino et al., 2009). Since most refrigeration temperatures are between 0°C and 7°C, chilling can be an effective control measure for most mesophilic pathogens. Psychrotrophic pathogens are a major concern however. Around 1960 the term psychrotroph was suggested for organisms that can

grow at 5°C or below but have their optimum growing temperature between 20°C and 30°C (Eddy, 1960). The three psychrotrophic organisms of concern to the meat industry are: *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Clostridium botulinum* Type E. In one study the effectiveness of chilling on a psychrotrophic pathogen was compared to that of a mesophilic pathogen. The occurrence of *Yersinia enterocolitica* on pig carcasses was compared to *Campylobacter spp.* *Yersinia enterocolitica* was isolated from 5 of 60 carcasses before chilling and also from 5 carcasses after 1 hour of blast freezing. In comparison, *Campylobacter spp.* was isolated from 34 of 60 carcasses prior to chilling and only 1 out of 60 after blast freezing. Blast freezing does not seem to have a significant effect on the human pathogenic *Yersinia enterocolitica* on pig carcasses (Nesbakken et al., 2008).

Irradiation

The first patent for the use of irradiation as a means of food preservation was issued in 1929. Serious consideration as a reliable method of food preservation was not given until after World War II. In 1983, the Food and Drug Administration (FDA) permitted spices and vegetable seasonings to be irradiated. Shortly after, the USDA granted permission in 1985 for the irradiation of pork to control *Trichinella spiralis*. The type of radiation of primary interest in the food industry is electromagnetic radiation. The various radiation types are separated on the basis of their wavelengths, with the shorter wavelengths being more damaging to microorganisms (Jay, 2000). The most effective radiations used in food preservation are ionizing radiations with wavelengths under 2000 Å. They consist of alpha particles, beta rays, gamma rays, X-rays, and cosmic rays (Jay, 2000). These methods of preservation are known as cold sterilization because they destroy microorganisms without raising the temperature of the product (Jay, 2000).

According to Jay, 2000, there are multiple influences on the effectiveness of irradiation. In general, Gram positive microorganisms are more resistant to irradiation

than gram negative. Cell numbers also have an effect. The higher the concentration of microbes the more resistant to a given dose of radiation they will be. Proteins have also been shown to have a protective effect against radiation. Oxygen also can influence the effectiveness of radiation. Complete removal of oxygen from the cell suspension of *E. coli* has been reported to increase its radiation resistance up to three fold (Jay, 2000). The lethal effects on bacteria subjected to varying A_w values follow much the same patterns as heat resistance. The radiation resistance for dried cells is considerably higher than that for moist cells (Jay, 2000). The position of a bacterial culture along its growth curve also effects sterilization efficiency. Bacteria tend to be most resistant in the lag phase just prior to active cell division (Jay, 2000).

The suggested terminology for radiation treatments in foods is broken down into three groups: radappertization, radicidation, and radurization. Radappertization is commonly used in the canning industry and is considered commercially sterile. Radicidation is similar to pasteurization in that the quantity of non spore-forming pathogens, such as *E. coli* O157:H7 and Salmonella are reduced to non detectable levels. Radurization refers to the reduction of cell numbers in order to enhance the shelf life of that product. The radurization effects of gamma ray and electron beam were tested on beef steaks inoculated with *S. typhimurium* and *P. fluorescens*. Both indicator organisms were not detectable after gamma irradiation but *P. fluorescens* was recovered after 2 days of incubation with the electron beam treatment (Chung et al., 2000). Both methods were effective at destroying *Salmonella* and potentially extending shelf life.

One major drawback to the irradiation of foods is the development of color changes and off-flavors. The effects of ionizing radiation on beef pigments were studied by determining the absorption of spectra of myoglobin solutions and the reflectance spectra of radiation sterilized beef slices. Results indicate that radiation reduces the heme iron of the brown pigment of cooked meat to an unstable red pigment, which

reverts back to a ferric pigment upon exposure to air (Kamarei et al., 1979). Off flavors tend to be a problem in some foods as well (Fan et al., 2004). By irradiating under anaerobic conditions, off-flavors are minimized due to the lack of oxygen to form peroxides (Jay, 2000). Slight nutritional changes also occur such as the breakdown of vitamin C, E, and B. However, these losses are comparable to those seen in other food processing methods (Holmes, 1984).

Consumer Acceptability

There are many traits that repetitively drive consumers to purchase cuts of meat. Some of the more important traits to consumers when deciding what to buy are meat color, fat content, and price. However, more often it is the eating experience that keeps consumers coming back to a specific product. Characteristics of meat that are involved in a good eating experience are tenderness, juiciness, flavor, and texture. Of these traits the easiest to extrinsically influence are tenderness and juiciness (Miller et al., 2001). Mechanical tenderization or injection of brine or marinade can create a more uniform supply of tender cuts in an area where tenderness is highly variable or typically tougher (Kolle et al., 2004).

Tenderness of steaks can be determined by using a Warner-Bratzler Shear (WBS) force value. According to a beef survey, consumer tenderness acceptability increased as WBS values decreased (Miller et al., 2001). The threshold value for consumer perception of tender to tough beef occurred between 4.3 and 4.9 kg of WBS (Miller et al., 2001). This means that consumers can accurately segregate differences in beef tenderness and that a consistent supply of tender beef may be the most important factor in meat production success.

Many companies have tried to market specialty brands to guarantee tenderness. A study was conducted to determine consumer impressions of Tender Select, a model

beef brand comprised of cuts from tender U.S. Select carcasses (Shackelford et al., 2001). A telephone survey was conducted in Denver to recruit 1,036 consumers for this study. Consumers who met minimal limits for household income, age, and beef consumption were invited to participate in a beef shopping and usage study in a local supermarket. Point-of-purchase material was developed that described Tender Select as "the only steak guaranteed tender and lean." When shown a copy of the Tender Select concept card, 89% of participating consumers (n = 759) indicated that they would definitely or probably buy that product. Of those consumers that said they would buy the product, 35% indicated that their purchases of Tender Select would be in addition to their current fresh meat purchases. Most consumers (54.1%) indicated that if Tender Select was available at their grocery store, 1 or 2 of their next 10 purchases of beef cuts would be Tender Select. In this particular survey, 65% of consumers indicated that if a grocery store carried a line of beef cuts guaranteed to be tender, they would buy all of their beef at that store.

There are a few processes that industry has implemented so that a uniform supply of tender beef can be produced. Whole muscle beef cuts can be tenderized through blade tenderization or injection of brine. Steaks blade tenderized one or two times received higher sensory panel ratings for myofibrillar and overall tenderness than steaks not tenderized (George-Evins et al., 2004). In a separate study, injecting round muscles with a salt and phosphate solution improved most palatability traits compared to blade tenderized or control steaks (Mueller et al., 2006). In either case, enhanced steaks or roasts were more acceptable than non-enhanced controls.

Blade Tenderization

One way to improve the perceived tenderness of a cut of meat is through mechanical blade tenderization. Blade tenderization involves a series of razor sharp blades or needles that are pressed into a cut of meat for a predetermined amount of repetitions. The transition in consumer perception from tender to tough beef occurs between 4.3 and 4.9 kg of WBS based on greater than or equal to 86% consumer acceptability (Miller et al., 2001). The industry goal is to create uniformity among beef cuts below the acceptable WBS values for tender beef. In strip loin and in sirloin steaks the WBS shear force was significantly lowered from 2.7 kg to 2.4 kg by needle tenderization (Pringle et al., 1998). In the same study, consumers were asked to rank the same steaks for tenderness. Needle tenderization did not affect sensory tenderness in strip steaks but improved sensory tenderness scores in sirloin steaks (Pringle et al., 1998). Results of this research indicate that blade tenderization is more important in beef cuts that typically contain higher levels of collagen. Mechanical tenderization improved initial and overall tenderness of, outside round, top sirloin, striploin, inside round, and chuck tender (Jeremiah et al., 1999). A study conducted by Bowker and others in 2007 evaluated the effects of blade tenderization, hydrodynamic pressure processing, and blade tenderization + hydrodynamic pressure processing on collagen solubility and tenderness related protein characteristics. They found that all three treatments increased collagen solubility and increased fragmentation of myofibrils by 35% compared with controls (Bowker et al., 2007). Results suggest that blade tenderization disrupts the muscle structure to improve tenderness.

Needle Injection

It is common practice to mechanically tenderize cuts of meat that are typically not perceived as very tender. One such method is injection of brine through multi-needle

injectors. The most common brine used is varying concentrations of CaCl_2 at varying percentages based on the green weight of the product.

Typically these practices have positive effects on beef quality traits. A study by Lansdell et al., 1995, evaluated the effects of CaCl_2 brine on beef quality traits. The un-injected control was compared to a 200 ppm CaCl_2 brine pumped at 5% of green weight and applied at 30 h postmortem. Subprimals used in the study were toploin and inside rounds sampled from 22 *Bos indicus* steers. Of the controls, 86% of top loin steaks and 78% of inside round steaks had WBS values > 4.5 kg. The injection of CaCl_2 reduced the percentage of steaks to 43% and 24% respectively (Lansdell et al., 1995). Trained sensory ratings for tenderness were also improved, while not affecting flavor intensity or causing any off-flavor problems compared with the controls. Lean color scores for top loin steaks were not affected by CaCl_2 , however inside round steaks had a lean color score lighter red than the controls.

Varying levels of CaCl_2 , pumping percentages, and time of injection can have different effects on the quality attributes of beef, specifically the tenderness. In one study by Wheeler et al., (1993), two experiments were conducted to determine the optimum protocol for maximizing meat quality with CaCl_2 injection. In experiment 1, the differing effects of injection time were evaluated with 175 ppm CaCl_2 or water injected at a level of 10% compared to the controls. Retail lean color of the CaCl_2 treatment was not affected at 24 h postmortem, but was slightly darker 30 min postmortem. Experiment 2 compared the effects of 24 h postmortem injection of 200 or 250 ppm CaCl_2 pumped at either 5 or 10% (wt/wt) to controls. All four treatments compared to controls reduced the variation in shear force with CaCl_2 (Wheeler et al., 1993). Small differences were seen in beef flavor intensity and off flavor ratings for CaCl_2 injected meat, but the small differences were of no practical significance. Injection of CaCl_2 is an effective way to increase tenderness

and decrease variation among cuts of meat without compromising other beef quality traits.

There is a potential concern for enteric pathogens being introduced to the center of a product when brine is injected into the whole muscle. Many factors are involved but the brine is considered a major factor in the transmission of pathogens. Consequently, non-intact, non-comminuted tissues should be cooked to a temperature sufficient to render these products safe to consume. These exact degrees of doneness were analyzed by Gill et al. (2008). Groups of 3 cm steaks were injected with 6 separate brines containing 2 or 5% of each of NaCl and sodium tripolyphosphate that were not supplemented or were supplemented with 2% soy protein or 2% emulsified sunflower oil. Brines were inoculated with a five cocktail strain of *E. coli* and *Listeria* at 8 log cfu/ml. Steaks were cooked to internal temperatures of 50, 55, 60, 65, or 70°C. Results indicated that *E. coli* was injured by the brines but not *Listeria*. Also, supplementation with protein and oil to brine did not protect the organisms (Gill et al., 2008). In addition it was established that cooking steaks to an internal temperature > 60 but ≤ 65°C was sufficient to inactivate all bacteria in the meat (Gill et al., 2008). In further narrowing down the temperature range of steaks, more tests were conducted using the same protocols but instead cooking to internal temperatures of 63, 64, or 65°C, with holding times of 0, 1, or 2 minutes. These results indicated that cooking to a temperature of 65°C without holding would be sufficient to reduced numbers of *E. coli* or *L. monocytogenes* by ≥ 7 log units (Gill et al., 2008).

Regulatory Background

There are many organisms that potentially exist on the outer surface of meat but the one of most concern in non-intact beef is *E. coli* O157:H7. One reason is that *E. coli* O157:H7 is an extremely virulent organism. *E. coli* O157:H7 was first recognized as a foodborne pathogen in 1982 when there were two confirmed outbreaks in Oregon and

Washington due to drinking water (Mead 1999). It wasn't until 1993 when *E. coli* O157:H7 caused an outbreak of illness that was attributed to meat. Eleven lots of beef patties produced on Nov. 29 and 30 of 1992 by Jack In The Box Corp. accounted for one of the biggest *E. coli* O157:H7 outbreaks in the meat industry (Duffy et al., 2006). There were 501 reported cases and 3 deaths attributed to this outbreak. Consumer concern about the safety of their food spurred a major regulatory risk assessment. In 1994, FSIS notified the public that raw ground beef contaminated with *E. coli* O157:H7 is adulterated under the Federal Meat Inspection Act unless the ground beef is further processed to destroy this pathogen. Also in 1994, FSIS began sampling and testing ground beef for *E. coli* O157:H7. *E. coli* O157:H7 did not show up in non-intact beef until 1995 at a graduation banquet in Wisconsin (Rodrigue et al., 1995). Sixty one out of 193 attendees developed a gastrointestinal illness traced back to a strain of *E. coli* O157:H7. It was mostly attributed to undercooked roast beef and salad cross contamination. In 1999 FSIS published a Federal Register notice explaining that all raw non-intact beef products, in addition to ground beef, that are found to be contaminated with *E. coli* O157:H7 must be further processed into ready to eat product, or they will be deemed to be adulterated. The notice also explained that if intact cuts of beef that are to be further processed into non-intact products prior to distribution for consumption are found to be contaminated with *E. coli* O157:H7, then they must be processed into ready to eat product or they will be deemed adulterated. FSIS would also consider it acceptable to irradiate these products prior to distribution for consumption.

Risk Assessment

Is *E. coli* O157:H7 a risk likely to occur in non-intact beef? There are four areas to evaluate in trying to answer this question. First, does *E. coli* O157:H7 exist on the surface of primal destined for tenderization processes? Second, are these known

concentrations being transferred to the inner portion of steaks and roasts? Third, if translocation of cells is occurring, are conventional cooking methods adequate to destroy cells? Fourth, how effective are current intervention strategies at lowering surface microbial numbers?

A study conducted in 2003 sampled six different processing plants over a 4 week period for the prevalence of *E. coli* O157:H7 on subprimals (Warren et al., 2003). Results showed that only two samples out of 1,014 (0.2%) were positive. A similar study conducted by the ABC Research Corp. sampled briskets, chucks, rounds, and middle meats from five different processing plants throughout the Midwest and Southeast (Kennedy and Badnaruk, 2004, 2005). One study was conducted from January to February and the other August to November. Results were very similar to those reported by Warren et al., 2003, which revealed that only 1 out of 1,200 (<0.083%) samples tested positive.

The degree of *E. coli* transfer from the surface of whole muscle products to the inner portions has been shown to be dependent on the number of microbes that exist on the surface of beef. In a study by Luchansky et al., 2008, initial inoculation levels ranged from 0.5 to 3.5 log CFU/cm². Core samples taken from top butts were separated into six different segments with the first four being 1 cm each originating from the inoculated surface. Regardless of inoculation level, transmission existed in segments 1 through 3. At the highest inoculation level, *E. coli* transmission existed in all six segments of the meat. There was no pathogen recovery from segments 4 through 6 from inoculation levels 1.5 log CFU/cm² or lower. Luchansky et al., 2008, also studied the degree of transmission depending on lean or fat side inoculation of top butts and single versus double passes through a blade tenderizer. They found no differences between lean or fat side inoculation or single versus double pass tenderization.

Conventional cooking methods are adequate to destroy pathogens that may exist on the surface of whole muscle products but may not be for internal translocation of pathogens in non-intact products. The survival of bacteria was studied by directly injecting a bacterial cocktail into the center of steaks and then cooking to different degrees of doneness. The temperatures being analyzed were 50, 55, 60, 65, 70°C while the organisms being evaluated were *E. coli* and *L. innocua* (Gill et al., 2008). Results indicated that possible recovery of both *E. coli* and *L. innocua* existed until an internal temperature of 65°C was obtained.

Several intervention strategies are currently being utilized by processing facilities. The effectiveness of these strategies was evaluated as a risk assessment for non-intact beef. The interventions studied were surface trimming, hot (82°C) water, warm (55°C) 2.5% lactic acid, warm (55°C) 5.0% lactic acid, activated lactoferrin + warm (55°C) 5.0% lactic acid. The application of all interventions reduced surface number 0.9 to 1.1 log CFU/ 100cm² (Heller et al., 2007).

To further evaluate the extent of *E. coli* transmission into non-intact beef, this study was conducted as a continuation of prior research in an effort to strengthen areas within the current risk assessment for non-intact beef that are lacking adequate data. This data will aid in the assessment as to whether *E. coli* O157:H7 is a hazard likely to occur in whole muscle non-intact beef.

Materials and Methods

Media Preparation

Petri dishes (Fisher 08-757-13) were prepared with 40 g/L of Tryptic Soy Agar (Neogen Corp. Acumedia 7100A, Lansing, MI) with 2.5 mL/L of stock Nalidixic Acid (Sigma-Aldrich N8878-25G, St. Louis, MO) at a concentration of 200 ppm. Petri dishes (n = 500) were filled using an automatic plate pourer (AES Laboratories, Combourg, France) with 18 mL of agar dispensed at 45°C. The petri dishes were stored at 10°C to reduce moisture loss over time.

Inoculum preparation

An *Escherichia coli* strain resistant to 200 ppm Nalidixic Acid was used as an indicator organism for the transfer of *E. coli* through blade tenderization. To prepare the inoculum, a single loopful of an isolated colony was transferred to four 10 mL test tubes of Tryptic Soy Broth (Neogen Corp. Acumedia 7164A, Lansing, MI) with 200 ppm Nalidixic Acid (Sigma-Aldrich N8878-25A, St. Louis, MO) and incubated for 24 h at 37°C. Following incubation, the inoculum was centrifuged (Adams Physicians) at 4300 rpm for 5 min. The supernatant was discarded and the cell pellets were suspended in 10 mL of sterile 0.009% saline solution and vortexed to evenly distribute cells within the test tube. Initial inoculum numbers ranged from 8.21 log CFU/mL to 10.06 log CFU/mL.

Inoculation of Strip Loins

Strip loins were purchased from a commercial beef processor and stored at 4°C for no more than 4 d. Forty mL of inoculum were then placed in a 200 mL aerosol spray bottle (VWR Innovations, West Chester, PA) to evenly distribute a range of 6 to 8 mL of

inoculum to the first loin to be tenderized. Inoculum was applied to the lean side of the strip loin under a Labconco Purifier Biological Safety Cabinet (Model 3440009, Kansas City, MO). The inoculated loin was then placed in a disposable foil serving tray, covered and placed in a coffin meat case at 4°C for 30 min to allow for cell attachment. The inoculated loin was then passed lean side up, anterior end first through a TC700M tenderizer (Ross Industries, Midland, Va.) followed by subsequent uninoculated loins (n = 5) run in the same manner. The tenderizer contained two blade sets with 7 rows and 32 blades per row. Each blade was 3 mm wide was set 10 mm apart within a row and also within a column. Each blade faced perpendicular to the direction of the conveyor belt. Each row alternated the angle of the blade. Each set of blades descended until the tips were within 2 mm of the conveyor belt. The conveyor belt advanced at a speed of 1.7 m/min. After being tenderized, loins were then placed lean side down in a clean foil serving tray and placed back into the meat case at 4°C until sampling.

Coring of Strip Loins

Starting with the last striploin tenderized, core samples were taken over a 1.5 h period. Striploins were cored in six different locations spanning the entire length of the loin starting with the anterior end. Cores (Humboldt MFG. CO, Schiller Park, IL) with a diameter of 19 mm (#12) were inserted from the fat side to the lean side (fat side up). Each core was removed aseptically from the loin and carefully removed the same way the sample entered the core (fat side forward) to prevent the sample from backing up on areas of the coring device suspected to have higher CFU's. Coring devices were then sterilized in 70% ethanol and flamed between each coring repetition. The cored sample was then placed on clean butcher paper and cut into six sections. The first four sections were 1 cm thick and represented the four sections closest to the lean side of the loin. Section 1 contained the first cm of the core including the lean side surface (inoculated side of loin). The remaining portion (after fourth cm) was then split in half and

represented sections 5 and 6, closest to the fat side or the uninoculated side of the loin. Sections 5 and 6 were about 1 cm in thickness each with section 6 including the fat side surface (dorsal side) of loin. Sections were measured with a ruler and cut using sterile surgical razors. Each section was put into a whirl-pak bag (Nasco, Atlanta, GA) and stored in a cooler with ice until maceration which was no longer than 1.5 h.

Maceration of Samples

Prior to conducting the experiment, 50 mL conical test tubes were filled with 45 mL of sterile Butterfields Phosphate buffer with an Omnisense (Wheaton Science Products, Millville, NJ) under a bio-safety hood. Whirl-pak bags (Nasco, Atlanta, GA) containing 5 g samples were then combined with 45 mL of phosphate buffer to achieve a 1/10 dilution (5g sample/45 mL buffer) and macerated in a stomacher (Seward 400, Bohemia, NY) for 2 min at 230 rpm. Sample cups were then filled with approximately 5 mL of dilute per sample (per core section).

Spiral Plating

Prior to spiral plating the petri dishes with media was removed from the refrigerator with sufficient time to allow the media to reach room temperature to prevent any shock to cells and allow for evaporation of any condensation. Each sample cup was then spiral plated (Don Whitley Scientific LTD, West Yorkshire, England). Petri dishes were plated with 50 μ L of sample. Serial dilutions of samples ranged from 1/10 to 1/10000. Once plated, the spiral plates were allowed time to dry before inversion to prevent spreading of surface moisture. Each plate was then inverted and incubated at 37°C for 24 h.

Plate Counts

Plate counts were done manually using a spiral plate counting grid. Areas within the grid were counted until a minimum number of 20 colonies were reached. That colony count and the corresponding area within the grid were recorded. The number of colonies

was then divided by the amount of mL plated corresponding to the specific area on the grid which yielded bacteria/mL.

Statistical Analysis

Data were analyzed as a split-, split-plot arrangement of a randomized block design. A run of an inoculated loin and five uninoculated loins served as a block. The loin served as the whole plot with the loin by run interaction as the error term for testing the loin effect. A core and core by loin interaction served in the first split plot with core within a loin and run as the error term for those main and interaction effects. The section and all remaining 2- and 3- way interaction effects served as the second split plot with the residual error being used to test these effects. Significance ($P \leq 0.05$) main effect and interaction means were separated by Fisher's protected LSD using the PDIFF option of LSMeans in SAS (SAS Inst., Inc., Cary, NC).

Results

Results for the contamination from one loin to the next are found in Figure 1. Total log CFU/mL for loin 1 (the inoculated loin) was more than 1 log higher ($P < 0.05$) than all other loins. Loin 2 did not differ ($P = 0.20$) from loin 3, but did have higher ($P < 0.05$) log CFU/mL than loins 4, 5, and 6. The remaining loins 3, 4, 5, and 6 did not differ ($P > 0.05$).

The interaction between loin and core acted independently ($P = 0.28$; Figure 2) of each other. The effects of core showed no differences ($P = 0.29$; Figure 3).

Results for the transmission of *E. coli* through core sections can be found in Figure 4. Total log CFU/mL for section 1 (the lean side) was much higher ($P < 0.0001$) than all other sections. Section 2 was higher ($P < 0.0001$) than for sections 3, 4, 5, and 6. Section 6 (the fat side) had higher ($P < 0.05$) log CFU/ mL than sections 3, 4, and 5. There were no differences ($P > 0.05$) between sections 3, 4, and 5.

The core and section acted independently ($P = 0.76$) of each other. The effects of loin was independent ($P = 0.22$) of section. The interaction between loin, core, and section was not significant ($P > 0.05$).

Discussion

Escherichia coli O157:H7 is a major food safety concern to today's food industries. *E. coli* O157:H7 fits into the Enterohemorrhagic group of *E. coli* and only affects the large intestine while producing large quantities of Shigella-like toxins (Jay, 2000). The prevalence of O157:H7 in foods is highly variable. As it applies to the meat industry, 3.7% of 164 beef, 1.5% of 264 pork, 1.5% of 263 poultry, and 2.0% of lamb samples tested positive for *E. coli* O157:H7 (Doyle and Schoeni, 1987).

The first well documented outbreak of *E. coli* O157:H7 happened in 1982 where there were 2 separate outbreaks attributed to drinking water throughout the Oregon and Washington regions (Mead, 1999). Since 1982, there has been an estimated 62,000 cases occurring annually with 1,800 hospitalizations and 52 deaths. As with most foodborne diseases the young, old, and immunocompromised are most at risk. The first major *E. coli* O157:H7 outbreak in the meat industry occurred in 1993 at the hands of the Jack in the Box Corporation. Eleven contaminated lots of beef patties were distributed throughout Washington, Idaho, Nevada, and California with a reported 501 cases and 3 deaths. It was then recognized that undercooked ground beef is a major food safety concern. Shortly after in 1994, FSIS notified the public that raw ground beef contaminated with *E. coli* O157:H7 is, "Adulterated," under the Federal Meat Inspection Act unless the ground beef is further processed to destroy the pathogen. Also in 1994, FSIS began testing and sampling ground beef for *E. coli* O157:H7. The first documented outbreak in non-intact beef was in 1995 at a university graduation banquet in Wisconsin (Rodrigue et al., 1995). Sixty one out of 193 attendees developed a gastrointestinal illness from a strain of *E. coli* O157:H7. The outbreak was attributed to undercooked

roast beef and salad cross contamination. It wasn't until 1999 that FSIS published a Federal Register notice explaining that all raw non-intact beef products, in addition to ground beef, that are found to be contaminated with *E. coli* O157:H7 must be processed into ready to eat product, or they will be deemed to be, "Adulterated." The justification for the 1999 policy is that *E. coli* O157:H7 is an extremely virulent organism, and *E. coli* O157:H7 may potentially survive conventional intact cooking methods.

A risk assessment for whole muscle non-intact beef products should be geared around the question, "Is *E. coli* O157:H7 a hazard likely to occur in whole muscle non-intact beef?" In doing this the major areas of question needing to be answered are: Does *E. coli* O157:H7 exist on primals destined for tenderization processes and if so, at what concentration do these cells exist at? Are these known concentrations being translocated to the inner portions of steaks and roasts? If so, are conventional cooking methods adequate to destroy cells? How effective are current intervention strategies at reducing surface microbial numbers?

Surface swabs of beef primals destined for tenderization processes are the best way to evaluate the presence of *E. coli* O157:H7. In one study, 6 different processing plants were sampled over a 5 week period (Warren et al., 2003). Of the 1,014 samples taken from subprimals, 2 samples tested positive with an incidence rate of 0.2%. In a similar study conducted by the ABC Research Corporation, briskets, chucks, rounds, and middle meats were swabbed for incidence of *E. coli* O157:H7 (Kennedy and Badnaruk, 2004, 2005). All together 1,200 samples were taken (200 per subprimal type) and of that one sample tested positive with an overall incidence rate of less than 0.083%. Although we are beginning to understand the incidence rate of *E. coli* O157:H7, there is a need for research examining the concentration of contamination. We are beginning to understand that the rate at which cells can be translocated to the inner

portions of steaks and roasts is dependent on the initial surface levels of *E. coli* O157:H7 on primals.

In 2008, a study was conducted on the effects of inoculation level on the translocation of *E. coli* O157:H7 (Luchansky et al., 2008). Top butts were used and regardless of the inoculation level (0.5, 1.5, 2.5, 3.5 log CFU/cm²) possible transmission existed between segments 1 and 3, which represent the top 3 cm of the top butts. It is important to notice that for inoculation levels up to 1.5 log CFU/cm², there was no pathogen recovery from segments 4 – 6. Also, there were no differences ($P > 0.05$) in recovery of pathogens in segments 2 – 6 regardless of inoculation levels. Results from the present study were similar with initial inoculum numbers above 8 log CFU/mL. There were no differences between segments 3 – 5 however; segment 6 did have higher *E. coli* numbers than segments 3, 4, and 5. The higher numbers in segment 6 are most likely due to cross contamination of the purge on the tenderizer conveyor belt and exist on the outer surface of segment 6. In phase II of the study, tenderization between the fat side and the lean side was tested as well as single pass versus double pass tenderization through the blade tenderizer (Luchansky et al., 2008). At 4 log CFU/cm² possible transmission to all segments existed. However, there were no differences ($P > 0.05$) between fat or lean side inoculation and single versus double pass tenderization.

The main focus of the current study was to evaluate the degree of contamination down the processing line from one contaminated striploin to subsequent uncontaminated striploins. The interaction between loins 1 – 6 acted much like the interaction between segments 1 – 6. Results indicated that after loin 3, initial inoculum numbers have been diluted down enough where there were no differences in loins from that point on. Data from this study, as well as by Luchansky et al., 2008, indicate that the limiting factor for internal contamination of non-intact beef is the amount of contamination present on the surface of primal prior to tenderization. Within the current risk assessment for non-intact

beef, there is lacking research on the condition and concentration of *E. coli* O157:H7 on contaminated whole muscle products immediately prior to tenderization processes. This is the area currently needing the most attention.

Research demonstrates that internal contamination of whole muscle non-intact beef is possible. The major concern is that conventional cooking methods are not adequate to destroy pathogens internally in non-intact beef if they are cooked to a, “Rare” or, “Medium rare” degree of doneness. In one study, a cocktail of *E. coli* and *Listeria* was injected into the center of tenderized steaks immediately prior to cooking (Gill et al., 2008). Steaks were cooked to internal temperatures of 55, 60, 65, 70, and 75°C. They found that by enumerating both *E. coli* and *Listeria* on resuscitating and non-resuscitating media that destruction of all pathogens did not occur until a minimum internal temperature of 65°C was reached.

There are many organic acid interventions that are in use today in processing facilities. As earlier mentioned, the concentration of pathogenic cells on the surface of primals is the limiting factor on the amount of cells that get translocated to the inner portions of steaks and roasts. Organic acid washes, baths, and sprays are commonly used to lower surface microbial numbers. In order to evaluate the risk assessment for non-intact beef we have to evaluate the efficiency of these solutions. In one study, 5 different treatments were tested against each other as well as a control. The treatments were: Surface trimming, Hot (82°C) water, Warm (55°C) 2.5% lactic acid, Warm (55°C) 5.0% lactic acid, Activated lactoferrin + Warm (55°C) 5.0% lactic acid (Heller et al., 2007). The mean pre-intervention sample numbers were 3.6 log CFU/100 cm². The application of all interventions resulted in a 0.9 to 1.1 log CFU/100 cm² reduction. All intervention treatments reduced ($P > 0.05$) surface numbers when compared to the control.

It is also important to notice that *E. coli* O157:H7 is a mesophile with an optimum growth temperature between 20 and 40°C (Jay, 2000). All processors today have adopted the HACCP program which was first developed by Pillsbury in the 1960's. The HACCP program sets critical control points and monitoring practices for all hazards biological, chemical, or physical. Temperature of the product and raw materials is one critical control point and is highly monitored. Most facilities today use forced air chilling rooms with subzero temperatures to quickly lower carcass temperatures. The surface of these carcasses can be rapidly cooled so that the enzymatic activities of microbes that may exist on the surface are slowed drastically (Jay, 2000). Once chilled all raw material and product are kept below 7°C to minimize microbial growth.

There are many factors to analyze when deciding whether or not *E. coli* O157:H7 is a hazard likely to occur in whole muscle non-intact beef. More research needs to be conducted on the prevalence and concentration at which *E. coli* O157:H7 that may exist on primals destined for tenderization processes. It is known that conventional cooking methods when non-intact beef is prepared to, "Rare" or "Medium rare," doneness are not adequate to destroy pathogens that may have been translocated to the inner portion of roasts and steaks. It is also known that current intervention strategies are effective at reducing surface microbial numbers by one log CFU/cm².

Implications

The purpose of this study was to examine extreme conditions in *E. coli* contamination of whole muscle non-intact beef and to evaluate the degree of internal translocation of cells as well as transmission of cells from one whole muscle product to another. The recovery level of indicator *E. coli* being below the detectable limit at the inner most location of striploins would suggest, that even at extreme conditions of contamination, the internal transmission of *E. coli* O157:H7 would be unlikely to occur. Contamination of striploins from one loin to another was detectable up to 5 loins. However, internal contamination was mostly attributed to the upper most 3 sections which would ultimately be heated to a higher degree of doneness than the inner most section, establishing a higher probability of the destruction of viable cells in the 3 uppermost sections.

Table 1: Solutions

1. Stock Nalidixic Acid
100 mL ddH₂O
0.8 g Pure Nalidixic Acid
Add Sodium Hydroxide (NaOH) until dissolved
2. Stock Butterfields Phosphate Buffer
34 g KH₂PO₄
500 mL ddH₂O
Adjust pH to 7.2 with 1 N NaOH
Bring volume to 1 L with ddH₂O
3. Butterfields Phosphate Buffer
1000 mL ddH₂O
1.25 mL Stock Butterfields Phosphate Buffer
4. Saline Solution
100 mL ddH₂O
0.9 g Sodium Chloride (NaCl₂)
5. 70% Ethanol
30 mL ddH₂O
70 mL Pure Ethanol
6. Tryptic Soy Agar w/ 200 ppm Nalidixic Acid
1000 mL ddH₂O
40 g Tryptic Soy Agar

25 mL Stock Nalixidic Acid (Added to agar after auto-claving and cooling to 45-50°C)

7. Tryptic Soy Broth w/ 200 ppm Nalidixic Acid

1000 mL ddH₂O

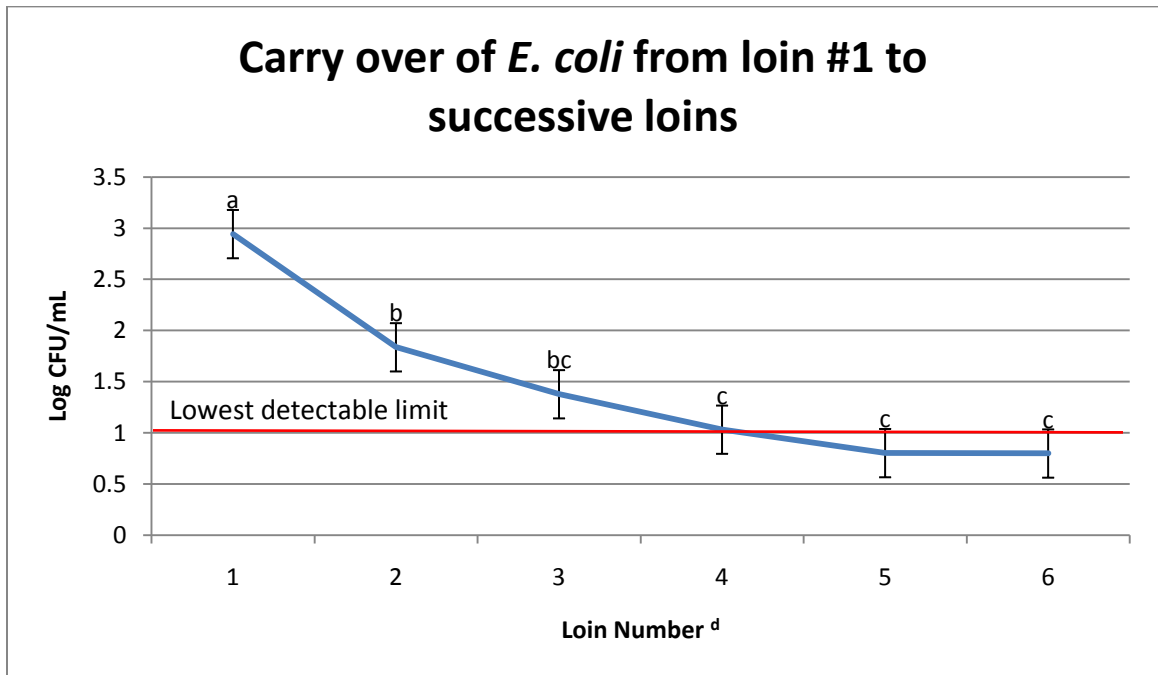
30 g Tryptic Soy Broth

25 mL Stock Nalidixic Acid ((Added to agar after auto-claving and cooling to 45-50°C)

Table 2		Loin, CFU/g					
		1	2	3	4	5	6
Core A	Section						
	1 cm	4.40	3.38	3.53	2.26	2.7	2.22
	2 cm	3.18	4.31	2.24	2.03	1.23	1.39
	3 cm	2.46	3.48	1.23	1.13	0	0
	4 cm	2.35	3.30	0.67	1.33	0	0
	5 cm	2.20	1.61	0.56	0	0	0.67
	6 cm	3.22	1.43	0.73	1.13	0	1.33
Core B	Section						
	1 cm	4.90	4.07	3.61	3.08	3.03	2.78
	2 cm	3.39	2.45	1.29	2.00	0.56	1.23
	3 cm	2.20	1.77	0.56	0	0.56	0.77
	4 cm	2.61	1.68	1.45	0.67	0	0
	5 cm	2.00	2.02	0	0	0.56	0
	6 cm	2.81	1.73	0	0.67	0.56	0.56
Core C	Section						
	1 cm	4.57	1.39	3.75	2.89	3.12	2.59
	2 cm	3.52	2.18	1.23	1.13	0.56	0
	3 cm	2.48	2	0.67	0	0	0
	4 cm	2.19	3.27	0.56	0	0	0
	5 cm	3.08	2.30	1.49	0	0	0
	6 cm	2.07	1.75	0.56	0	0.56	1.13
Core D	Section						
	1 cm	4.97	4.10	3.43	3.22	3.09	2.92
	2 cm	3.21	1.77	1.20	1.23	1.13	0.77
	3 cm	2.64	1.56	0	0.67	0	0.56
	4 cm	1.81	2.18	0	0	0	0.56
	5 cm	2.15	2.30	0.56	0.88	0	0
	6 cm	2.88	1.51	0.56	1.39	0	0.83
Core E	Section						
	1 cm	4.74	3.76	3.78	2.52	2.87	1.98
	2 cm	3.38	2.24	1.99	0.95	1.39	0
	3 cm	2.44	1.55	0.56	1.29	0	0
	4 cm	2.11	1.41	1.69	0.76	0	0
	5 cm	2.44	2	0.56	0	0	0
	6 cm	2.54	2.37	0.56	0.82	0	0.83
Core F	Section						
	1 cm	4.90	4.28	3.72	3.24	2.80	2.16
	2 cm	2.96	1.78	1.84	1.23	0.56	1.29
	3 cm	1.83	1.33	0.77	0	0.56	0
	4 cm	1.83	1.45	1.54	0	0.67	0
	5 cm	2.23	1.70	1.45	0.56	0.56	0
	6 cm	3.27	1.43	1.23	0	1.13	2.16

Table 2; Means for loin*section*core. Loin 1 was contaminated loin followed by five successive uncontaminated loins. Core A represents core location most anterior on loin. Section 1 represents uppermost 1 cm nearest and including the inoculated surface followed by successive 1 cm sections.

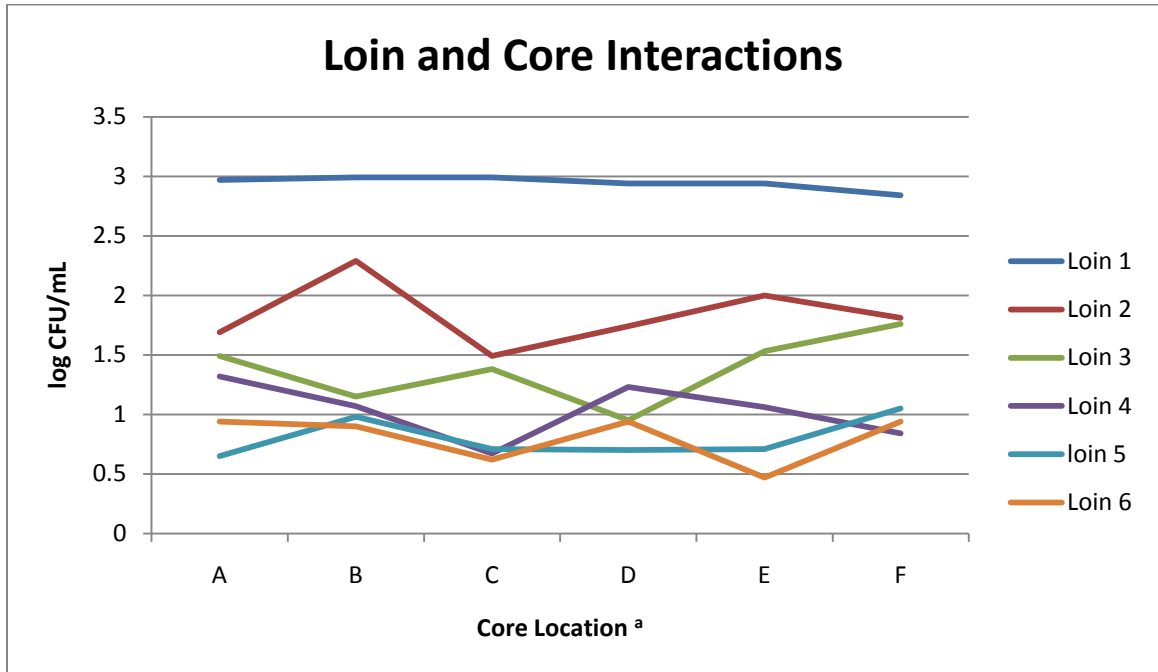
Figure 1. Effect of transmission of indicator *E. coli* resistant to 200 ppm nalidixic acid to subsequent loins.



^{abc} Means with a different superscript differ ($P < 0.05$).

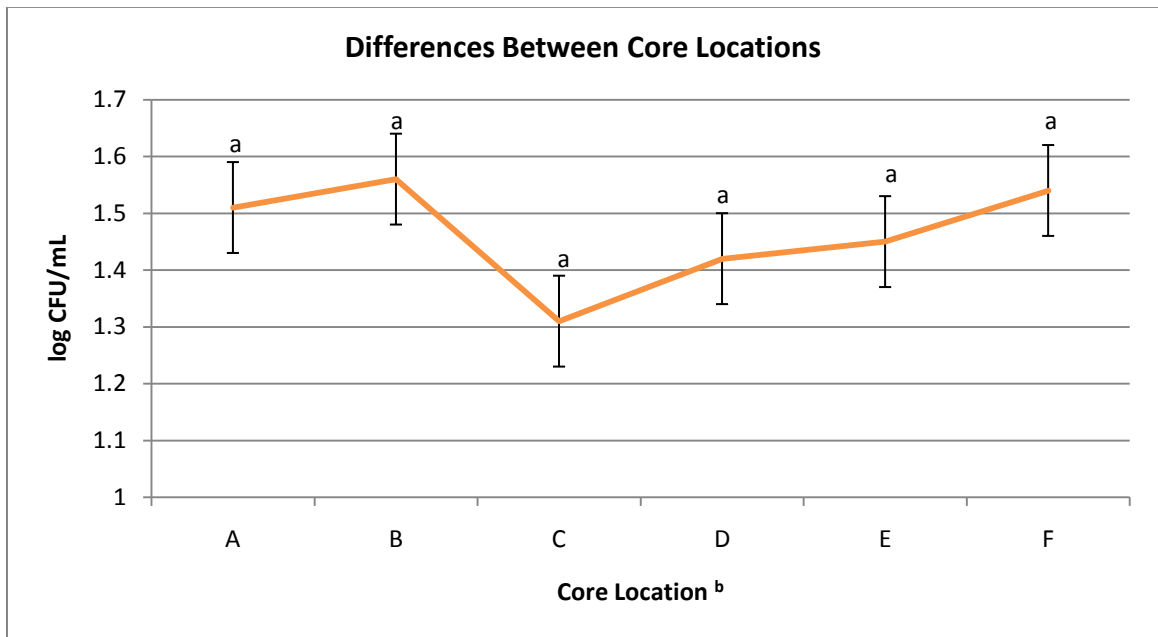
^d Lean side surface of loin 1 was inoculated with 8.21 to 10.06 log CFU/mL acid resistant *E. coli*.

Figure 2; The effects of loin and core interactions.



^a Core A represents core location mostly anterior on loin. Core F represents core location mostly posterior on loin.

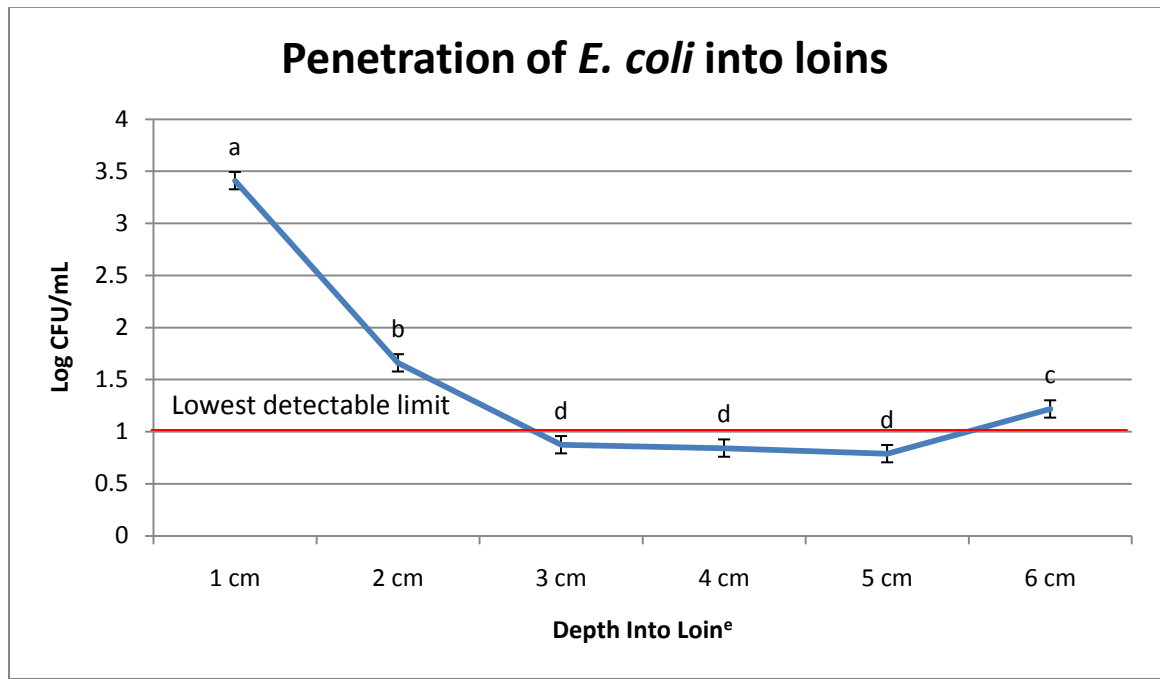
Figure 3; Effect of transmission of indicator *E. coli* resistant to 200 ppm nalidixic acid on core location of loin.



^a Means lacking common superscripts differ ($P < 0.05$).

^b Core A represents core location mostly anterior on loin. Core F represents core location mostly posterior on loin.

Figure 4. Effect of transmission of indicator *E. coli* resistant to 200 ppm nalidixic acid to inner segments of loin.



^{abcd} Means with a different superscript differ ($P < 0.05$).

^e Depth into loin is represented by lean side (1 cm) to fat side (6 cm) of loin.

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