Chapter 2
Fate of *Escherichia coli* O157:H7 in Meat

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Introduction

In the United States, the Center for Disease Control and Prevention (CDC) estimates that the number of foodborne illnesses annually is approximately 76 million cases, resulting in 325,000 hospitalizations and 5,000 deaths. Of those, almost 14 million cases of foodborne illness, 60,854 hospitalizations, and 1,800 deaths are caused by known foodborne pathogens (Mead et al., 1999). The cost of human illness, medical expenses, and productivity losses associated with the six most dominant foodborne pathogenic bacteria has been estimated to be between $2.9 and $6.7 billion dollars per year (Buzby et al., 1996). For decades the meat industry has been the center of some of the most costly outbreaks in world history.

*Escherichia coli* O157:H7 has been a major concern in the meat industry for decades and has increasing concerns with the development of new processing techniques. *E. coli* O157:H7 has been associated with food since 1982, but *E. coli* O157 is naturally found in the intestinal tract of cattle and in cattle feces (Rodrı́quez & McLandsborough, 2001). A cascade effect of *E. coli* O157:H7 can be seen during the slaughter and production process. *E. coli* O157:H7 in the feces of cattle can be transferred to the hide. The feces on the hide are transferred to the carcasses during the de-hiding process and from the carcass the knives and saws become a vector to transfer *E. coli* O157:H7 onto other cuts of meat. The contaminated cuts of meat are then ground and added to other animal’s cuts of meat. This is a possible cascade of events that can lead to massive amounts of ground products contaminated with *E. coli* O157:H7. The Hazard Analysis and Critical Control Points (HACCP) system and other quality programs have been established to reduce the risk of possible pathogenic contamination during manufacturing process. According to Food Safety and Inspection Service (FSIS) in 2007, *E. coli* O157:H7 was linked to 21 recalls.

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of meat products resulting in 90 infected persons with a foodborne illness and 33,358,521 pounds of product lost (Food Safety and Inspection Service, 2008).

How specifically *E. coli* O157 interacts with meat provides a greater understanding of areas within the production process that require an intervention step applied. In this chapter we will explore the different factors that influence “The Fate of *Escherichia coli* O157 in Meat”. This topic has been explored in Food Microbiology, Food Science, Biology, and Meat Science with the goal of providing a piece of this complex puzzle to create a safer food supply. Evaluation of patterns of recalls provides more details into where food safety needs to focus, the physiology and conditions of survival of *E. coli* O157 can aid in the development of intervention techniques as well as exploration of vectors that can be used to transfer *E. coli* from objects/instruments or equipment to meat products. Basic knowledge of the physiology of whole muscle cuts can provide insight into the specific locations where pathogens can be transferred. New food safety considerations with new techniques of processing like needle injection and need tenderization must be considered as well as an evaluation of current interventions can detail methods that have been shown to be successful in the reduction of *E. coli* O157:H7 on carcasses and in the meat. All these topics help to clarify the pieces to this massive puzzle.

**Escherichia coli O157:H7**

*E. coli* is a gram-negative, facultative anaerobic, non-spore-forming rod, which belongs to the Enterbacteriaceae family. Theodor Escherich first cultured ‘Bacterium coli’ in 1885 from the feces of a healthy individual. It was renamed *Escherichia coli* in 1919 in a revision of bacteriological nomenclature (Law, 2000). Many benefits have been found from *E. coli* in human medicine, food industry, and the water industry. Some studies suggest that *E. coli* can serve as a benefit to the human body by synthesizing vitamin K and by using competitive inhibition to out compete other bacteria that might enter the intestinal tract.

Differences between strains of *E. coli* lie in the combination of different antigens they possess. There are three types of antigens: the somatic lipopolysaccharide antigen (O), the flagellar antigens (H), and the capsular antigens (K). There are approximately 174 O antigens, 56 H antigens, and 103 antigens that have been identified. There are several stains of *E. coli* that have been isolated. The enteric *E. coli* are divided on the basis of virulence properties into enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), verotoxigenic (VTEC), entero-hemorrhagic (EHEC), and enteroaggregative (EaggEC). ETEC can be found in humans, pigs, sheep, goats, cattle, dogs, and horses; EPEC is found in humans, rabbits, dogs, cats, and horses; EIEC and EAggEC are only found in humans; VTEC is found in pigs, cattle, dogs, and cats; while EHEC is found in humans, cattle, and goats and attack porcine strains that colonize the gut in a manner similar to human EPEC strains (Fratamico et al., 2002).
There are several differences between the *E. coli* O157:H7 and other strains of *E. coli*. *E. coli* O157:H7 has a genome size of 5.4 Mb, Uropathogenic *E. coli* 5.2 Mb, and K12 5.2 Mb. *E. coli* O157:H7 and *E. coli* K-12 have many similarities in their genomes. It has been reported that 4.1 Mb are shared commonly on the backbone. Differences lay within the genes that code for the O strain and the K strain. There are 1.34 Mb that code for 1,378 genes in the O strain and 0.53 Mb coding for 528 genes in the K strain. Within the backbone there are 106 islands in the same location that have either O or K islands. K12 has a rough colony type because it has a partial LPS, while O157:H7 has a smooth colony type because it has a capsule and a full LPS. K12 does not have any toxins, adhesion factors, iron transport systems, capsule, or plasmids while O157:H7 does (Riley & Saier, 2007).

**Human Health Concerns with *E. coli* O157:H7**

*E. coli* O157:H7 was first described in 1975 in California after it was isolated from a woman with bloody diarrhea, but its identification as an enteropathogen was not until two, nearly simultaneous, U.S. outbreaks during 1982 (Ingham et al., 2006; Wells et al., 1983). It is considered a serious threat to public health in developed countries. In the United States alone, it is the single greatest cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Andreoli et al., 2002). *E. coli* O157:H7 causes the majority and most severe outbreaks of gastrointestinal illnesses related to *E. coli* (Peacock et al., 2001) from infections that range from asymptomatic conditions to mild bloody diarrhea or even severe hemorrhagic colitis. Severity of symptoms usually depends on status of the person infected with the pathogen, with the very young or immunocompromised suffering the most severe episodes.

Infection with *E. coli* O157:H7 can cause a wide variety of outcomes (Food Safety and Inspection Services, 2008; Ingham et al., 2006; Paton & Paton, 1998), with cases being reported worldwide. Bloody diarrhea (or hemorrhagic colitis, HC) caused by *E. coli* O157:H7, where infection of the large intestine occurs, is clinically different from that produced by other gastrointestinal pathogens. Clinical symptoms range from 1 to 8 days, with an average incubation period of 3 days (Peacock et al., 2001). Initially, patients develop abdominal cramps and watery diarrhea, with a varying percentage of these patients’ diarrhea resolving without further complications. The cramps can be very severe, with the cecum and ascending colon as the most affected areas that can mimic an acute abdomen inflammation and lead to exploratory laparotomy. Fever is usually absent or mild but occasionally can exceed 102°F (38.9°C). In mild disease without bloody diarrhea, patients have less abdominal cramps, vomiting, and fever and are less likely to develop systemic sequelae, hemolytic uremic syndrome (HUS), or to die (Su & Brandt, 1995). The occurrence of bloody diarrhea can happen as often as 15–30 minutes. Vomiting is also reported in...
about 30–50% of cases. Approximately 95% of the cases of HC resolve completely without further complication, however, the remaining 5% develop hemolytic uremic syndrome.

Hemolytic uremic syndrome, a term used for the first time in 1955, is defined as a disorder where kidney failure, hemolytic anemia, and thrombocytopenia (platelet deficiency) develops, usually after 7 days of the onset of diarrhea (Elliott & Robins-Brown, 1993). These symptoms are also accompanied by coagulation defects and variable nervous system signs (Hilborn et al., 1999; Elliott & Robins-Brown, 1993). The pathogen avoids expulsion from the body through its virulence factors causing an attachment that induces the transfer of verotoxin to the mucosa where it is transported by the epithelial cells and absorbed by the gut wall (Hilborn et al., 1999; Law, 2000; Paton & Paton, 1998).

History of *E. coli* O157:H7 in Meat Industry

Meat inspection was practiced in France as early as 1162, in England 1319 and in Germany 1385. In the United States meat inspection has been noted in the 1800s but mandatory inspection did not occur until 1906 with The Meat Inspection Act (Aberle et al., 1975). The government was stimulated by the release of the book *The Jungle* written by Upton Sinclair published in 1904. *The Jungle* reported poor food safety practices observed in the meat industry. The book outlined several areas during slaughter and manufacturing that needed further food safety implementation methods. The Meat Inspection Act of 1906 began the recognition and new regulatory standards that are mandatory to meat industry even today.

In 1981 and 1985 Congress passed several laws that focused on the inspection system focusing on the transmission of disease from animal to humans during consumption (Aberle et al., 1975). The need for wholesome products and the evaluation of live animals prior to slaughter for health concerns which included small butchers and farmers. The poultry industry soon followed with inspection regulations in 1957 with the Poultry Products Inspection Act. In 1967 and 1968 congress passed the Wholesome Meat Act and the Wholesome Poultry Products Act to ensure that processing plants would be held liable for the products being produced in their facilities (Aberle et al., 1975).

Many other laws were granted in years to follow, but education and training of employees were lacking in government documents. In 1986 the Processed Products Inspection Improvement Act provided a resource to the meat inspectors on how to allocate training and increase the overall effectiveness of product inspection. These measures were evaluated in the early 1990s when there was several highly publicized food poisoning with *E. coli* O157:H7 and meat products. In 1996 the USDA mandated the implementation and use of the HACCP in meat and poultry plants to help with food safety and aid as a prevention method. HACCP is designed to identify safety hazards that can by controlled
and monitored during the food production process. HACCP was first introduced by Pillsbury Company in 1959 to assure that food produced for NASA astronauts had a safe food supply during space travel (Aberle et al., 1975). The concept of prevention instead of reaction of hazards in the food resulted in a renewal of training and education to meat suppliers and producers. Sanitation practices, pre- and post-operational procedures, flow diagrams of all products produced and methods of slaughters as well as all forms used to monitor critical control points are documented in a record keeping section. Critical control points are steps within the production process that can reduce or eliminate the potential for a hazard (chemical, biological, or physical) to enter into the food product (Aberle et al., 1975).

HACCP and other programs (i.e., ServSafe) along with the USDA continual renewal of rules for the meat industry have re-ensured the consumers that the United States provides one of the safest supplies of food. FSIS along with universities throughout the world have collaborated to keep the latest information of pathogens and meat products current. *E. coli* O157:H7 has been a reoccurring problem in the meat industry.

The presence of *E. coli* O157:H7 in feedlots along with the presence in cattle feces has provided a challenge for cattle producers and packing plants. It is inevitable that cross-contamination will occur in these conditions. From 1998 until May 31, 2008, the pattern of outbreaks with *E. coli* O157:H7 and meat products reflects both changes in meat processing and increase in sampling in the processing plants. Figure 2.1 displays the pattern of recalls associated with *E. coli* O157:H7 and meat products over the past 10 years. From 2000 to 2002 there was a pattern of higher amount of recalls in meat products with *E. coli* O157:H7 in the United States. During this period a higher amount of smaller recalls with grocery food chains were reported. In many cases of these recalls less than 200 lbs of product was included in the grocery recall.

In October 2002, FSIS ordered all beef plants to re-examine their food safety plans, based on evidence that *E. coli* O157:H7 is a hazard reasonably likely to occur and to implement interventions to prevent it (Food Safety and Inspection Service, 2002). Scientifically trained FSIS personnel then began to systematically assess those food safety plans for scientific validity and to compare what was written to what was taking place in daily operations. A majority of the meat processing plants have made major changes to their operations based on the directive, including the installation and validation of new technologies specifically designed to combat *E. coli* O157:H7. Many plants have also increased their testing for *E. coli* O157:H7 in order to verify their food safety systems (Food Safety and Inspection Services, 2002).

Beginning in January of 2003 the Beef Industry Food Safety Council (http://www.bifsco.org/BestPractices.aspx) took representatives from every sector of the beef industry and diligently working together on unified best practice documents that will serve as a blueprint for making beef an even safer product. These documents are available to animal producers, slaughter facilities, and retail stores on the best practices to use to ensure a safer meat product. These
Fig. 2.1 Number of recalls and total amount recalled from 1998 to May 2008 in the United States associated with meat products and *Escherichia coli* O157:H7 according to the Food Safety and Inspection Services
documents are regularly updated with the newest research findings (Beef Industry Food Safety Council, 2008).

As a result of these actions, in 2003 the U.S. Department of Agriculture’s Food Safety and Inspection Service released data showing a drop in the number of *E. coli* O157:H7 positive samples in ground beef collected compared with previous years (Food Safety and Inspection Services, 2002). A noticeable reduce was observed in both number of recalls and the number of recalls resulting in more than 100,000 lbs requiring recalling status was observed (Food Safety and Inspection Services, 2008).

In 2007 there was another spike in recalls in the United States. In October 2007, the second largest meat recall of 21.7 million pounds of meat was announced. Forty cases of *E. coli* O157:H7 infections have been identified with PFGE patterns that match at least one of the patterns of *E. coli* strains found in Topp’s brand frozen ground beef patties. The ill persons from this outbreak resided in eight states [Connecticut (2), Florida (1), Indiana (1), Maine (1), New Jersey (9), New York (13), Ohio (1), and Pennsylvania (12)]. Two patients developed a type of kidney failure called hemolytic uremic syndrome (HUS) with no deaths reported (Food Safety and Inspection Services, 2007a). On the heels of this massive recall, Cargill Meat Solutions Corporation, a Wyalusing, Pennsylvania, firm, was voluntarily recalling approximately 1,084,384 pounds of ground beef products because they may be contaminated with *E. coli* O157:H7, the U.S. Department of Agriculture’s Food Safety and Inspection Service announced November 3, 2007. This recall affected over 30 different ground beef products distributed throughout the United States (Food Safety and Inspection Services, 2007b).

Immediately the FSIS responded to these high volume recalls investigating the food safety practices observed in these plants. Six days after this recall was issued, after 67 years of business Topps Meat Company was closed. This conclusion was indefinite after such a large recall. Hudson Foods Co. closed its plant in Columbus, Neb., after it agreed in 1997 to destroy 25 million pounds of hamburger in the largest U.S. meat recall after *E. coli* was found in the ground beef. The plant later reopened with new owners (Associated Press, 2007). The Food Safety and Inspection Services (FSIS) continues to monitor recalls and conducts research with many universities to ensure that the newest technologies and intervention tactics are used.

**Sources of *E. coli* O157:H7 Cross-Contamination**

There are many vectors that can be used to transfer *E. coli* O157:H7 on and/or into meat products. The feces of the animal can be transferred on the hides and carcass, the equipment can be contaminated, personnel might not use proper hygienic practices, airborne contamination, and rodents, insects, and other animals are all potential sources.
Depending on the sample size of hides and carcasses and the location of the study a range of between 1 and 40% prevalence of *E. coli* O157:H7 has been reported (Arthur et al., 2002; Bonardi et al., 2001). In a study with 355 beef cattle in the United States the scientist reported a 17% prevalence of positive samples for *E. coli* O157:H7 and a strong correlation between the fecal and hide prevalence with the carcass contamination (Elder et al., 2000). Several steps within the de-hiding process have been identified as causing the most cross-contamination of fecal matter. Hygienic practices in a slaughter facility can influence the presence of *E. coli* O157:H7 on carcasses and in the environment. Heuvelink, Roessink, Bosboom, and Boer (2001) visited several slaughter facilities and sampled the brisket, flank, and back surfaces and reported that 39% of the 27 slaughter plants visited had inadequate hygiene practices, resulting in 50% of the carcasses having visible contamination of feces that must be cut away. Arthur et al. (2002) reported that 56% of the hide samples in three abattoirs were positive for *E. coli* O157 with 41% of these being below 60 MPN/100 cm$^2$. In this same study 14.7% of the carcasses were positive for *E. coli* O157 with 83% of these below 1.3 MPN/cm$^2$. In this study the researches concluded that the rump region on the carcass was identified as having the most contaminated with fecal organisms than the other sites in one study and was linked to the skinning process and the presence of more fecal and dirt matter prior to slaughter (Bell, 1997; Gill et al., 1996). Besides the rump site, the hindquarter and flank were identified in three beef slaughtering facilities in a Canadian study (Gill et al., 1998).

Some researches believe that during transporting cattle to the slaughter facility that cattle *E. coli* O157:H7 might be shred and aid as a method for cross-contamination. Minhan et al. evaluated the influence of lairage and transportation in fecal shedding of *E. coli* O157 in cattle. No increase in the prevalence of *E. coli* O157 from farm to dressed carcass was observed. None of the 168 samples were positive for the dressed carcass. This study demonstrated that even positive cohorts of cattle may be slaughtered and processed to produce clean carcasses when hygienic practices are followed (Minihan et al., 2003). Madden et al. found similar results with cattle harvested in Northern Ireland with no positive *E. coli* O157 samples on 780 carcasses (Madden et al., 2001).

Equipment is also another vector for transferring bacteria from surface to meat products. Pathogenic bacteria transfer rates from contact surfaces to food items can be influenced by many factors. Rodriguez and McLandsborough (2001) found that the transfer rate of *Listeria monocytogenes* onto bologna was lowest with less pressure and shorter time on stainless steel surfaces. Oliveira et al. (2006) found that the ability of *Salmonella* to adhere to polyethylene and polypropylene was dependent on strain. *Salmonella Typhimurium* and *Campylobacter jejuni* have also been shown to have the ability to transfer from stainless steel to romaine lettuce after 10 seconds (Moore et al., 2003). Dawson, Han, Cox, Black, and Simmons (2007) also found that *S. Typhimurium* can be transferred almost immediately on contact and can survive up to 4 weeks on a dry tile surface.
while maintaining high enough populations to transfer to foods. Ingham et al. (2006) reported that *Streptococcus pyogenes* adhered almost immediately to various plastic, ceramic, and stainless steel utensils and were present at similar inoculated levels for at least 2 hours. Although these studies are not of *E. coli* O157:H7 they do provided evidence that equipment can be a source of cross-contamination due to attachment.

Personnel can also be a source of transfer of *E. coli* O157:H7 to meat products. If personnel do not wash their hands properly or do not change gloves often, then *E. coli* O157:H7 can be transferred from restrooms or from other uncooked meat products. Food safety training can aid in the reduction of potential occurrences with cross-contamination. Many studies have been conducted to determine what influences the retention of food safety training. In 2002 food handlers were given a survey to evaluate their beliefs and it was determined the best way to train. Sixty-three percent of the 127 participants admit to sometimes not carrying out food safety behavior because of lack of time, lack of staff, and lack of resources. It is recommended that risk-based approach and demonstrations can change the behavior of the food handlers (Clayton et al., 2002). In Florida comparison between outbreaks prior to training (1997–2000) and after training (2001–2003) determined that insufficient time or temperature during cooking, cross-contamination, bare-hand contact, insufficient cold and hot holding times were still the major causes of foodborne outbreaks before and after training, while the number of cases reduced from 5,671 prior to 3,568 after training. There conclusion is that training does help with food safety but knowing trends in contributing factors can help to determine areas of focus needed for food safety training (Hammond et al., 2005).

Food industry has the constant challenge of controlling rodents, insects, and other animals out of their plants and storage units. Most plants have adopted multiple hurdles and procedures to minimize entry of such animals. An active and aggressive rodent control plan is necessary to maintain continual control. Along with rodent control, airborne transfer is a potential source of contamination in the plant. The ventilation system needs to be maintained and included in the sanitation plan daily. Bird droppings and animals can live in the ventilation system and result in animal feces and parts to be sprayed during the ventilation of the plant. Also during the cleaning process, *E. coli* O157:H7 can be aerosolized and remain in the air for some time depending on the droplet size. Fans and other air circulation systems must be used with caution in environments where raw products can interact with cooled or further processed products.

There are many sources where contamination can occur such as airborne transfer, rodent contamination, hide/carcass transfer of pathogen, and personnel poor habits. Education to personnel and proper sanitation standard operating procedures (SOPs) can aid in the reduction in the transfer of *E. coli* O157:H7 and other pathogens throughout the plant and into the product.
Survival of *E. coli* O157:H7

Studies have focused specifically in the growth, survival, and inactivation characteristics of this pathogen (Bell, 1998; Juneja & Marmer, 1999; McClure & Halls, 2000). Studies have been performed to understand the behavior of *E. coli* O157:H7 in different substrates and foods for varying periods of time, as well as the effect that different intrinsic and extrinsic factors such as hot and cold temperatures, pH, organic acids, water activity (Aw), salt, control of reductio-n–oxidation potential (RO), fat content, irradiation, and preservatives will have on this specific pathogen. *E. coli* O157:H7 can be controlled by proper cooking of the food product to a specific temperature and time.

*E. coli* O157:H7 is a cause for concern especially if present in foods that do not go through a treatment process to eliminate the pathogen, or that could be contaminated after such process and before packaging as in the case of ready-to-eat (RTE) products. After the outbreak in 1993 (Anonymous, 1993; Bell et al., 1994), the USDA considered *E. coli* O157:H7 an adulterant if present in ground beef, setting for the first time in the United States’ history a zero tolerance policy for the presence of a microorganism in raw meat product (Heuvelink et al., 2001; Hollingsworth & Kaplan, 1997; Todd, 2004; Tuttle et al., 1999). Some examples of the foods involved with *E. coli* O157:H7 outbreaks include ground beef (Anonymous, 1993; Bell et al., 1994; Brandt et al., 1994) and meat products (Anonymous, 2007; Jay et al., 2004; Laine et al., 2005); apple juice (Anonymous, 1996; Besser et al., 1993; Cody et al., 1999); radish (Michino et al., 1999); raw sprouts (Anonymous, 1997); lettuce (Ackers et al., 1998; Hilbourn et al., 1999); and other types of fresh vegetables including baggy salads (Ackers et al., 1998; Anonymous, 2007; Sivapalasingam et al., 2004), as confirmed by the recent spinach outbreak in California.

Microorganisms are not killed instantly when exposed to a lethal agent, but rather, the population decreases exponentially. The D value or “decimal reduction time” is used in food microbiology to describe at any given temperature the time required in minutes to reduce 90% (or 1 log) of a specific microbial population in a specific food, and it is affected by factors such as pH, water activity (Aw), content of preservatives, product composition, and the size of the microbial population, among others. Studies have revealed that cooking ground beef with 17–20% fat at 57.2°C and 62.8°C have D values of 4.5 and 0.40, respectively. Cooking hamburgers to an internal temperature of 71.1°C (160°F) for 15 seconds is required to assure adequate cooking and prevent outbreaks (Doyle et al., 1997; Pflug & Holcomb, 1977; Stumbo, 1973; Wojciechowski et al., 1976).

Pasteurization is also an accepted heating method to destroy this pathogen in milk, fruit juices, and ciders. Treatment of milk for 15 seconds at 71.7°C (161°F) allows a 5-log reduction of *E. coli* O157:H7 and the same reduction is achieved in apple cider when it is pasteurized at 68.1 for 14 seconds (Al-taher & Knutson, 2004; Lawrie, 1998). Other studies have shown recovery of *E. coli* O157:H7 in
artificially inoculated foods after frozen storage. In one study, *E. coli* O157:H7 was recovered from inoculated strawberries, radishes, and cabbage after 2 and 4 weeks of storage at –20°C (Hara-Kudo et al., 2000).

Ground beef used in the manufacturing of hamburger patties is often produced in a central location and distributed under frozen conditions to fast food restaurants in different locations. In the 1993 *E. coli* O157:H7 multistate outbreak involving undercooked hamburgers, contaminated frozen patties produced by a single plant in California were involved with illness 6 weeks after the production date (Bell et al., 1994; Tuttle et al., 1999). Studies performed after that outbreak in inoculated ground beef patties (20% fat) revealed that *E. coli* O157:H7 can survive for up to 4 weeks after storage at –2°C with a 1.5 log reduction in the population. Storage of ground beef at –20°C for 12 months established recovery of the pathogen with an approximate reduction of 1.0 log (Ansay et al., 1999), demonstrating the ability of *E. coli* O157:H7 to survive in hamburgers for long periods of time at frozen temperatures with little decline in numbers of viable cells.

As seen in the examples above, *E. coli* O157:H7 displays a unique ability to survive in a wide variety of products subjected to different process conditions for long periods of time, allowing the foods to serve as vehicles in the transmission of infections.

**Physiology of Whole and Ground Meat Products**

The physiology of the meat product influences the likelihood of pathogens to be able to adhere and survive over time on the product. Whole muscle cuts have different areas of concerns with *E. coli* O157:H7 then further processed meat products. With intact whole muscle cuts, the interior of the cuts is sterile to vegetative pathogens. Internalization of any pathogenic microorganisms can only occur when the external surface is penetrated exposing the interior by the destruction of the myofibrillar structure of the meat. Meat tenderization methods such as brine injection and basic needle tenderization can place pathogens from the surface or from a contaminated needle into the interior of the whole muscle. The presence of water can also influence the transfer of pathogens internally.

The ability of meat to retain inherent or added water affects such eating attributes as toughness, juiciness, appearance, and the firmness of the bit (Lonergan, 2005). Water is held either inside the muscle cells or in the extracellular space. Largest amount of water is in the myofibrils and between the myofibrils. About 10% of the total water is held in the “I” band. There are many factors that influence the water holding capacity of a meat product. These include pH, protein structure alterations, alterations in the structural components, development of rigor mortis, and addition of substances. The absorption of contaminated water can place *E. coli* O157:H7 into the interior of the meat (Lonergan, 2005).
Also when a product is ground or sliced pathogens can be spread through the meat (Lonergan, 2005). Ground products have a greater probability of exposure to \textit{E. coli} O157:H7 than intact product because ground products have more exposure to equipment and personnel handling. Ground products must be cut up, which is mostly done manually, and then ground in a machine with meat from other animals. Equipment, tables and personnel add to the increased exposure of \textit{E. coli} O157:H7. Ground products are also sold in a raw state that requires consumers to properly cook the product to reduce the chances of illness. The lethality step is placed into the hands of consumers who rarely use thermometers during the cooking process. If this process is performed properly then raw products will be generally free from vegetative pathogens and most spores (survival of spores depends on the specific microorganism that produces it) (Buzby et al., 1996).

**Enhanced and Mechanically Tenderized Meat Concerns**

Sensory and quality attributes of tenderized meat have been studied extensively by many authors before; however, the microbiological aspects of this process have not received much attention until very recently. It is generally accepted that bacteria associated with meat are derived from the ingesta, the environment and the instruments used in the fabrication of the carcass, occurring only in the surface of the meat (Co, 1979). The internal muscles and deep tissues of the carcass are sterile unless they are subjected to a considerable breakdown of the connective tissue structure and muscle fibers. Similarly, during the process of carcass fabrication, Mechanical tenderization processes (such as blade tenderization, brine injection or marination) can introduce bacteria into the deep tissues of the subprimals (Gill & Penney, 1979; Gill et al., 2005a; Gill et al., 2005b; Sporing, 1999), which can become a problem if the meat is undercooked.

In an \textit{E. coli} O157:H7 risk assessment for blade-tenderized beef conducted at Kansas State University, beef top sirloin subprimals were inoculated with high levels of the pathogen (106 cfu/cm²) and subjected to one pass through a needle tenderization unit (Phebus et al., 2000). After evaluation of core samples, the needle tenderization process resulted in about 3.0 logs of the pathogen being translocated into the deep tissues (6 cm from the surface). Samples inoculated at low levels also resulted in a similar trend, with approximately 1.8 logs of the pathogen being transferred into the center of the meat cut. When determining adequate cooking temperatures for the steaks using an oven, the authors also reported that internal temperatures of 140°F and higher were needed to eliminate \textit{E. coli} O157:H7 by broiling.

In another study conducted by Gill and others (Gill et al., 2005), the microbiological conditions of the surface and deep tissues of beef mechanically tenderized at a packing plant were determined. The authors reported that the
tenderizing process did not significantly alter the numbers of bacteria (aerobes, coliforms, and *E. coli*) on the surfaces of strip loins and that none of them were recovered from the deep tissues of treated cuts. When these results are compared to those obtained by Gill and others (Gill & McGinnis, 2004), the results of the packing plant study revealed that the surface counts at retail stores were ≥2.0 log10 units more than those obtained in the plant. The authors suggested that not only storage was a factor on the high surface numbers obtained at retail stores, but that the cleanliness of the tenderizing equipment at the packing plant was a major aspect affecting the numbers of bacteria recovered from deep tissues. Other studies have also confirmed that the numbers of bacteria recovered from deep tissues of needle tenderized meat are significantly affected by the number of bacteria in the surface and the penetration depth but not by the number of “incising events” (passes) to which the meat is subjected (Gill et al., 2005a).

As well as with needle tenderization, injection of meats can pose the risk of translocation microbial flora and pathogens that are in the surface of the meat into sterile deep tissues of the cut. Some studies have tested the survival of different pathogens in the brine, a solution that is usually re-circulated and that if contaminated can subsequently inoculate additional cuts; however, just a few studies have focused on the surface-deep tissues translocation levels that can occur while enhancing meat products. Introduction of pathogenic microorganisms into the deep tissues of meat can result in a shorter shelf life and an increase in the risk of foodborne illness (Johnston, 1978).

In a study conducted in Canada, the brine used to pump moisture-enhanced pork was microbiologically analyzed for up to 2.5 hours after recirculation (Greer et al., 2004). The authors reported significant increases in the numbers of bacteria obtained from the brine after 1.75 hours of recirculation. After 2.5 hours of recirculation, the reported log CFU/ml counts were 4.50 (total plate count), 2.99 (lactic acid bacteria), 3.95 (*pseudomonas*), 2.79 (*Brochothrix thermosphacta*), and 3.01 (enterics); indicating that these solutions can harbor significant numbers of spoilage bacteria and can be distributed easily in the meat. In a recent study, the impact of a commercial injection process in the microbial flora of pork loins was studied. Aerobic bacteria recovered from re-circulated brine were >3.5 log10 units more than those obtained from the preparation tank after 30 minutes of processing (Gill et al., 2005c).

Similarly, other authors have tested the survival of pathogens in the brine and its effects on enhanced products. In one study, brine used to enhance eye of round primal cuts was inoculated with cultures of *L. innocua*, and portions of meat and brine analyzed after injection (Gill et al., 2008). The authors reported that the levels of this pathogen in the meat were about 0.72 log10 units less than those obtained in the brine. The authors also suggested that factors such as pumping pressure and number of strokes per minute can also affect the amount of brine (and therefore, pathogens) retained by the meat. Additionally, the authors suggested that if a meat product is subjected to both needle
tenderization and injection with brine, the enhancement process must be performed prior to the tenderization process to reduce the levels of possible contamination retained by the meat.

In a study conducted at Colorado State University, decontamination methods for *E. coli* O157:H7 were tested on beef subprimal cuts intended for moisture enhancement. Inoculated meat cuts were treated with hot water, lactic acid, and activated lactoferrin among other interventions and then injected with a brine solution containing 0.5% sodium chloride, 0.25% sodium tripolyphosphate, and 2.5% sodium lactate (Heller et al., 2007). The authors reported that treatment of the meat cuts with the interventions resulted in $0.9–1.1 \log_{10} \text{cfu}/100 \text{ cm}^2$ reduction (a significant reduction when compared to the control samples); however, no significant differences among treatments were found. When internal swab surfaces were analyzed, the process resulted in $<1.05\% \text{ cfu/cm}^2$ of surface pathogen transferred into the meat.

**Intervention Strategies**

Microbial contamination of meat with pathogens such as *E. coli* O157:H7 and *Salmonella* is a public health concern due to the outbreaks of foodborne illness commonly associated with the consumption of these products. The need to prevent these unfortunate incidents has prompted the incorporation of different types of control measures in the processing facilities in order to reduce and eliminate these pathogens from the food products and to prevent them from entering the food supply. Contamination of the carcasses can occur in different steps during the slaughter process, especially during de-hiding and evisceration of the animal.

As part of the adoption of the HACCP system, all beef processors and plants need to develop a plan that identifies the hazards that are associated with their respective process and the control measures that can be implemented in each step to reduce their likelihood in the food product. In the U.S. meat industry some of these control measures are known as interventions, proven procedures that significantly reduce microbial contamination from the meat surfaces, with many of them being used in sequence as part of a multiple hurdle approach. These control measures can be categorized into (a) physical (hot water spray, steam pasteurization, steam-vacuuming, water wash cabinet, and knife trimming); (b) chemical (organic acids, polyphosphates, chlorine, acidified sodium chlorite, ozone, peroxyacetic acid, nisin, and lactoferrin); (c) emerging technologies (hydrostatic pressure, irradiation, pulsed electric fields, and microwaves) (Samelis, 2005); and (d) biological (lactic acid bacteria and bacteriophages). The use of the previous interventions and their effectiveness on beef hides (Acuff, 2005; Koohmaraie et al., 2005), carcasses (Keeton & Eddy, 2004) beef trim/variety meats, and ground beef (Snijders et al., 1985; Koohmaraie et al., 2005) have been reported by previous authors, and they are often used in
addition to other procedures, such as inspection of the carcass and knife trimming of any visible feces, ingesta, hair, lesions, or bruises (USDA-FSIS, 1996). The effectiveness of the interventions and the levels of bacterial reduction that are obtained vary according to the testing methodologies that are used and the type of meat surface that has been tested, often leading to diverse results. Additionally, the concentration of the acid and its pH also determines the effectiveness of the compound against bacterial loads (Snijders et al., 1985). Examples of the effectiveness of different spray interventions in beef carcasses and meat products are summarized on Table 2.1. It is worth noting that even though interventions can reduce the risk of pathogens to be transferred to meat surfaces and their final products, they do not provide 100% assurance of safety. In addition, the use of interventions should not be viewed as a way to “clean” unwholesome products and in no case they can be a substitute for strict hygienic manufacturing practices and good cleaning and sanitation procedures in the processing facility.

In different studies conducted at Texas Tech University, the effectiveness of lactic acid producing bacteria ($\sim 10^7$ cfu/ml), acidified sodium chlorite (1,000 ppm), and lactic acid (3%) as intervention strategies to control *E. coli* O157:H7 and *Salmonella* Typhimurium DT 104 in non-intact beef products have been evaluated and proved effective against these pathogens (Echeverry, 2007). In one of the studies, inoculated boneless beef strip loins sprayed individually with the interventions and subjected to mechanical tenderization after 14 or 21 days of aging presented significantly lower *E. coli* O157:H7 counts in the internal muscle (between 1.2 and 2.0 logs) by the application of lactic acid and lactic acid bacteria (Echeverry et al., 2008a). In an additional study, inoculated strip loins sprayed with the interventions after 14 days of aging and followed by injection with a brine solution also presented lower internal *E. coli* O157:H7 counts (up to 2.0 logs) after the application of lactic acid bacteria and acidified sodium chlorite (Echeverry et al., 2008b).

**Conclusion**

*E. coli* is the most researched bacteria in the microbiological field. *E. coli* O157:H7 has been the cause of multiple outbreaks of food borne illness in the United States for more than a decade. The financial impact of producers and health concerns has caused an increase in research and acknowledgement of need. A growing population of people creates a need for food production that provides quality and safe products manufactured efficiently.

There are several areas within the meat industry that require further research to help in the continual goal of providing the safest product to consumers. Continual monitoring of *E. coli* and meat products, updating information about the physiology of *E. coli* O157:H7 and mutational changes.
Table 2.1 Selected studies on the effectiveness of different interventions sprays and dipping solutions used in the US meat Industry to control and eliminate different microbial pathogens in beef tissues

<table>
<thead>
<tr>
<th>Interventions</th>
<th>Concentration (temperature)</th>
<th>Microorganism</th>
<th>Log$_{10}$ reduction</th>
<th>Tissue/product</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot Water Wash</td>
<td>N.A (76–80°C)</td>
<td>Aerobic Plate Counts</td>
<td>4.3 CFU/cm$^2$</td>
<td>Beef tissue</td>
<td>Anderson, Marshall, Stringer, and Naumann (1979)</td>
</tr>
<tr>
<td>Trimming + HWW$^1$</td>
<td>N.A (35°C)</td>
<td>E. coli O157:H7</td>
<td>4.7 CFU/cm$^2$</td>
<td>Beef carcass</td>
<td>Phebus et al. (1997)</td>
</tr>
<tr>
<td>Hot Water Spray</td>
<td>N.A (95°C)</td>
<td>E. coli O157:H7</td>
<td>3.7 CFU/cm$^2$</td>
<td>Beef carcass</td>
<td>Castillo, Lucia, Goodson, Savell, and Acuff (1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella</td>
<td>3.8 CFU/cm$^2$</td>
<td>Beef carcass</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Typhimurium</td>
<td>2.9 CFU/cm$^2$</td>
<td>Beef carcass</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aerobic Plate Counts</td>
<td>3.3 CFU/cm$^2$</td>
<td>Beef carcass</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Coliforms</td>
<td>2.5 CFU/cm$^2$</td>
<td>Beef carcass</td>
<td></td>
</tr>
<tr>
<td>Water Wash</td>
<td>N.A (35°C)</td>
<td>E. coli O157:H7</td>
<td>3.3 CFU/cm$^2$</td>
<td>Outside beef rounds</td>
<td>Castillo et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella</td>
<td>3.4 CFU/cm$^2$</td>
<td>Outside beef rounds</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Typhimurium</td>
<td>2.9 CFU/cm$^2$</td>
<td>Outside beef rounds</td>
<td></td>
</tr>
<tr>
<td>Water Wash + Lactic Acid</td>
<td>2.0% (55°C)</td>
<td>E. coli O157:H7</td>
<td>5.3 CFU/cm$^2$</td>
<td>Outside beef rounds</td>
<td>Castillo et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella</td>
<td>5.4 CFU/cm$^2$</td>
<td>Outside beef rounds</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Typhimurium</td>
<td>&gt;0.9 CFU/cm$^2$</td>
<td>Beef Short Plate Pieces</td>
<td>Rose et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aerobic Plate Counts</td>
<td>&gt;1.0 CFU/cm$^2$</td>
<td>Beef Short Plate Pieces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Plate Counts</td>
<td>&gt;1.0 CFU/cm$^2$</td>
<td>Beef Short Plate Pieces</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Total Coliforms</td>
<td>&gt;0.8 CFU/cm$^2$</td>
<td>Beef carcasses</td>
<td>Dormedy, Brashears, Cutter, and Burson (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Mesophilic Bacteria</td>
<td>~1.0 CFU/cm$^2$</td>
<td>Beef Trim</td>
<td>Harris, Brashears Brooks, and Miller (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total Psychrotrophic Bacteria</td>
<td>~1.0 CFU/cm$^2$</td>
<td>Ground Beef</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Total Coliforms</td>
<td>~0.7 CFU/cm$^2$</td>
<td>Ground Beef</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Total E. coli</td>
<td>&gt;0.8 CFU/cm$^2$</td>
<td>Ground Beef</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella spp.</td>
<td>&gt;0.5 CFU/cm$^2$</td>
<td>Ground Beef</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.4 CFU/g</td>
<td>Ground Beef</td>
<td></td>
</tr>
<tr>
<td>Interventions</td>
<td>Concentration (temperature)</td>
<td>Microorganism</td>
<td>Log$_{10}$ reduction</td>
<td>Tissue/product</td>
<td>References</td>
</tr>
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</tr>
<tr>
<td>Lactic Acid</td>
<td>2.0% (N.A)</td>
<td><em>E. coli</em> O157:H7</td>
<td>&gt; 2.0 CFU/g</td>
<td>Beef Trim</td>
<td>Harris, Miller, Loneragan, and Brashears (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>Typhimurium</em></td>
<td>&gt; 1.0 CFU/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>4.0% (N.A)</td>
<td><em>E. coli</em> O157:H7</td>
<td>&gt; 2.0 CFU/g</td>
<td>Beef Trim</td>
<td>Harris et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td>&gt; 1.2 CFU/g</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>Typhimurium</em></td>
<td>&gt; 0.5 CFU/cm$^2$</td>
<td>Beef Trim</td>
<td>Harris et al. (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em> spp.</td>
<td>0.6 CFU/g</td>
<td></td>
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</tr>
<tr>
<td>Acidified Sodium Chlorite</td>
<td>1,000 ppm (N.A)</td>
<td></td>
<td></td>
<td>Beef Trim</td>
<td>Harris et al. (2005)</td>
</tr>
<tr>
<td>Acidified Sodium Chlorite</td>
<td>1,200 ppm (NA)</td>
<td><em>E. coli</em> O157:H7</td>
<td>&gt;1.1 CFU/g</td>
<td>Beef Trim</td>
<td>Harris et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td><em>Typhimurium</em></td>
<td>0.6–0.8 CFU/cm$^2$</td>
<td>Ground Beef</td>
<td></td>
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</tr>
<tr>
<td>Acidified Sodium Chlorite</td>
<td>1000 ppm (N.A)</td>
<td><em>E. coli</em> O157:H7</td>
<td>0.6–0.8 CFU/cm$^2$</td>
<td>Beef Briskets</td>
<td>Hajmeer, Marsden, Fung, and Kemp (2004)</td>
</tr>
<tr>
<td>Acidified Sodium Chlorite</td>
<td>1,200 ppm (22.4–24.7°C)</td>
<td>Staphylococcus aureus</td>
<td>0.8 CFU/cm$^2$</td>
<td>Beef Carcasses</td>
<td>Castillo, Lucia, Kemp, and Acuff (1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em> O157:H7</td>
<td>3.8–4.5 CFU/cm$^2$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Salmonella</em></td>
<td>3.9–4.6 CFU/cm$^2$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Interventions: HWW (Hot Water Wash)

$^2$ N.A: Not applicable/not available
Updating food safety concerns with new meat products (i.e., meat tenderized and \textit{E. coli} internalization) and development and evaluation of intervention strategies are some areas that need continual research and monitoring. The Food Safety and Inspection continues monitoring recalls and conducts research with various universities to ensure that the newest technology and intervention tactics are used.

The fate of \textit{E. coli} O157 in meat includes many sectors of the food science/meat science discipline which provide pieces to a complex puzzle of factors. Evaluation of recall patterns, the physiology/survivability of \textit{E. coli} O157, identification of vectors of transfer of \textit{E. coli} O157, the physiology of meat products, non-intact meat products concerns (enhancement, mechanical tenderization), and evaluation of current intervention methods all aid in the exploration of the fate of \textit{E. coli} O157:H7 in meat.

Food safety will continue to be the number one concern of the United States government and through the collaboration of many disciplines in food science the goal for the safest food supply in the world will continue to be reached.

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