THESIS

LONGITUDINAL STUDY OF *SALMONELLA ENTERICA*, *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN A SMALL AND VERY SMALL FRESH MEAT PROCESSING PLANT ENVIRONMENT

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ABSTRACT

LONGITUDINAL STUDY OF *SALMONELLA ENTERICA*, *ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN A SMALL AND VERY SMALL FRESH MEAT PROCESSING PLANT ENVIRONMENT

Small and very small fresh meat processing facilities have scarce resources to monitor foodborne pathogen contamination patterns and transmission dynamics in their premises. Environmental control of *Salmonella enterica, Escherichia coli* O157:H7, and *Listeria monocytogenes*, is important to prevent cross-contamination of meat products by pathogens that may persist in a facility. Although *Listeria* spp. are non-pathogenic, a high prevalence in a meat processing environment indicates failures in the cleaning and sanitation procedures. The purpose of this study was to conduct a six month longitudinal study to monitor *Escherichia coli* O157:H7, *S. enterica, L. monocytogenes* and other *Listeria* spp. contamination patterns, and to identify potential harborage sites in a small and very small fresh meat plant. Additionally, in order to gain insights about the facilities, manufacturing practices, and other relevant practices, managers from the two participating plants were asked to complete a questionnaire. Feedback of the study results was given to plant staff in a bilingual session, along with a basic training in food safety topics.

Both plants were sampled during mid-shift operation on a monthly basis. Environmental site ($n \leq 54$) and beef carcass composite samples were collected mid-shift. Samples collected included food contact surfaces (e.g., tables, scales, bins), and non-food contact surfaces (e.g., walls, drains, sinks). Overall, 1,979 environmental sponge samples were collected and microbiologically analyzed to detect and isolate *S. enterica*, *E. coli*

O157:H7 and *L. monocytogenes*. Further characterization of the recovered pathogen isolates by molecular subtyping (e.g. PFGE, ribotyping) was performed to gain insight in contamination transmission within the facilities.

S. enterica was isolated from 15 (4.5%) and 8 (2.4%) samples from Plant 1 and Plant 2, respectively. Characterization by PFGE using XbaI generated 6 different patterns in Plant 1, whereas all isolates from Plant 2 had the same pattern. *S. enterica* was recovered more than once from two sites in Plant 1, but only 2 isolates recovered from a drain in the slaughter area yielded the same PFGE pattern. *E. coli* O157:H7 was detected in 1.2% of samples in Plant 1; PFGE using XbaI generated 2 different patterns, and none was recurrently isolated from a single site. *E. coli* O157:H7 was not isolated from Plant 2.

Over the course of the study, roughly 28% and 6% of the samples tested positive for *Listeria* spp. other than *L. monocytogenes*, in Plant 1 and 2, respectively. *Listeria innocua* was the predominant *Listeria* spp. in both plants. *L. innocua* allelic type AT-1 was recovered from 15% of samples collected across Plant 1, whereas in Plant 2, type AT-6 was found mostly contained in the slaughter area. *L. monocytogenes* was isolated from 17% of the samples from Plant 1 and 1.2% of samples from Plant 2. Roughly 97% (54/56) of *L. monocytogenes* isolates recovered from Plant 1 belonged to ribotype DUP-1042B, which was recovered up to five times from 15 different sampling sites across the facility; the remaining two isolates belonged to ribotype DUP-1057B. Noteworthy, ribotype DUP-1042B belongs to a major human outbreak-associated clonal group known as Epidemic Clone I, posing a high risk for meat product contamination in this facility. Conversely, in Plant 2, *L. monocytogenes* DUP-1030B was recovered from three samples and only one isolate belonged to DUP-1030A; no single site in Plant 2 repeatedly tested positive for *L. monocytogenes*.

In light of the bacteriological results from this study, good manufacturing practices for the control of environmental contamination practiced by Plant 2 seem to be effective in the prevention of contamination spread and pathogen persistence; e.g. sanitizer dip stations at entry points, use of foam to clean equipment, use of quaternary ammonium compounds (QAC) or chlorine for sanitation of floors and other food contact surfaces, and QAC for sanitation of drains. Some high risk procedures practiced by the cleaning crew in Plant 1 may contribute to contamination spread; e.g., use of high pressure water for daily cleaning of drains, no sanitation step after cleaning drains, and lack of designated cleaning tools for drains.

Although economic resources may be limited, microbiological monitoring of the plant environment is useful from a risk assessment standpoint. In this study, the prevalence of *L. monocytogenes* was high and widespread in Plant 1, and a predominant strain belonging to an Epidemic Clone group was elucidated. This information increases awareness and provides the plant management with valuable information for decision making, and motivates the implementation of new policies and targeted interventions in problematic areas. Our findings suggest that *L. monocytogenes* and *L. innocua* have higher prevalence than *S. enterica* and *E. coli* O157:H7 in the fresh meat processing plant environments. While *L. monocytogenes* may persistently contaminate the environment of fresh meat processing plants, *E. coli* O157:H7 and *S. enterica* contamination appears to be mostly sporadic.

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LITERATURE REVIEW

AN OVERVIEW OF *SALMONELLA ENTERICA, ESCHERICHIA COLI* O157:H7, AND *LISTERIA MONOCYTOGENES* IN FOOD PROCESSING ENVIRONMENTS

1.1. Overview of foodborne illness

Foodborne pathogens, including 31 major pathogens and unspecified agents, pose a recurrent problem estimated to cause 48 million human foodborne illnesses, 128,000 hospitalizations and 3,000 deaths annually in the United States (Scallan et al., 2011a, b). Combined, *Listeria monocytogenes,* non typhoidal *Salmonella* spp.*, E. coli* O157:H7 and other shiga-toxin producing *E. coli,* are attributed about 17% of the total estimated number of episodes of domestically acquired foodborne illness; noteworthy, *Listeria monocytogenes* had the highest hospitalization rate (94%). In the United States and other developed countries, the food safety regulations and surveillance technologies associated with tracking foodborne illnesses have improved over the years. Nevertheless, there is a high risk of a large human death toll as a result of outbreak investigation delays due to novel strains, unexpected food sources and the greater difficulty for the consumer to be aware of often numerous ongoing food product recalls. A wide range of factors influence contamination, growth, persistence, and survival of pathogens throughout the food production process

from farm to table (Batz et al., 2007). An increasing number of vehicles for human exposure to foodborne pathogens have emerged, including foods that have not been previously associated with particular foodborne illness pathogens (i.e. listeriosis outbreak associated with cantaloupe consumption) (CDC, 2011b). Moreover, many emerging strains have been found to possess a combination of genetic traits that enhance virulence to cause disease in humans, as seen in the large-scale *Escherichia coli* O104:H4 outbreak which occurred in Germany from May to July 2011 (Cheung et al., 2011) and rising concern for antibiotic and antimicrobial resistance found in certain pathogen strains, particularly in biofilms (Høiby et al., 2010; Bridier et al., 2011).

Food processing companies, regardless of their production capacity and type of product, face the challenge of delivering safe food to their consumers. Increasing awareness of the public towards food safety topics and the numerous unfortunate episodes of foodborne illness and deaths, represent an increasing drive to take action in the prevention of contamination.

1.2. *Listeria monocytogenes*

Listeria genus and taxonomy

The genus *Listeria* includes Gram-positive, non-sporeforming, catalase positive rod shaped bacteria (Murray et al., 1926). The genus *Listeria,* until recently, only had six recognized species, including *L. monocytogenes*, *L. innocua, L. ivanovii, L. welshimeri, L. seeligeri,* and *L. grayi,* with only two species (*L. monocytogenes* and *L. ivanovii*) considered pathogenic. Two novel species have been described and included in the *Listeria* genus, *L. rocourtiae* first isolated from lettuce in 2002 (Leclercq et al., 2010), and *L. marthii* isolated from a natural environment in upstate New York in 2009 (Graves et al., 2010).

Listeria monocytogenes is commonly associated with listeriosis in more than 40 species of animals and humans. Genes encoded in the *Listeria* pathogenicity island 1 (Kreft

& Vásquez-Boland, 2001), and internalin genes (Ireton & Cossart, 1997) are key for *L. monocytogenes* virulence. Interestingly, the occurrence of atypical hemolytic *L. innocua* strains that carry the *Listeria* pathogenicity island I have been reported (Johnson et al., 2004). *L. ivanovii* shares certain characteristics with *L. monocytogenes* (i.e. hemolysis) and is occasionally associated with spontaneous abortions in ruminants, particularly sheep (Gyles, 2010).

Genetic diversity of *Listeria monocytogenes*

Listeria monocytogenes strains are grouped in four genetic lineages (lineages I, II, III, and IV). Lineage I includes major epidemic clone strains implicated in multiple listeriosis epidemics worldwide and it encompasses isolates belonging to serotypes 1/2b, 3b, 3c, and 4b. Lineage II contains isolates belonging to serotypes 1/2a, 1/2c, and 3a; these have been isolated from human cases and are overrepresented among food isolates. Recently, isolates previously classified as lineage III were shown to have specific phenotypical and genetic characteristics suggesting two divergent groups recently designated lineages III and IV (Ward et al., 2008; den Bakker et al., 2010; Orsi et al., 2010).

All *L. monocytogenes* strains are equipped with virulence genes encoded by its pathogenicity island (LIPI-1). However, as previously stated, the species is composed of three main lineages that are often associated either with clinical cases and outbreaks, or to food and environmental samples (Gyles, 2010). *L. monocytogenes* serovar 4b has been most commonly associated with human infection, followed by 1/2a, 1/2b and 1/2c strains (McLauchlin, 1990b). Those strains that have been frequently associated with outbreaks have been grouped in four epidemic clones (ECI, ECIa, ECII and ECIII); ECIa is overrepresented among isolates from outbreaks and sporadic human clinical suggesting those isolates have high probability of causing disease when exposure occurs (den Bakker et al., 2010); ECII are 4b strains associated with outbreaks in US and Europe; ECIII

represents a lineage II (1/2a) strain that persisted for at least 12 years in a processing facility and was also associated with a multistate outbreak (Kathariou, 2002; Olsen et al., 2005).

Listeria in nature

Listeria spp. are ubiquitous in the environment and described as saprophytic organisms. Weis and Seeliger (1975) reported it was readily isolated from soil, wood, decaying plant material, and feces of wild animals. Both *Listeria* spp. and *L. monocytogenes* have been isolated from plant-soil environment (Weis and Seeliger, 1975), water (Watkins and Sleath , 1981; Luppi et al., 1986; Colburn et al., 1990; Frances et al., 1991; Bernagozzi et al., 1994; Arvanitidou et al., 1997), silage animal feed (Wiedmann et al., 1996; Ueno et al., 1996), cut-grass from cattle feedlots (Mohammed et al., 2010), and farm environments (Fenlon et al., 1996; Nightingale et al., 2004).

Given the wide range of habitats and hosts where *Listeria* spp. can grow and multiply, it is easily spread through multiple transmission routes, finding its way to contaminate raw agricultural commodities and food processing environments (Beuchat, 1996). The bovine farm ecosystem maintains a high prevalence of *L. monocytogenes*, including subtypes linked to human listeriosis cases and outbreaks (Nightingale et al., 2004; Esteban et al., 2009). Prevalences of *L. monocytogenes* up to 24% have been reported in bovine farms, and up to 32% in goats and sheep farms (Nightingale et al., 2004); in cow-calf operations, herd prevalence ranges from 0-23% (Mohammed et al., 2010); prevalence of roughly 10% in a milking sheep farm and 3% in the associated dairy plant (Ho et al., 2007); a wider range (6-70%) has been found in cow and bull hides and bovine carcasses (Akkaya et al., 2007; Guerini et al., 2007). Moreover, *Listeria* spp. and *L. monocytogenes* have been isolated from pig carcasses and tonsils (Autio et al., 2000), cold-smoked pork (Bērziņš et al., 2010) as well as fresh water fish (Jallewar, 2007), lake whitefish, sablefish, farm-raised

Norwegian salmon, farm-raised Chilean salmon, wild-caught salmon (Hoffman et al., 2003), raw tropical seafood and smoked fish (Parihar et al., 2008; Thimothe et al., 2004).

Listeria in food

The ability of *L. monocytogenes* to adapt to different stress conditions found in food processing environments has been studied. Research studies have established that the pathogen is able to multiply in a wide range of temperatures, from -1.5°C through 45°C and at pH values from 4.0 through 9.6, growing optimally in the range 30–37°C (Petran and Zottola, 1989; Farber and Peterkin, 1991; Phan-Thanh et al., 2000). These characteristics bring direct implications for food safety, in food processing technologies used to prolong shelf life and in the event of cross-contamination of food, given that *L. monocytogenes* has also been reported to grow in solutions of up to 39.4% sucrose (a_w of 0.92-0.90) and high salt concentrations (up to 10%) (Petran and Zottola, 1989; Farber and Peterkin, 1991), and acid tolerance adaptation allows for survival even in high acid products (Gahan et al., 1996).

Given that ready-to-eat (RTE) meat products, seafood, salads, and soft cheese have instrinsic characteristics that support the survival of *L. monocytogenes,* and are not thoroughly cooked before consumed, these product are considered higher risk foods (CDC, 2011a). In addition to products that have been previously associated with listeriosis outbreaks, research studies have successfully recovered the pathogen from a wide range of ready-to-eat products, among others, seafood salad, smoked seafood, soft cheese, bagged salads, luncheon meats and deli salads (Gombas et al., 2003), vacuum-packed processed meats (Grau and Vanderlinde, 1992), dried sausage (Thévenot et al., 2005), boiled and partially cooked crawfish (McCarthy, 1997), and paté (Morris and Ribeiro, 1989). In 2003, the Food Safety and Inspection Service (FSIS) published in the Federal Registry a final rule to declare *L. monocytogenes* as an adulterant in a RTE product (Federal Registry, 2003).

Listeria in food-processing environments

Continuous incoming raw materials or live animals to the food processing location contribute to the constant introduction of the pathogen to the environment, thus *L. monocytogenes* might be considered endemic in food processing environments (McLauchlin et al., 2004). *L. monocytogenes* can survive for a long time under appropriate conditions of temperature and soiling (i.e. food residues), and might even survive to dry conditions (Palumbo and Williams, 1990). *Listeria* spp. can be isolated from food-processing facilities even when standard hygienic practices are in place (Farber and Peterkin, 1991). Some authors have suggested that adherence properties of *Listeria* may allow persistence and recurrence in plant environments (Kushwaha and Muriana, 2009).

Depending on the combination of various factors such as plant design and effectiveness of the hygienic hurdles and good practices in place, there might be transmission of the pathogen to food contact surfaces and ultimately to the product. Transmission of contamination in processing environments has been studied in different food processing facilities including: poultry and pork processing plants (Chasseignaux et al., 2001), milking facilities (Fox et al., 2009), catfish filleting plants (Chen et al., 2010), latin-style fresh cheese processing plants (Kabuki et al., 2004), ready-to-eat meat processing plants (Kushwaha and Muriana, 2009; Williams et al., 2011), restaurant food processing areas (Lakicevic et al., 2010), dried sausage processing plant (Thévenot et al., 2005), smoked fish processing plants (Lappi et al., 2004; Thimothe et al., 2004), and fresh mixed sausage processing line (von Laer et al., 2009).

With the objective of elucidating contamination patterns and prevalence of foodborne pathogens, researchers use a longitudinal sampling approach in food processing facilities. Williams et al. (2011) investigated contamination patterns of *L. monocytogenes* in RTE meat processing environments; a one year longitudinal study was conducted in six

small or very small RTE meat processing plants in Colorado, Kansas, and Nebraska. Analysis of a total of 688 samples that included environmental sponge samples collected from food contact surfaces (i.e., tables, knives), non-food contact surfaces (i.e., drains, floors, sinks, door handles), and finished product were performed for *L. monocytogenes* contamination. Overall, prevalence of the pathogen across different plants ranged from 1.7 to 10.80%. Similarly, von Laer et al. (2009) conducted a longitudinal study in a fresh mixed sausage processing facility in Brazil. Sixty-eight samples, including raw material, environment, food contact and non-food contact surfaces, workers' hands, and final product were analyzed over a 5 month period. The results showed the most prevalent serotype was 1/2c, however, presence of isolates from serotype 4b and 1/2b was found in the final product (von Laer et al., 2009). Findings were in agreement with previous studies that reported higher prevalence of 1/2c serotype, presumably associated with enhanced capacity for *L. monocytogenes* serotype 1/2c to attach to stainless steel surfaces that are commonly used in food processing activities (Lundén et al., 2000).

Other studies have found that serotype 1/2a was the predominate serotype of *L. monocytogenes* food and environmental isolates. O'Connor et al. (2010) performed subtyping characterization of 145 *Listeria* isolates from several food processing plants in Ireland over a three year period (2004–2007). The isolates came from a variety of food categories, including raw and cooked meat, dairy products, fruits and vegetables, cereal and other food ingredients and the food-processing environment (internal drains, floors, walls and door handles from several food-processing plants). The most common serovar was 1/2a (57.4%), followed by 4b (14.1%), 1/2b (9.7%), 4c (4.4%) and 1/2c (6.7%).

Pathogenicity and virulence of *Listeria monocytogenes*

Listeria monocytogenes has multiple strategies to invade a large panel of mammalian cells; host cell invasion is critical for several stages of listeriosis pathogenesis such as the

initial crossing of the host intestinal barrier, and nonphagocytic cell invasion (Seveau et al., 2007). The pathogen may cause systemic or central nervous system infections with high mortality rate and host survival depends on the host adaptive immune response; however, *L. monocytogenes*' ability to replicate in the cytosol of infected host cells and cell-to-cell spread enables it to avoid humoral immune responses (Pamer, 2004).

Recent studies using genome sequencing analysis have elucidated that about five percent of the *L. monocytogenes* genome is dedicated to encode surface proteins which provide anchoring systems possibly related to the ability to survive and interact with a variety of cell types (Cabanes et al., 2002). A number of bacterial proteins, including surface proteins internalins InlA and InlB, have been shown to contribute to bacterial invasion of host cells (Seveau et al., 2007). Similarly, Milohanic et al. (2001) presented evidence that the autolysin enzyme Ami plays a direct role in the addition of *L. monocytogenes* to eukaryotic cells via its cell wall binding-domain. Upon initial internalization in the cell, the pathogen forms a double-membrane vacuole and uses Listeriolysin O (LLO), a secreted pore-forming protein, to escape and further infect other cells (Gedde et al., 2000).

Risk population and disease

L. monocytogenes is capable of causing serious invasive disease including abortion, septicemia, meningitis, and meningoencephalitis in human and animals. Among humans, some of the predisposing conditions which are often associated with listeriosis include neoplastic disease, immunosuppression, pregnancy, extremes of age, diabetes mellitus, alcoholism, cardiovascular and renal collagen diseases, and hemodialysis failure (Conly and Johnston, 2008).

The primary route of infection is across the intestinal epithelium after consumption of contaminated food products by the host (Schlech et al., 1983; Linnan et al., 1988; Pamer, 2004). In pregnant women, a possible route of infection is intrauterine transmission to fetus

and neonatal infection (McLauchlin, 1990a). Other possible, but very rare, routes of transmission for human listeriosis are direct contact with the environment (O'Driscoll et al., 1999), contact with naturally infected animals (McLauchlin and Low, 1994), and cross infection between newborn infants (McLauchlin et al., 1986).

Outbreaks of human foodborne illness

Ready-to-eat meat products contaminated with *L. monocytogenes* have been associated with major outbreaks, as well as large volume food product recalls (Farber and Peterkin, 1991; Linnan et al., 1988; Graves et al., 2005; Conly and Johnston, 2008). Other foods implicated in documented outbreaks include homemade Mexican style cheese in 1985, 2000 and 2009 (Linnan et al., 1988; MacDonald et al., 2005; Jackson et al., 2011), delicatessen turkey meat in 2000 (Olsen et al., 2005), cheese in Germany 2006 (Koch et al., 2010), as well as sporadic listeriosis cases associated with various contaminated food products (Pinner et al., 1992).

The first documented multi-state outbreak of listeriosis associated with fresh whole cantaloupe took place in late 2011 (CDC 2011b). The outcome was at least 146 persons infected, 30 deaths and one miscarriage as reported to CDC from 28 states. Illnesses were associated with four strains of *L. monocytogenes*. After conducting an inspection of the implicated farms, FDA released an overview of factors that potentially contributed to the contamination of fresh, whole cantaloupe with the pathogen *L. monocytogenes* (FDA, 2011). The following were identified as possible contributors of contamination: from the growing environment, it is presumed that sporadic *L. monocytogenes* in the agricultural environment and incoming cantaloupe, as well as a truck used to haul culled cantaloupe to a cattle operation, may have contributed to the introduction of the pathogen into the packing facility. Futhermore, the plant had floors and equipment that were not easily cleaned; specifically, washing and drying equipment previously used for another agricultural

commodity; additionally, the process flow lacked a pre-cooling step to remove field heat from cantaloupes (CDC 2011b).

1.3. *Salmonella*

Salmonella genus and taxonomy

Salmonella is one of the most extensively studied bacterial species. *Salmonella* are facultative anaerobic, Gram-negative, non-sporing rod-shaped bacteria belonging to the family *Enterobactericeae*. The latest taxonomy convention of *Salmonella* groups two species, *S. enterica* and *S. bongori* (Reeves et al., 1989). *S. enterica* includes six subspecies, namely, enteric, salamae, aroynae, diarizonae, houenae and indica; and a subdivision of 2,521 serovars is presented in Table 1.1.

Table 1.1. Species and subspecies within *Salmonella* genus.

***Salmonella* species and subspecies**		**No. of serovars**
S. enterica	subspecies *enterica* (I)	1,504
	subspecies *salamae* (II)	502
	subspecies *arizonae* (IIIa)	95
	subspecies *diarizonae* (IIIb)	333
	subspecies *houenae* (IV)	72
	subspecies *indica* (VI)	13
S. bongori	(V)	22
TOTAL		**2,541**

Adapted from D´Aoust and Maurer (2007)

For epidemiological purposes, Jay (2005) suggests the following grouping: (i) those that infect humans only (*S.* Typhi, *S.* Paratyphi A, *S.* Paratyphi C); (ii) host-adapted serovars, including some human pathogens that may be foodborne [*S.* Gallinarum (poultry), *S.* Dublin (cattle), *S.* Abortus-ovis (sheep), and *S.* Choleraesuis (swine)]; and (iii) unadapted serovars, pathogenic for humans and other animals, including most foodborne serovars.

Salmonella in nature

Salmonella can be found in nonsymptomatic animals, animal feeds, and even air. The primary habitat is the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects (Jay, 2005). Bacteria are excreted in feces and can be spread in the environment. *Salmonella* has been isolated from water, soil, plant surfaces, animal feces, eggs, raw meats, raw poultry, and raw seafood, among other sources (FDA, 2009).

Research studies have gained insight in the cycle of contamination of *Salmonella* and dissemination patterns. The transmission of *Salmonella* spp. between production animals (pigs and cattle) and wildlife in Denmark was investigated by Skov et al. (2008). Samples from birds (n=2,567), rodents (225), insects and other animals (141) that lived in surrounding areas near farms were analyzed. *Salmonella* was isolated from insects (22.6%), rodents (5.2%), cats and dogs (6.5%), and wild birds (1.5%) living close to the infected herds. Phenotypical and genotypical typing of the isolates suggested that *Salmonella* was transmitted from infected herds of production animals to wildlife; detection of *S.* Typhimurium indicated that birds feeding on insects or invertebrates were at a higher risk of infection compared to birds feeding on seeds and grains.

Steneroden et al. (2011) estimated the prevalence of *Salmonella* in the environment in animal shelters in Colorado. Thirty-two animal shelters were sampled and 28% were positive for environmental *Salmonella* contamination. All animal shelters sampled in the Eastern plains (3/3) and one-third of the shelters on the Front Range showed environmental contamination with *Salmonella*. The authors hypothesized that increased prevalence of *Salmonella* in those areas could be due to presence and proximity of livestock facilities such as beef feed lots or dairy operations.

Humans that have an occupational exposure to *Salmonella* might spread the pathogen to their household if they fail to observe good sanitary practices. Rice et al. (2003) conducted a study to assess household contamination with *Salmonella enterica* where any of the residents had an occupational exposure. Exposure could take place on cattle farms with known salmonellosis in cattle, a *Salmonella* research laboratory, or a veterinary clinic experiencing an outbreak of salmonellosis. The authors analyzed vacuum cleaner bags and observed 27% (15/55) of samples from households with occupational exposure to *S*. enterica were positive versus 4.2% (1/24) without known exposure. These findings highlight the risk of transmitting contamination to the household, posing a health risk to the residents (i.e. crawling babies on contaminated carpets).

Food animal production environments have been analyzed to elucidate contamination patterns and persistence. Callaway et al. (2005) studied the effects of two production systems on *Salmonella* of sows housed indoors in farrowing stalls (n=52) compared to sows housed outdoors (n=52). No differences were detected in *Salmonella* between indoor and outdoor farrowing huts. Interestingly, the authors found some *Salmonella* genotypes persisted within some wallows for over 5 months, and genetically indistinguishable *Salmonella* isolates were found in multiple wallows. Gotter et al. (2012) showed that areas in the indirect environment, including ceilings, aisles and other surfaces, are possible major but often underestimated causes of residual *Salmonella* in swine farms.

Salmonella **in food and food processing environments**

Poultry meat and eggs are commodities that have high prevalence of *Salmonella*. Extensive research and regulation efforts have been oriented to the reduction of prevalence and control of *Salmonella* in poultry and egg production. The "*Salmonella* Verification Sample Result Reporting: Agency Policy and use in Public Health Protection" was issued by FSIS (2006). It announced the *Salmonella* verification sampling program for meat and

poultry establishments to be scheduled on risk-based criteria, focusing on those companies with previously reported higher *Salmonella* positive samples; furthermore, establishments are grouped in three categories based on consistent process control.

FSIS (2010) reported results on *Salmonella* testing of 29,734 verification samples across eight meat and poultry product classes. The following percent positive rates of *Salmonella* per product class were found: broilers (6.7%), turkey (4.6%), market hog (2.4%). In contrast, cattle positive samples were below 0.5% whereas processed fresh meat had positives: ground beef (2.2%), ground chicken (18.8%) and turkey (10.2%). In light of these reports, it is evident that control of the pathogen is a challenge for the industry and processed meat products show higher contamination, which could be due to cross-contamination or from bacteria harbored in the processing premises.

Salmonella endemic in farm premises may reach food processing facilities via transportation trucks, wildlife or other routes, and pose a risk of contamination to food. Kich et al. (2011) investigated the distribution and types of *Salmonella* in 12 swine finishing herds and a slaughter facility in Brazil. A total of 1258 samples (environmental, feed, carcass, lymph node, and feces) were collected. The distribution of positive samples was as follows: finishing pen floors, feed, pre-chilled carcass surfaces, and post-chilled carcass surfaces ranged from 24-29%, feces 44%, lymph nodes 46% and slaughter holding pens 90%. The authors observed correlations between pulsotypes from shedding pigs (feces), herd environment (pen floors), and lymph nodes. Furthermore, pulsotypes from the lairage correlated with carcass surface samples before and after chilling. These results illustrate the transmission contamination from environment to carcasses in the processing facilities. Similarly, Magistrali et al. (2008) reported that environmental contamination may have represented a major source of infection for pigs both on farm and during transport to the slaughterhouse (abattoir) in Italy. In their study, *Salmonella* was isolated from cleaned pens,

individual fecal samples, the truck used to transport the pigs to the abattoir and after slaughter (cecal contents, mesenteric lymph nodes and carcasses). *S.* Typhimurium was the most prevalent serovar at the farm level and other *Salmonella* were most prevalent during transportation and slaughtering stage. Swanenburg et al. (2000) reported *Salmonella* was isolated in 70 to 90% of samples from lairage areas collected when pigs were present. The usual cleaning and disinfection protocol led to a reduction of the contamination to 25% positive samples, whereas improved intervention achieved 10% positive samples.

Pathogenicity and virulence of *Salmonella*

Schmidt and Hensel (2004) reviewed in detail the pathogenicity islands (PAI) and their role in the virulence of bacterial pathogens. Most virulence factors of *S. enterica* are determined by chromosomal genes, and many of these are located within pathogenicity islands (PAI). The PAI of *S.* Typhimurium are known as *Salmonella* pathogenicity islands (SPI). SPI-1 and SPI-2 are essential for invasion and intracellular life of the pathogen; both encode a type III secretion system (T3SS) which aids in translocation of virulence proteins into eukaryotic target cells. The SPI-1 encodes a T3SS that delivers proteins into host cells. These proteins modify the regulation of the cell´s cytoskeleton, and finally result in the internalization of the pathogen into the cell. On the other hand, SPI-2 is essential to cause systemic infections and proliferation within host organs. The SPI-2-encodes a T3SS required for the protection of the pathogen within the *Salmonella*-containing vesicle against the host´s innate immunity. There are other SPIs and further details can be found in Schmidt and Hensel (2004). A schematic representation of the pathogenesis of *Salmonella* and its molecular basis is reviewed by Wallis and Galyov (2000).

Salmonella serotypes differ significantly in their pathogenic potentials. Jones et al. (2008) analyzed the confirmed cases of *Salmonella* infection (excluding *S.* Typhi and *S.* Paratyphi) reported in the surveillance network FoodNet from the period 1996–2006.

Interestingly, *S.* Choleraesuis was significantly more likely to cause hospitalization (60%) than the other 12 serotypes, including *S.* Typhimurium (24%), *S.* Enteritidis (21%), and *S.* Javiana (21%). According to CDC (2010), *S.* Typhimurium has decreased in incidence, whereas the incidence of serotypes Newport, Mississippi, and Javiana have increased.

Recently, Hendriksen et al. (2011) analyzed the global distribution of the 15 most frequently identified serovars of *Salmonella* isolated from humans from 2001 to 2007 in laboratories from 37 countries. Overall, 43.5% were *S.* Enteritidis and 17.1% were *S.* Typhimurium. Interestingly, serovars reported by developed countries showed a spectrum and distribution that remained relatively consistent over the years, as opposed to developing countries, where considerable year-to-year variability in both the serovar spectrum and distribution was observed.

Antimicrobial resistance of *Salmonella*

The use of antimicrobials for prophylaxis in food producing animals has been a great concern, because it is believed to be an important factor in the emergence of strains with resistance to certain antimicrobials (Threlfall et al., 2000). *Salmonella* remains present in the animals under the selective pressures of administered antibiotics. Increasing evidence supports the hypothesis that *Salmonella* bacteria exchange resistance genes among themselves when passing through the intestine (Butaye et al., 2006).

Marrero-Ortiz et al. (2011) conducted a study to identify and characterize antimicrobial resistance, plasmids and resistance genes in *Salmonella* isolates obtained from dairy cattle in Wisconsin. Results were compared to the human cases of salmonellosis reported in Wisconsin in 2006; there were a number of instances where the serovars isolated from dairy cattle were among the top fifteen associated with human infections, including *S.* Typhimurium. Kich et al. (2011) reported antimicrobial resistance profiles from *Salmonella* isolates from swine finishing herds and a slaughter facility in Brazil. A total of 59

different antimicrobial resistance profiles were observed in 572 *Salmonella* isolates. From these isolates, 17% were susceptible to all 15 antibiotics tested, 83% were resistant to at least one, and 43% were resistant to four or more antibiotics (multi-resistant).

Human consumption of antimicrobial medications has been associated with an increased risk of non-typhoid *Salmonella* infections. The increasing use of antibiotics is also believed to increase incidence of foodborne infections with drug-resistant *Salmonella.* Koningstein et al. (2010) conducted a study to determine the risk of salmonellosis attributable to human consumption of antimicrobial drugs. The case–control study surveyed 22,602 laboratory-confirmed *Salmonella* infections, diagnosed in Denmark between 1997 and 2005. The results showed that increasing use of antibiotics, particularly fluoroquinolones, was associated with increased incidence of foodborne infections with drug-resistant *Salmonella*.

Mølbak (2005) described the susceptibility of humans to pathogenic bacteria could be separated into a "competitive effect" and a "selective effect" that offer advantages for certain resistant pathogens. The competitive effect takes place by depleting the normal gut flora, thus the host is susceptible to a smaller dose of ingested *Salmonella.* The selective effect gives advantages to the resistant *Salmonella* strains that already colonized the gut when the antimicrobial is taken.

An important factor associated with the increase in multidrug resistance among particular *Salmonella* spp. is the international spread of certain clonal genotypes, i.e. the global epidemic spread of multidrug-resistant *S.* Typhimurium DT104, since the early 1990s (Butaye et al., 2006). *S*. Typhimurium DT104 is characterized by its resistance to five antimicrobials—ampicillin, chloramphenicol, streptomycin, sulfa drugs, and tetracyclines, and has acquired resistance to trimethoprim and the fluoroquinolones (Jay, 2005). Researchers hypothesize that the resistance may be associated with increased virulence,

and several epidemiological studies have demonstrated an association between infections with drug-resistant non-Typhi *Salmonella* and increased mortality and morbidity (Mølbak, 2005).

Risk population and disease

Salmonellosis results from the intake of foods contaminated with *Salmonella* spp. containing significant numbers of non-host specific strains, around 10^7–10^9/g (Jay, 2005). The route of infection is the internalization of *Salmonella* spp. bacterial cells from gut lumen into epithelium of the small intestine where inflammation occurs. Acute symptoms are nausea, vomiting, abdominal cramps, self-limiting diarrhea, fever, and headache. Chronic consequences may appear 3-4 weeks after acute symptoms. Individuals from all age groups are susceptible, but illness is generally most severe in the elderly, infants, and the infirm (FDA, 2009).

Outbreaks of foodborne illness

One of the major outbreaks of *S.* Enteritidis occurred in 1994 and it was estimated that more than 224,000 persons in 41 states developed gastroenteritis after eating the implicated ice cream product (Hennessy et al., 1996). Investigation determined cross-contamination had occurred during transportation of milk in tanker trucks that had previously hauled liquid eggs. Selected outbreaks documented by CDC (2012a) the last 5 years have been associated with a variety of food products: *S.* Typhimurium in ground beef, african dwarf frogs; *S.* Heidelberg in kosher chicken livers, ground turkey; *S.* Enteritidis in turkish pine nuts, alfalfa sprouts; and other serotypes in whole papayas, chicks and ducklings, turkey burgers, and cantaloupe.

An illustration of an ingredient-driven outbreak was documented by Cavallaro et al. (2011). A nationwide outbreak of human *S.* Typhimurium infections was linked to the eating of contaminated peanut butter, peanut paste, and roasted peanuts produced at

facilities in Georgia and Texas, and resulted in one of the largest food recalls in U.S. history (3,918 peanut butter–containing products). Inadequate peanut roaster temperatures or cross-contamination were possible sources of introduction of *Salmonella* through rainwater leakage into storage areas and storage of raw peanuts near roasted peanuts.

1.4. *Escherichia coli O157:H7*

Pathogenic *Escherichia coli* taxonomy

E. coli are Gram-negative, rod-shaped bacteria belonging the family Enterobacteriaceae. Five virulence groups of *E. coli* are recognized, according to their effect on certain cell cultures, serological groupings, and to the disease syndromes and characteristics: enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC) (Jay 2005). Meng et al. (2007) included the diffuse-adhering *E. coli* (DAEC). Selected characteristics of each one of the groups is presented in Table 1.2.

Table 1.2. Selected characteristics of pathogenic *E. coli* groups.

	Foodborne	**Disease**	**Invasion**	**Enterotoxin**	**Infectious dose**
EPEC [1]	Any food exposed to fecal contamination	Infantile diarrhea	Locus of enterocyte effacement (LEE), induce AE lesions	Unrelated to the excretion of typical *E. coli* enterotoxins	Low in infants, but $>10^6$ in adults
EHEC[1]	Various food implicated	Hemorrhagic colitis, complications can lead to Hemolytic Uremic Syndrome	Locus of enterocyte effacement (LEE)induce AE lesions	Large qty of Shiga toxin (Stx)/ verotoxin	Suspected as few as 10 cells
STEC[2]	Yes	Hemolytic Uremic Syndrome	Absence of locus of enterocyte effacement (LEE)	Large qty of Shiga toxin (Stx)/ verotoxin	
EIEC[1]	Any food exposed to human feces from an ill individual	Bacillary dysentery	Invades and proliferates in colonic epithelial cells similar to shigellae	No	10 cells
ETEC[1]	Unclear	Gastroenteritis in infants and adult travelers	Fimbrial attachment and colonization of colonic cells	Yes (heat labile LT and heat stable ST)	10^6-10^9
EAHEC[4]	Yes	Hemolytic Uremic Syndrome	Absence of locus of enterocyte effacement (LEE)	Large qty of Shiga toxin (Stx)/ verotoxin	
DAEC[4]	?	Yes, young children	No invasion to epithelial cells, no adherence factor		
EAEC[5]	Unclear	Persistent diarrhea infants and children	Aggregative adherence to Hep-2 cells in stacked brick fashion		

[1](FDA, 2009) [2](Kaper et al., 2004) [3](Brzuszkiewicz et al., 2011) [4](Meng et al., 2007) [5](Jay, 2005)

EPEC and EHEC strains share the ability to elicit attaching and effacing (AE) lesions. EPEC's *eaeA* gene was first associated with AE lesion formation and later, McDaniel et al. (1995) localized a chromosomal locus called locus of enterocyte effacement (LEE) that encodes determinants of the AE phenotype. The key virulence factor for EHEC is Stx, which is also known as verocytotoxin (VT). The toxin damage can lead to hemolytic uremic syndrome (HUS), which is characterized by hemolytic anemia and potentially fatal acute renal failure. Stx also mediates local damage in the colon, which results in bloody diarrhea, hemorrhagic colitis, necrosis and intestinal perforation (Kaper et al., 2004). Shiga toxin stx_2 variant is associated with the most pathogenic strains belonging to seropathotype A (*E. coli* O157:H7 strains) (de Sablet et al., 2008).

More than 200 serotypes of *E. coli* can produce shiga-like toxin, however most of these serotypes do not contain the LEE pathogenicity island and are not associated with human disease. This led to the use of the term Shiga toxin-producing *E. coli* (STEC) or verotoxin-producing *E. coli* (VTEC) for any strain that produces Stx and the term EHEC is used to denote only the subset of Stx- positive strains that also contain the LEE (Kaper et al., 2004). Coombes et al. (2008) reported the identification of three genomic islands encoding non-LEE effector (*nle*) genes and 14 individual *nle* genes in non-O157 STEC strains that correlate independently with outbreak and HUS potential in humans.

Several highly adapted *E. coli* clones have acquired virulence attributes which present increased adaptability to new niches and cause a broad spectrum of illnesses. These virulence traits are frequently encoded on genetic elements that can be mobilized into different strains to create novel combinations of virulence factors, or become 'locked' into the genome (Kaper et al., 2004). Such is the case of the recently proposed new pathotype Entero-Aggregative-Haemorrhagic *E. coli* (EAHEC) isolated in a large-scale *E. coli* O104:H4 outbreak which occurred in Germany from May to July 2011 (Brzuszkiewicz et al., 2011).

Numerous cases of hemolytic-uremic syndrome (HUS) and deaths were caused by O104:H4 strains that, according to genome sequencing results, were found to be more closely related to typical enteroaggregative *E. coli* (EAEC) than to enterohemorrhagic *E. coli* (EHEC) strains (Cheung et al., 2011). Brzuszkiewicz et al. (2011) further analyzed the sequencing results, comparing it with selected *E. coli* genomes. Their results indicated that a number of horizontal gene transfer events took place to create the genome of the German outbreak strain. Researchers hypothesized that this strain probably originated from an EAEC pathotype, as suggested by the absence of the LEE island and high similarity to the genome of EAEC strain 55989. Furthermore, the German outbreak strain had the *Stx*-phage which is typical feature of EHEC strains and a plasmid-encoded drug resistance.

STEC in food processing environments

Given that ruminants are natural reservoirs of STEC strains, high prevalence is common in areas where fecal contamination is continuous, namely, farms, transportation trucks used to deliver animals to slaughtering houses, slaughtering halls, etc. Small et al. (2002) reported prevalence of *E. coli* O157:H7 and *Salmonella* spp. in cattle lairages were 27.2, and 6.1%, respectively, and in sheep lairages 2.2, and 1.1%, respectively. Genotypic matches have been found between *E. coli* O157:H7 isolates obtained from transport trailer side walls and those from cattle hide samples within the packing plant (Childs et al., 2006). Transportation trucks may play a role in the transmission of pathogens while transporting animals from farms to processing facilities. Barham et al. (2002) found prevalence levels for EHEC O157 and *Salmonella* spp. on the trailers were 5.43 and 59%, respectively. Interestingly, increased shedding of *Salmonella* was observed during transportation.

Even when cleaning and sanitation procedures are in place, the complete elimination of pathogens is not achieved. Studies have found increased tolerance of some strains to the commonly applied control methods. Dlusskaya (2011) reported heat resistant

isolates from a slaughtering plant, and hypothesized that resistance may be attributable to the selective pressure exerted by steam pasteurization in the processing environment. Similarly, Aslam et al. (2004) recovered more *E. coli* isolates during the summer than the winter months and PFGE patterns suggested persistence of the pathogen on processing equipment. Survival of *Escherichia coli* in the environment was reviewed by van Elsas et al. (2011) and effectiveness of interventions for decontaminating meat was reviewed by Aymerich et al. (2008) and Kaspar et al. (2010).

STEC in food

E. coli O157:H7 bacteria resist acid stress, and tolerate a minimum pH for growth of 4.0 to 4.5; however, induced-acid resistant strains have been found to increase tolerance to heating, irradiation and antimicrobials (Meng et al., 2007). STEC has been isolated from raw milk, meat, and spinach, among a great variety of food products. Ground beef is a high risk product and FSIS legislation declared *E. coli* O157:H7 an adulterant in ground beef (Federal Registry 2009). However, increasing scientific evidence supports that non-O157 shiga-toxin producing strains have a high prevalence in meat products, and are equally capable of causing severe foodborne illness outbreaks.

Hussein (2007) reviewed reported levels of non-O157 STEC in whole cattle carcasses, ground beef, retail beef cuts, and sausage and found 1.7–58%, 2.4–30%, 11.4–49.6%, and 17–49.2%, respectively. The prevalence rates of *E. coli* O157 ranged from 0.1 to 54.2% in ground beef, from 0.1 to 4.4% in sausage, from 1.1 to 36.0% in various retail cuts, and from 0.01 to 43.4% in whole carcasses. A large study by Hill et al. (2011) reported the results from 971,389 samples (trim, ground beef, and variety meats) from commercial beef production plants, collected during 2005–2008. Samples were screened for *Salmonella* or shiga toxin–producing *E. coli*–specific genes. Overall, 4.6%, 4.6%, and 0.8% samples were positive for EPEC, EHEC, and *E. coli* O157, respectively. The authors suggested that more

prevalent strains STEC, EPEC and EHEC, serve as more sensitive indicators of contamination than *E. coli* O157 strains alone.

Recently, FSIS proposed a rule about STEC (Federal Registry, 2011) and has announced the intention to implement sampling and testing for additional STEC strains (O26, O45, O103, O111, O121, and O145). The agency has determined that the mentioned six STEC strains, as well as O157:H7, are adulterants of non-intact raw beef products and product components within the meaning of the Federal Meat Inspection Act (FMIA).

Risk population and disease

It is well established that *E. coli* is the predominant facultative anaerobe of the human colonic flora and usually is harmless. However, with immunosuppressed hosts or when gastrointestinal barriers are violated, even strains that are not considered pathogenic may cause infection. Pathogenic *E. coli* clones may cause urinary tract infections, sepsis/meningitis and enteric/diarrheal disease (Nataro and Kaper, 1998; Mead and Griffin, 1998).

Outbreaks of foodborne illness

E. coli O157:H7 is the most infamous STEC strain, well known by food processors and the public due to its association with many foodborne illness outbreaks. Selected outbreaks in past years documented by CDC (2012b) include romaine lettuce, hazelnuts, cheese, beef, pre-packed cookie dough, and fresh spinach, among other products. However other STEC strains are equally able to cause severe illness. In 2008, a large outbreak of diarrheal illness and hemolytic uremic syndrome was attributed to *E. coli* O111:NM, causing illness in at least 314 people, HUS in 17 cases, and one death (Oklahoma State Department of Health, 2009). The first documented outbreak linked to O145 was reported in 2010, associated with shredded lettuce, causing hospitalization of 26 confirmed patients from 5 states. Three patients developed HUS, and no deaths were reported (CDC, 2012b).

1.5. *Persistent foodborne pathogen contamination in processing plants*

Scientists have faced a major complexity in determining the criterion to classify certain bacteria strains as persistent. Several authors (Pan et al., 2006; Carpentier and Cerf, 2011) have commonly referred to the repeated isolation of a strain from a food processing plant as persistent. However, a recurrent isolation may not occur in every sampling event, even though the strain is present; alternatively, a recurrent isolation of specific strains may be an indicator of incessant incoming contamination from an outside source, rather than persistence in the premises.

Some authors support the theory that the efficiency of cleaning and disinfection is lower on surviving bacterial cells attached for a long time than on recently attached cells, given that surviving bacteria are able to adapt to low residual concentrations of chemicals used for cleaning and disinfection (Pan et al., 2006; Marouani-Gadri et al., 2010). Carpentier and Cerf (2011) summarized the factors that predispose the presence of bacterial cells of one specific strain in a harborage site after cleaning and disinfection as follows: (1) the efficiency of cleaning, (2) the efficiency of disinfection and (3) the number of cells prior to cleaning and disinfection. The author argues that no matter the number of attached surviving cells, if growth conditions are met, in terms of temperature, water, pH and nutrients, growth resumes between two cleaning and disinfection activities, regardless of virulence, and bacterial cells could persist.

On the contrary, other authors suggest that persistent strains might have specific genetic traits which provide an improved ability to persist. Fox et al. (2011) compared persistent and non-persistent strains and found that transcription of many genes (including three gene operons: pdu; cob-cbi; and eut) was upregulated among persistent strains. The upregulation of these genes by persistent isolates suggests a possible role in persistence of *L. monocytogenes* outside the human host and indicates that internalization represents only

part of the life cycle of persistent strains utilized as a means of increased disinfectant resistance. Another proposed consequence of the expression of these three gene operons may be the virulence capacity of persistent strains. The researchers concluded that a persistent strain could increase the risk of human infection with an invasive virulent strain, since it poses increased risk of recontamination of a final product and could potentially lead to cases of sporadic infection or outbreaks.

L. monocytogenes may persist in food plants for months and up to several years (Tompkin, 2002). Chasseignaux et al. (2001) reported finding up to four different genotypes of *L. monocytogenes* after cleaning and sanitation steps in a food processing environment. Authors present two hypotheses to explain recurrent isolates, either cleaning operations are not effective or the pathogens are continuously entering the processing environment and find appropriate conditions to survive and grow. Several factors influencing the survival of *L. monocytogenes* strains in food processing plants are recognized: complexity of structure of processing machines, poor hygienic properties, strain-specific properties such as differences in adherence to stainless steel surfaces, and susceptibility to disinfectants (von Laer et al., 2009).

Different properties of *L. monocytogenes* cells have been studied aiming to elucidate mechanisms that may facilitate persistence. Vatanyoopaisarn et al. (2000) reported significantly different initial adherence was associated with the presence of flagella; while Meylheuc et al. (2001) did not observe a difference in attachment between flagellated and nonflagellated *L. monocytogenes*, but reported that at 20°C the cells were more electronegative. Briandet et al. (1999) hypothesized about hydrophobicity and increased attachment ability, whereas Smoot and Pierson (1998) found no correlation between cell hydrophobicity and attachment, and further hypothesized that proteins play a key role after achieving reductions of 99.9% in the presence of trypsin.

Wong (1998) reported that *L. monocytogenes* survived for prolonged periods adhered to stainless steel and rubber, and even could multiply on stainless steel when favorable conditions were met. Those findings are in agreement with Gamble and Muriana (2007) who reported strongly adherent strains of *L. monocytogenes* adhered equally well to four different substrates found in food-processing environments (glass, plastic, rubber, and stainless steel) and showed high-level attachment at different temperatures (10, 20, 30, and 40°C). The authors reported that a high percentage of the moderately to highly adherent strains were those isolated from raw or processed meats, whereas weakly adherent isolates were mostly those recovered from ready-to-eat meat processing facilities' environments. Similarly, numerous other studies agree that stainless steel is a highly favorable surface for *L. monocytogenes* biofilm formation (Smoot and Pierson, 1998; Blackman and Frank, 1996; Meylheuc et al., 2001; Schwab et al., 2005). Alternatively, Chavant et al. (2002) observed biofilm formation of *L. monocytogenes* on polytetrafluoroethylen (PTFE) noting minimal biofilm formation in cold storage conditions, which was attributed to the hydrophobic nature of the surface.

Regarding other materials, Paiva et al. (2010) studied *L. monocytogenes* infiltration in concrete blocks and the benefits of using a sealant to prevent harborage in cracks or capillary compartments available in concrete structures. Their findings proved that bacterial cells have the ability to infiltrate concrete blocks, increasing concern of potential harborage sites in non-food-contact surfaces, such as ceilings, walls, and floors. In light of these results, it is widely acknowledged that *L. monocytogenes* has unique advantages to become established in a food processing environment.

Vestby et al. (2009) analyzed 111 *Salmonella* isolates from product samples and the environment of Norwegian feed and fish meal factories. The strain collection included persistent and sporadic strains recovered in the period 1991–2006. A biofilm is defined as a

microbial community characterized by cells that are irreversibly attached to a substratum, interface, or each other. The biofilm potential was evaluated at room temperature (20°C) on polystyrene and on the air-liquid interphase. Overall, the strongly persistent *S.* Agona and *S.* Montevideo were good biofilm producers and the non-persistent *S.* Typhimurium displayed the weakest biofilm forming abilities. These results suggest that biofilm forming ability may be an important factor for persistence of *Salmonella* in the factory environment.

Marouani-Gadri et al. (2010) assessed the potential of *E. coli* O157:H7 to persist in a processing environment by inoculating polyurethane coupons. Cleaning and sanitation products were applied at half concentration recommended, and further soiling was applied daily to the coupons to mimic harborage site conditions. Findings suggest that for *E. coli* O157:H7 to persist, there would have to be a large biofilm population before the cleaning and disinfection, and favorable growth factors (i.e. temperature). Interestingly, after repeated chemical treatments, viable but not culturable (VBNC) *E. coli* O157:H7 were able to divide on the coupons.

Overview of selected foodborne pathogen biofilm formation abilities

It is well established that bacteria have the ability to adhere to surfaces and form biofilms. Increasing scientific evidence has elucidated that formation of a biofilm is influenced by the characteristics of the attachment surface, availability of suitable conditions and also by the characteristics of the bacterial cell and by environmental factors. It is recognized that the attachment and biofilm-forming capabilities of bacteria are multifactorial. Factors include the attachment surface, the presence of other bacteria, the temperature, the availability of nutrients, and pH. Furthermore, in food-processing environments, bacterial attachment is additionally affected by food matrix constituents, presence of a mixed microbial community and the features of the bacterial cell surface; such as flagella, surface appendages and polysaccharides that play a role in this process, in

particular for bacteria linked to food-processing environments (van Houdt and Michiels, 2010).

It is widely accepted that in a biofilm, the cells are enclosed in matrix which forms multiple layers. The estimated composition is about 15% cells and 85% matrix by volume (Agle, 2002) protected by extracellular polysaccharides and proteins (Sutherland, 2001; Stewart and Costerton, 2001), extracellular DNA, and dead cells (Webb et al., 2003; Yarwood, et al., 2004). In the case of foodborne pathogens of interest, such as *S.* Typhimurium and STEC *E. coli*, cellulose has been shown to be a crucial component of the extracellular matrix (Zogaj et al., 2001; Solano et al., 2002). *L. monocytogenes* produces extracellular polymeric substances (EPS) and can readily use EPS produced by other bacteria species to form biofilms (Hanna and Wang, 2003).

The complex composition of the biofilm creates a compact structure that prevents biocides from penetrating and inactivating all cells within the biofilm (Chae and Schraft, 2000; Agle, 2002; Branda et al., 2005; Uhlich et al., 2006; Bridier et al., 2011). The presence of organic matter and potential interactions between antimicrobials and biofilm components can impair the effect of disinfectants and disinfection procedures (Lambert and Johnston 2001). Likewise, potential interactions between antimicrobials and biofilm components are likely to restrict infiltration into the biofilm (Bridier et al., 2011). Additionally, research studies have demonstrated stratified activity of growth, protein synthesis and metabolic activity within biofilm layers (Werner et al., 2004).

Recent scientific reviews (Høiby et al., 2010; Bridier et al., 2011) have scrutinized latest findings regarding resistance of bacterial biofilms to disinfectants. Altogether, elucidated biofilm conditions and exposure of bacterial cells to concentration gradients of disinfectants has been hypothesized to trigger various processes within bacterial cells, namely, adaptation responses of specific phenotypes, upregulation of bacterial genes

involved in the oxidative stress response, efflux pumps, and cell-to-cell communication (quorum sensing) mechanisms. Furthermore, biofilms may constitute an optimum environment for bacterial cells to exchange genetic elements at an increased rate, possibly allowing for the acquisition of new genes for antibiotic or biocide resistance, virulence, and other environmental survival abilities (Watnick and Kolter, 2000). In short, it is believed that once bacteria adhere to a surface and form biofilm, they become more resistant to cleaning and sanitation treatment and removal strategies; furthermore, cells detaching from the biofilm could further turn into the source of persistent contamination (Chae and Schraft, 2000; Hanna and Wang, 2003). It remains unclear how the interactions that take place in multi-species biofilms might contribute to synergistic relationships among bacterial species.

Biofilm formation abilities of selected foodborne pathogens

The biofilm formation process has been studied for foodborne pathogens of concern to the food processing industry. Researchers aimed to gain insight about presumed associations between biofilm formation, persistence ability, and increased virulence of foodborne pathogens. Some limitations of comparing different results are due to different growth conditions, surfaces, and media within experiments and may play a role in the observed mixed results, since all these environmental conditions can affect *L. monocytogenes* biofilm formation (Hanna and Wang, 2003).

Chae and Schraft (2000) found that some *L. monocytogenes* strains varied significantly in their ability to adhere and produce biofilm, but no trends could be observed when serotypes and source of the isolates were compared. Similarly, Hanna and Wang (2003) reviewed numerous research studies that have reported mixed evidence and there is no definite correlation between *L. monocytogenes* strain, serotype, or source and the ability to form biofilm. Overall, it seems that serotypes less commonly implicated in disease (such as 1/2c) may be better biofilm formers, and persistent strains appear to form more

biofilm than sporadic strains in most of the studies, but the opposite has also been shown (Hanna and Wang, 2003).

Numerous single culture experiments have provided evidence of the ability of *Salmonella* to form biofilm on materials commonly found in the food processing environment. Jun et al. (2010) evaluated microbial biofilms on common food contact surface materials including stainless steel, white high-density polyethylene, formica-type plastic, and polished granite. The authors reported that both *E. coli* and *Salmonella* adhered and grew well on stainless steel, high-density polyethylene, and granite. Furthermore, researchers have identified characteristics of low sensitivity to commonly used sanitizers. Joseph et al. (2001) studied sensitivity of the biofilm cells of *Salmonella* isolates from poultry to hypochlorite and iodophor. Biofilm was tested on plastic, cement and stainless steel and cells were exposed to the sanitizers at different concentrations. Biofilm cells on stainless steel were most sensitive to the sanitizers whereas those on plastic were most resistant.

Stepanović et al. (2004) tested a panel of 122 *Salmonella* spp. and 48 *L. monocytogenes* strains, isolated either from humans, animals or food, to determine the influence of the growth media on biofilm formation. All tested strains produced biofilm in suitable media and *Salmonella* spp. produced more biofilm in nutrient-poor media, while *L. monocytogenes* produced more biofilm in nutrient-rich media. In a previous study, results obtained by Stepanović et al. (2003) suggest that microaerophilic and CO2-rich conditions provide the best environment for *Salmonella* biofilm formation, while the least biofilm was formed under anaerobic conditions. Moreover, resistance of *Salmonella t*o dry conditions has been reported. Iibuchi et al. (2010) analyzed survival of *Salmonella* on polypropylene discs under desiccation conditions. Survival for more than 200 days was observed at 28°C,

suggesting that strains with high biofilm productivity can survive under dry conditions longer than those with low biofilm productivity.

Silagyi et al. (2009) studied biofilm formation, quorum sensing and a simulated transfer of *E. coli* O157:H7 from surfaces with biofilms to food products. Findings suggest that biofilm formation may not be directly affected by the bacterial growth in tested broths. *E. coli* O157:H7 formed biofilm in meat, poultry broths and certain produce broth (i.e. spinach, cantaloupe). Quorum sensing (AI-2) signals by *E. coli* O157:H7 strain did not always result in the accumulation of strong biofilm under the tested condition. Biofilm-forming *E. coli* O157:H7 strain was able to strongly attach on produce products, including cantaloupe. Dourou et al. (2011) evaluated the effect of soiling substrates and temperature (4°C and 15°C) on attachment and biofilm formation by *E. coli* O157:H7 on stainless steel and high-density polyethylene (HDPE) surfaces. Surface material did not affect adherence or biofilm formation ability, and the pathogen was able to adhere and multiply at 4°C.

Multi-species culture biofilm

However, it is more likely that biofilms in natural environments are composed of multiple species of organisms. Watnick and Kolter (2000) addressed biofilms as a multispecies microbial community, more likely to be found in nature than single culture ones. The authors discussed advantages of bacteria associated with biofilms, highlighting the possible symbiotic or detrimental relationships between groups of bacteria. For instance, an example of cell-cell signals that result in lethal interspecies interactions are the bacteriocins (Riley, 1998), which are proteins produced by some bacteria with lethal action against closely related species. Conversely, bacterial cells could benefit from the opportunity to acquire transmissible genetic material at accelerated rates. It has been shown that even between very distantly related organisms, horizontal gene transfer events are capable of introducing completely novel physiological capabilities and complex

phenotypes in a single step (Lawrence, 2002). Thus, multi-species biofilm might provide ideal conditions for emergence of new pathogens by acquisition of antibiotic resistance, virulence factors, and environmental survival capabilities.

Foodborne pathogens of major concern, such as *L. monocytogenes,* have been proven capable of integrating into EPS and biofilm formed by other bacteria (Sasahara and Zottola, 1953; Hassan et al., 2004). Scientific literature suggests that resident microflora in food processing premises play an important role in foodborne pathogen persistence. Interestingly, although *L. monocytogenes* may be a minority in the initial ecosystem, it has the potential to outcompete other dominating organisms in stress conditions such as refrigeration (Hanna and Wang, 2003). *E. coli* O157:H7 co- cultured with selected strains of resident microbiota recovered from a slaughter hall to form dual-organism biofilms, showed an increased colonization of polyurethane coupons (Marouani-Gadri et al., 2009).

Control of bacterial biofilms in food processing premises

Researchers have evaluated different methods aiming to reduce foodborne pathogen adhesion to surfaces and prove effectiveness of sanitizers on established biofilms. Various treatment approaches have been tested, including the use of competitive exclusion microorganisms, the adsorption of proteins to a food processing surface and the optimal combination of different cleaning and sanitation products or methods. Zhao et al. (2004) found anti-*Listeria*l activity among *Enterococcus durans*, Lc. *lactis* subsp. *lactis* strains due to the production of enterocin and nisin, correspondingly. Leriche et al. (1999) noted that nisin-producing bacteria can reduce or even eliminate *L. monocytogenes* biofilms. Direct adsorption of nisin onto a surface has also been shown to reduce *L. monocytogenes* colonization (Bower et al., 1995) as well as the use of *Pseudomonas fluorescens* biosurfactants (Meylheuc et al., 2001). These findings led to the accepted application of

nisin to polymer packaging materials; however it has not yet ben applied to general cleaning and sanitation procedures.

Regarding cleaning and sanitation products, general recommendations highlight the importance of using products at recommended lethal doses and implementation of standard operational procedures. The sequential application of acid and alkaline products with heat is one of the recommended approaches to reduce established biofilms. Arizcun et al. (1998) tested different decontamination treatments to eliminate *L. monocytogenes* biofilm formed on glass surfaces; biofilms were not found susceptible to high osmolarity (10.5% NaCl), the interaction of sodium chloride and acid did not show a significant effect inactivating the bacteria. The authors reported the most effective treatment to remove the pathogen from biofilms (4.5 to 5.0 log CFU/cm^2 reduction) was achieved by combining NaOH (ph 10.5) and acetic acid (pH 5.4) applied sequentially at 55°C for 5 minutes each.

Pan et al. (2006) conducted a study to examine the resistance of biofilms of *L. monocytogenes* to sanitizing agents under simulated food processing conditions of combined starvation, washing, and sanitation conditions with a mixture of peroxyacetic acid, hydrogen peroxide, and octanoic acid (pH 3.8). Results showed that biofilms repeatedly exposed to the peroxide sanitizer developed resistance to the peroxide sanitizer as well as other sanitizers (quaternary ammonium compounds and chlorine). Interestingly, no significant difference in resistance to sanitizing agents was found between cells removed from the biofilms on peroxide-treated and control, suggesting that the resistance to sanitizing agents may be due to attributes of extracellular polymeric substances and is not an intrinsic attribute of the cells in the biofilm. Fatemi and Frank (1999) tested peracid sanitizers (peracetic (PAA) and peroctanoid acid POA) and chlorine for the inactivation of *L. monocytogenes* biofilms. Results showed peracid sanitizers were consistently more effective than chlorine for inactivating both *Listeria* and *Pseudomonas*. POA had a better performance

attributed to its hydrophobic nature and presumed better ability to cross biofilm matrix and bacterial cell membranes.

Typically applied strategies in the food industry include sanitizer foot baths at entry points, positively pressurized rooms, drain system sanitation, no mid-shift cleanup with high-pressure water hoses, prohibition or minimization of transit of personnel from raw to post processing areas, prevention of contaminants to enter the post process area, and incorporating separate drainage and ventilation systems for raw versus post cooked areas (Suslow and Harris, 2000; Kushwaha and Muriana, 2009). Although environmental sampling studies have proven that plant specific control programs are helpful to reduce contamination, regardless of various sources of incoming contamination, the control of pathogens such as *Listeria* pose a major challenge for food processors (Lappi et al., 2004).

In light of emerging knowledge about bacterial cell activity within biofilms and multi-species interaction, prospective strategies to eradicate biofilm suggest the use of combined treatments with diverse modes of action. A promising strategy may be the use of combined enzymes that can dissolve the biofilm matrix, which will enable biocides to diffuse to cells buried in lower levels (Oulahal-Lagsir et al., 2003; Gamble and Muriana, 2007; Høiby et al., 2010). Depending on the composition of the biofilm (cellulose, polymers, DNA residues), particular enzymes could be used, i.e. proteases, cellulases, polysaccharide depolymerases, or DNAse; the enzymatic processes have the advantage of disaggregating biofilm (Bridier et al., 2011).

1.6. Molecular subtyping methods

Conventional microbiological culture methods provide quantitative data of pathogen contamination but do not discriminate unrelated strains, thus are not useful for outbreak investigation or to elucidate environmental contamination patterns. Subtyping of bacteria and viruses is important from the epidemiological perspective for recognizing

outbreaks of infection, detecting the cross-transmission of pathogens, determining the source of the infection, recognizing particularly virulent strains of organisms, and monitoring vaccination programs (Olive and Bean, 1999). In the food processing environment, subtyping approaches are useful to identify bacterial strains relatedness, persistence in food processing facilities, contamination transmission patterns, etc. (Giovannacci et al., 1999; Wiedmann, 2002; Zhang et al., 2004; Fox et al., 2011).

Based on the cellular components targeted for classification of the organisms, subtyping methods can be grouped into two categories: (i) phenotypic methods and (ii) molecular subtyping methods. Phenotypic methods target cell molecules such as proteins and other metabolites. Phenotypic methods include serotyping (based on a reaction with an antibody, phage typing, antimicrobial resistance profiling, among others (Carlson and Nightingale, 2010). The serotype can only be assigned if the particular serological marker is present and this marker may not be present across the species, posing a limitation (Olive and Bean, 1999).

Molecular subtyping techniques target nucleic acids in cells. These methods can be further grouped in two categories: (i) band base and (ii) nucleic acid sequence based methods (Wiedmann, 2002; Boxrud, 2010). The band based methods compare one or many DNA fragments produced from an isolate by use of restriction enzymes or amplification of specific DNA fragment using primers. Some methods include: multiplex PCR, pulsed-field gel electrophoresis (PFGE), polymerase chain reaction (PCR), ribotyping, plasmid typing, multilocus variable-number tandem-repeat analysis (MLVA), rep-PCR, random amplified DNA polymorphism (RAPD) among others.

Multiplex PCR is the simultaneous amplification of genes or gene fragments using multiple primers. It is used to screen for the presence or absence of genes belonging to specific subtype, typically virulence genes (Carlson and Nightingale, 2010). Ribotyping is

the analysis of band pattern differences obtained through hybridization of labeled ribosomal RNA (rRNA) or ribosomal DNA (rDNA) with DNA fragments produced by cleavage of total DNA with an endonuclease (Bouchet et al., 2008). PFGE compares chromosomal and plasmid DNA fragment patterns obtained with rare-cutters restriction enzymes. PFGE is the gold standard used across international PulseNet laboratories for surveillance and outbreak investigation (Swaminathan et al., 2001; Ribot et al., 2006). For a detailed description of methods, refer to review articles (Wiedmann, 2002; Carlson and Nightingale, 2010; Boxrud, 2010; Nightingale, 2010).

Bacteria and viruses can also be subtyped by determining the sequence of nucleic acids for one or more loci of an organism. Sequence based subtyping methods include single-locus sequencing, multiple-locus sequence typing (MLST), and multiple-virulence-locus sequence typing (MVLST), and single-nucleotide polymorphisms (SNPs) (Boxrud, 2010). DNA sequencing–based subtyping methods such as multi- locus sequence typing (MLST) are valuable tools to probe evolutionary changes in an organism, identify clonal complexes and better understanding of global epidemiology (Maiden et al., 1998; den Bakker et al., 2010). However, as MLST typically targets conserved sequences of multiple housekeeping genes, it has low discrimination for foodborne outbreak investigation (Boxrud, 2010; den Bakker et al., 2010). Other methods have been developed to address local epidemiology issues. Multi-virulence locus sequence typing (MVLST) targets more diverse virulence loci and provides increased pattern diversity compared to MLST (Zhang et al., 2004; Chen et al., 2007).

Maiden et al. (1998) discussed the epidemiological uses of the molecular subtyping methods. Methods that give maximal variation within the population (ribotyping, PFGE and PCR) indicate rapid evolving variation, and are useful for investigation of localized outbreaks or short term epidemiology.

CHAPTER II

Longitudinal Study of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in a Small and Very Small Fresh Meat Processing Plant Environment

2.1. Summary

Salmonella enterica, Escherichia coli O157:H7, and *Listeria monocytogenes* are major foodborne pathogens that represent a permanent challenge to the meat industry. Although *Listeria* spp. are non-pathogenic, a high prevalence in a food processing environment indicates failure in cleaning and sanitation procedures. The purpose of this study was to conduct a six month longitudinal study to monitor *Escherichia coli* O157:H7, *S. enterica*. , *L. monocytogenes* and other *Listeria* spp. contamination patterns, and to identify potential harborage sites in a small and very small fresh meat plant. Additionally, plant profiles were analyzed to identify good practices or potential risk factors for contamination spread and pathogen persistence. Feedback of the study results was given to plant staff in a bilingual session, along with a basic training in food safety topics.

Plants were sampled during mid-shift operation on a monthly basis. Samples collected included food contact surfaces (e.g., tables, scales, bins), non-food contact surfaces (e.g., walls, drains, sinks) and beef carcass surface. Overall, a total of 1,979 sponge samples were collected, microbiologically analyzed and further characterization by molecular

subtyping (e.g. PFGE, ribotyping). *S. enterica* was isolated from 15 (4.5%) and 8 (2.4%) samples from Plant 1and Plant 2, respectively; in Plant 1, only two isolates recovered from a single site yielded same PFGE pattern in different sample collections. *E. coli* O157:H7 was detected in 1.2% of samples in Plant 1; PFGE using *XbaI* generated two different patterns, and none was recurrently isolated from a single site. *E. coli* O157:H7 was not isolated from Plant 2. *L. monocytogenes* was isolated from 17% of the samples from Plant 1 and 1.2% of samples from Plant 2. Roughly 97% (54/56) of *L. monocytogenes* isolates recovered from Plant 1 belonged to ribotype DUP-1042B, which was recovered up to five times from 15 different sampling sites across the facility. Noteworthy, ribotype DUP-1042B belongs to a major human outbreak-associated clonal group. Conversely, no single site in Plant 2 repeatedly tested positive for *L. monocytogenes*. Over the course of the study, roughly 28% and 6% of the samples tested positive for other *Listeria* spp. in Plant 1 and 2, respectively. *Listeria innocua* was the predominant species in both plants.

In light of the results from this study, Plant 2 practices seem effective in prevention of contamination spread and pathogen persistence. Conversely, high risk procedures identified in Plant 1 may contribute to high prevalence of *L. monocytogenes,* and persistence. Our findings suggest that *L. monocytogenes* may persistently contaminate the environment of fresh meat processing plants, while contamination of *E. coli* O157:H7 and *S. enterica* seems to be mostly sporadic.

2.2. Introduction

Foodborne illness is a considerable burden for public health and a major concern for the food industry. In the United States, it is estimated that foodborne illnesses cause about 48 million human illnesses, 128,000 hospitalizations and 3,000 deaths each year (Scallan et al., 2011a,b). *Listeria monocytogenes,* non typhoidal *Salmonella* spp.*, E. coli* O157:H7 and

other shiga-toxin producing *E. coli*, are attributed about 17% of the total estimated number of episodes of domestically acquired foodborne illness; moreover, it is estimated that over 75% of the deaths attributed to major foodborne bacterial pathogens each year are caused by these pathogens (Scallan et al., 2011a, b).

Major meat safety issues related to consumer health problems and product recalls are associated with bacterial pathogens; e.g. *E. coli* O157:H7 and *Salmonella* in fresh meat, while *L. monocytogenes* is a concern in ready-to-eat meat and poultry products (Sofos, 2008). Substantial efforts, both from regulatory agencies and food processors, have been made to prevent foodborne pathogen contamination along the food production and distribution chain. For instance, in 1994, the Food Safety and Inspection Service (FSIS) under the Federal Meat Inspection Act, declared *E. coli* O157:H7 an adulterant in raw ground beef (FSIS, 1999). Shortly after, the final rule on Pathogen Reduction and Hazard Analysis and Critical Control Point Systems (HACCP) was released (FSIS, 1996a). In order to verify pathogen contamination reduction in meat plants, performance indicators were set: (i) a performance standard, testing of *Salmonella* in raw products (FSIS, 2010c), and (ii) performance criteria, testing of generic *E. coli* to verify effective prevention of fecal contamination. Later, *L. monocytogenes* was declared an adulterant in ready-to-eat products (FSIS, 2003). Recently, in response to scientific evidence regarding additional strains of Shiga-toxin producing *E. coli*, the FSIS proposed a new rule and announced the intention to implement sampling and testing for additional STEC strains, including, O26, O45, O103, O111, O121, and O145 (Federal Registry, 2011).

Based on the company size, meat processing plants are classified as: (i) large plants, those with 500 or more employees, (ii) small plants, with more than 10 but fewer than 500 employees, and (iii) very small plants, those with fewer than 10 employees or less than $2.5 million in annual sales (FSIS, 1996b). In fact, continuous incoming live animals, raw

materials, etc., may introduce bacterial pathogens to the plant premises, and pose a challenge to control environmental contamination (McLauchlin et al., 2004).

Scientific evidence supports that *Salmonella* endemic in farm premises may reach food processing facilities via transportation trucks, wildlife or other routes, and pose a risk of contamination of food (Swanenburg et al., 2000; Callaway et al., 2005; Magistrali et al., 2008; Kich et al., 2011). Numerous scientific studies agree that *L. monocytogenes* may survive for a long time in food processing environments where suitable conditions are found (Palumbo and Williams, 1990; Tompkin, 2002; McLauchlin et al., 2004; Kushwaha and Muriana, 2009). High prevalence of EHEC *E. coli* has been found in lairages premises, transportation trucks, holding pens and related environments (Barham et al., 2002; Small et al., 2002; Aslam et al., 2004; Childs et al., 2006).

Once bacteria reach a food processing environment, failure of cleaning and sanitation (C&S) procedures in the control of harborage sites in the premises may lead to persistence of pathogen; e.g., *Salmonella* in fish-meal plants (Vestby et al., 2009); *E. coli* in high-risk, chilled-food factories (Holah et al., 2004); and *L. monocytogenes* in various food processing environments, including: fresh cheese (Kabuki et al., 2004), fish (Thimothe et al., 2004; Lappi et al., 2004), fresh sausage (von Laer et al., 2009) and RTE meat plants (Williams et al., 2011).

The use of microbiological environmental testing paired with molecular subtyping is useful to identify bacterial strains relatedness and transmission patterns in food processing premises (Giovannacci et al., 1999). A parallel effort should be made through education of meat handlers, as key strategy for prevention and control of contamination (Gome-Neves et al., 2011). Limited scientific literature is available regarding food safety training and evaluation of meat industry employees. Egan et al. (2007) reviewed 46 studies conducted worldwide regarding efficacy of food safety training in food industry, mostly foodservice;

questionnaires with multiple-choice format were the primary mechanism used to assess knowledge; topics addressed included high-risk foods, foodborne pathogens, cross-contamination and C&S; a key feature for improved effectiveness was performing the training in the workplace. MacAuslan (2001) addressed the need to provide training programs oriented to English as a Second Language (ESOL) speaking food handlers; elimination of the language barrier allows a better understanding of the training content. However, food safety training alone does not necessarily translate into improved food safety behavior (Seaman and Eves, 2010) and long-term interventions and evaluations are needed to assess behavioral change (Egan et al., 2007).

Small and very small meat processing plants may have the largest knowledge gap concerning foodborne pathogen prevalence and persistence; moreover, managers and employees may have limited access to customized food safety training. The current study was conducted to (i) determine the prevalence of three significant foodborne pathogens, including *L. monocytogenes*, *Salmonella enterica*, and *E. coli* O157:H7 in the environment of small and very-small fresh meat environments located in Colorado, (ii) characterize the recovered pathogen isolates by molecular subtyping and compare subtypes to identify presumed persistent or transient contamination, (iii) identify operational practices and policies that may act as risk factors for environmental contamination, and (iv) provide a facility-specific bilingual training addressing general food safety topics, results from the bacteriological analysis and recommendations.

2.3. Materials and Methods

Facility enrollment and initial site visits. To enroll facilities for voluntary participation in the study, meat processors located throughout Colorado and Southern Wyoming were initially contacted via mail. One small and one very-small facility that primarily process fresh meat, both located in Colorado, were chosen as final participants. Following enrollment, initial site visits to the facilities were arranged to identify environmental sampling sites and to construct sampling scheme maps (Appendices 1 and 2).

Sample collection. Six monthly sample collections were performed from June to December 2011. Environmental site ($n \leq 54$) and beef carcass composite samples ($n=1$) were collected mid-shift (9:00 AM – 12:00 PM) using a pre-moistened (10 ml Neutralizing Broth) sterile sponge-handle sampling apparatus (Solar Biologicals Inc., Ogdensburg, NY). Three adjacent 2 ft x 2 ft areas at each environmental site were sponge-sampled to complete isolation protocols for *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enterica*. Sites with limited surface area (drains, cracks, etc.) were sampled by dividing the total area into 3 portions. Food-contact surface samples included equipment (band saws, hand saws, circular saws, grinders, slicer, tubs, buckets, carts, hooks, vacuum packer), and tables (fabrication, cutting and packing tables and hoppers). Non-food-contact surfaces included doors, walls, floor, drains, employee boots and aprons, stress mats, squeegees, sinks, hoses, and offal collection barrels). Beef carcass composite samples were created for each pathogen isolation protocol by sponge-sampling 3 beef carcasses with a single sponge. The sampled areas of the carcasses included necks, chucks, briskets, frontal shanks, plates and flanks surfaces. Immediately after collection, bagged sponges were placed on ice or ice packs and transported to the laboratory for processing within 36 hours.

Isolation of *Salmonella* spp. Procedures used to detect and isolate *Salmonella enterica* (serovars other than *S. enterica* serovar Typhi) from sponge samples were adapted from the USDA-FSIS MLG protocol 4.05 (FSIS, 2010a). Briefly, each sample was combined with 90 ml of Buffered Peptone Water (BPW; BBL, BD), stomached for 2 minutes, and incubated 22 ± 2 h at 35°C ± 2°C. An aliquot (0.5 ml) of BPW was transferred into tubes containing 10ml of Tetrathionate Broth, Hajna (TTB): TT (Tetrathionate) Broth Base, Hanja (Difco, BD) supplemented with Iodine (Fisher Chemical, Thermo Fisher Scientific Inc., Waltham, MA or Mallinckrodt Chemicals, Avantor Performance Materials Inc., Phillipsburg, NJ) and Potassium Iodide (Acros Organics or Mallinckrodt Chemicals, Avantor Performance Materials Inc.). Simultaneously, an aliquot (0.1 ml) of BPW was transferred to tubes containing 10 ml of Rappaport-Vassiliadis Soya Broth (RVB; formulation from: EMD Science, Merck KGgA). TTB and RVB tubes were then incubated in a static water bath at 42 ± 0.5°C for 21 ± 3 h. After incubation, aliquots (0.01 ml) of each TTB and RVB tubes were streaked for isolation onto both Brilliant Green Sulfa Agar (BGS; Difco) and XLT4 Agar (XLT4): XLT4 Agar Base (Difco, BD) supplemented with XLT4 Supplement Tergitol 4 (Dalynn Biologicals, Calgary, Canada). All plates were incubated at 35 ± 2°C for 42 ± 6 hours. Plates were examined for typical *Salmonella* enterica morphology. *S. enterica* appear as white-opaque colonies surrounded by a pink background on BGS and as red, halo-surrounded colonies with or without a distinctive black center from H_2S production on XLT4. Up to 8 typical colonies were picked from any combination of the 4 agar plates for each sample and were sub-streaked to CHROMagar *Salmonella* (CHROMagar). CHROMSAL plates were incubated at 37 ± 2°C for 21 ± 3 h. Plates were examined for colonies with typical mauve colored morphology; growth on CHROMSAL that was typical of *S. enterica* was sub-streaked to BHI Agar, incubated at 37 ± 2°C for 22 ± 2 h, and further stored at 4°C pending PCR confirmation. Both a positive control sample, *S. enterica* serovar Typhimurium (ATCC

700408; Noah et al., 2005), and a negative control sample, were processed in parallel with the test samples.

Isolation of *E. coli* O157:H7. Samples were processed for isolation of *E. coli* O157:H7 following procedures adapted from FSIS (2010b). Briefly, each sponge sample was combined with 90 ml Modified Tryptone Soya Broth (Oxoid Ltd., Hampshire, United Kingdom) supplemented with Casamino Acids (Bacto, BD) and 20 mg/L Novobiocin Sodium Salt (MP Biomedicals, Solon, OH or Calbiochem, Merck KGaA, Darmstadt, Germany) (mTSB+N). Sponge samples were stomached for 2 min and incubated at 42 ± 1ºC for 18.5 ± 3.5 h. An aliquot (1 ml) of mTSB+N was subjected to immunomagnetic separation (IMS) according to either the manual or automated IMS protocol for Dynabeads anti-*E. coli* O157 (Invitrogen, Life Technologies, Foster City, CA). Following IMS, a 0.1 ml aliquot of the final Dynabead suspension was streaked for isolation. For the first sample collection, the initial medium used for plating the Dynabeads was MacConkey Sorbitol Agar (Difco, BD) supplemented with 20 mg/L Novobiocin Sodium Salt (MP Biomedicals or Calbiochem, Merck KGgA) and 2.5 mg/L Potassium Tellurite (MP Biomedicals) (mSMAC) and incubated at 37 ± 2°C for 21 ± 3 h. Up to 8 typical colonies from each mSMAC plate were sub-streaked to CHROMagar O157 (CHROMagar, Paris, France) incubated for 24 ± 2 h at 37 ± 2°C, examined for the mauve-colored morphology typical of *E. coli* O157:H7 and sub-streaked onto BHI Agar plates and incubated at 37 ± 2°C for 22 ± 2 h. mSMAC was the initial plating medium for Dynabeads for sample collections 1 and CHROMO157 was the initial plating for sample collections 2-6. CHROMO157 was supplemented with 20 mg/L Novobiocin Sodium Salt (MP Biomedicals or Calbiochem, Merck KGgA) and 2.5 mg/L Potassium Tellurite (MP Biomedicals) in sample collections 5-6. Incubation conditions and examination of CHROMO157 were not changed, and typical colonies were sub-streaked to BHI Agar. Following incubation of BHI Agar, plates were stored at 4°C until PCR confirmation was

performed. A positive control for *E. coli* O157:H7 (ATCC 43895; Noah et al., 2005), along with a negative control sample, were processed in parallel with the samples from the facility.

Isolation of *L. monocytogenes* and *Listeria* spp. Sponge samples were processed to isolate *L. monocytogenes* following protocols adapted from USDA/FSIS (FSIS, 2009). Briefly, for the primary enrichment, each sample was combined with 90 ml of UVM Modified *Listeria* Enrichment Broth (UVM; Difco, BD, Franklin Lakes, NJ), stomached for 2 minutes (Stomacher 400 Lab Blender or Circulator (Seward Ltd., West Sussex, United Kingdom) or a Smasher AESAP1064 (AES Chemunex, Bruz, France)) and incubated 22 ± 2 h at 30°C ± 2°C. A 0.1ml aliquot of the mUVM primary enrichment was streaked for isolation to Modified Oxford Agar (MOX): Oxford Medium Base (Difco, BD) supplemented with Modified Oxford Antimicrobic Supplement (Difco, BD). Plates were incubated at 35 ±2°C for 52 ± 4 h. A secondary enrichment was prepared transferring a 0.1ml aliquot of the mUVM enrichment to 10 ml Morpholinepropanesulfonic Acid-buffered *Listeria* Enrichment Broth (MOPS-BLEB): Buffered *Listeria* Enrichment Broth (Difco, BD) supplemented with Morpholinepropanesulfonic Acid (Sigma-Aldrich, St. Louis, MO or Acros Organics, Morris Plains, NJ) and Morpholinepropanesulfonic Acid Sodium Salt (Sigma-Aldrich or Acros Organics). Tubes were incubated at 35 ± 2°C for 21 ± 3 h followed by a secondary plating (0.1ml) to MOX as described above. MOX plates were examined for colonies with typical *Listeria* spp. morphology: gray-green crater-like colonies, surrounded by a zone of darkening in the medium due to esculin hydrolysis). Up to eight presumptive positive colonies from the combination of the 2 MOX plate sets were sub-streaked for isolation onto *L. monocytogenes* Chromogenic Plating Medium (LMPM; R&F Laboratories, Downers Grove, IL) and incubated for 24 ± 2 h at 37 ± 2°C. LMPM plates were examined to differentiate *L. monocytogenes* (light blue colonies) from other *L.* spp. (white colonies) based on hydrolysis

of a colorimetric phospholipase substrate (Restaino et al., 1999). Growth on LMPM was sub-streaked to Brain Heart Infusion Agar (BHI Agar): Brain Heart Infusion (Bacto, BD) supplemented with Granulated Agar (Difco, BD), incubated at 37 ±2°C for 22 ± 2 h for further PCR confirmation and stored at 4°C until PCR confirmation was performed. Positive controls for *L. monocytogenes* (10403S Δ*actA;* Roberts, 2004) and other *L.* spp. (*L. innocua* ATCC 3090; Seeliger, 1981), as well as a negative control sample, were processed in parallel with samples.

PCR confirmation. PCR assays targeting DNA sequences specific to each organism were used for confirmation of presumptive positive isolates. A portion of an isolated presumptive positive colony from each BHI plate was transferred to a sterile 0.2 ml tube (Eppendorf, Hamburg, Germany) or a well on a Thermo-Fast 0.2 ml semi-skirted 96-well PCR plate (Thermo Scientific, Thermo Fisher Scientific Inc.) using a sterile toothpick. To lyse bacterial cell and release of DNA, tubes or plates were microwaved at 1000W for either 4 min (Gram-positive) or 30 s (Gram-negative). All PCR reagent master mixes were set at 25 µl 1X, following the formulations suggested for GoTaq Green Master Mix (Promega Corp., Madison, WI). Thermal cycling was carried out in either a GeneAmp PCR System 2700 or a 2720 Thermal Cycler (Applied Biosystems, Life Technologies). A portion of the *invA* gene was targeted to confirm presumptive *S. enterica* colonies (Nucera et al., 2006). Presumptive *E. coli* O157:H7 positive samples were analyzed using a multiplex PCR protocol targeting the following genes *hlyE*, *fliC*$_{h7}$, *stx2*, *eaeA*, *rfbE*, and *stx1* (Hu et al., 1999). Confirmation of *Listeria* spp. and *L. monocytogenes* was performed targeting fragments of genes *sigB* and *hlyA*, respectively, as previously described (Norton et al., 2001; Nightingale et al., 2005). Primer sequences, concentrations, and references, along with thermal cycling conditions and resulting fragment sizes for all assays are described in Appendix 3. Cultures used as

positive controls for detection and isolation procedures were also used for PCR confirmation.

Preservation of positive isolates. Confirmed positive isolates were preserved for further subtyping analyses. Up to 8 *S. enterica,* 8 *E. coli* O157:H7, 4 *L. monocytogenes*, and 2 other *Listeria* spp. isolates were preserved from each sample. The remainder of the colony used for confirmatory PCR was transferred to 5 ml Brain Heart Infusion Broth (BHI Broth; Difco, BD), incubated at 37 ± 2°C for 21 ± 3 h with continuous agitation, combined with Glycerol (Acros Organics) at 15% v/v and frozen in cryogenic vials (Corning Inc., Corning, NY) at –80°C.

Subtyping of *S. enterica* and *E. coli* O157:H7. Single isolates of *S. enterica* and *E. coli* O157:H7 from samples that tested positive for a monthly sample collection were selected for subtyping by Pulse Field Gel Electrophoresis (PFGE) following the Centers for Disease Control and Prevention (CDC) PulseNet International Protocol for PFGE of *E. coli* O157:H7, *S. enterica*, *Shigella sonnei*, and *Shigella flexneri* (CDC, 2009). Resulting PFGE patterns were analyzed and compared using BioNumerics software (Applied Maths, version 6.6, Saint-Matins-Latem, Belgium). Similarity clustering analyses were performed using the unweighted pair group matching algorithm and the Dice correlation coefficient as described previously by Hunter et al. (2005) with a band matching tolerance of 2% and relaxed double matching.

Molecular serotyping of *L. monocytogenes*. A single *L. monocytogenes* isolate from each sample that tested positive for a monthly sample collection was selected for subtyping, using a modified version of the molecular serotyping multiplex PCR assay by Doumith et al. (2004) which classifies *L. monocytogenes* isolates into 1 of 4 serogroups. Each serogroup contains 1 of the 4 major serotypes associated with human disease (1/2a, 1/2b, 1/2c, and 4b).

Ribotyping of *L. monocytogenes*. A single *L. monocytogenes* isolate from each sample that tested positive for a monthly sample collection was selected for ribotyping. Automated *Eco*RI ribotyping was performed at Cornell University's Laboratory of Molecular Subtyping (Ithaca, NY) using the automated Riboprinter (DuPont Qualicon) as previously described by Williams et al. (2011).

Speciation and subtyping of other *Listeria* spp. Speciation and subtyping through DNA sequencing of a portion of the *sigB* gene was performed on a single other *Listeria* spp. isolate for each sample that tested positive for a monthly sample collection. Amplification by PCR, amplicon purification were prepared at Texas Tech; forward and reverse sequencing master mixes were submitted to the Life Sciences Core Laboratories Center at Cornell University (Ithaca, NY) where DNA sequencing was performed using Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase (Invitrogen, Life Technologies) on an Automated 3730 DNA Analyzer (Applied Biosystems, Life Technologies). Sequence assembly, proofreading, and alignment, along with speciation by performing BLAST searches and assignment of allelic types were performed as previously described by Doumith et al. (2004).

Manager questionnaire. Managers from the two participating plants were asked to complete a confidential questionnaire to gain insights about the facilities, manufacturing practices, and other relevant practices in order to better understand measures used to control introduction and spread of contamination within the facility. Questionnaire was adapted from surveys used previously by our research group (Appendix 4). Information on unique facility characteristics, routine activities, and employees was also captured to enhance development of intervention strategies aimed at mitigating pathogen persistence and spread throughout the facility.

In-plant bilingual training. Upon completion of isolates subtyping, an in-plant training session was developed for each plant using facility-specific subtyping results and responses from the manager questionnaire. In order to provide a knowledge background for better assimilation of our study purpose and results from bacteriological analysis, a set of bilingual (English-Spanish) presentation handouts were developed. The training materials addressed three main components: (i) foodborne illness (e.g., pathogens, source foods, and illnesses) (ii) general food safety practices for food processors (i.e. C&S) and (iii) facility-specific suggestions and focus areas derived from subtyping results, including color-coded maps. Effective cleaning and sanitation procedures were summarized from recommendations from FSIS (HACCP Consulting Group, LLC., 2009). The presentation handout concerning the former two topics was generic for both plants, and the latter designed separately for each plant (Appendices 5, 6 and 7).

A questionnaire was prepared for knowledge assessment and to estimate the impact of the training (Appendix 8). The questionnaire comprised a total of 28 questions, including 19 multiple choice, 8 true or false and 1 employee opinion question. In light of the small number of employees in each plant and to maintain anonymity, no demographic information was collected. Pre and post results were tracked using a corresponding number. The questionnaire forms were delivered electronically to the plant managers, who administered it to the employees prior to the training session. Participation of the employees in the evaluation was voluntary. The same evaluation was administered after the training. The goal of the pre-training evaluation was to assess the baseline of general knowledge of the foodborne pathogens targeted in this study, possible environmental contamination routes of the meat plant premises and the potential risks involved. The post-training evaluation aimed to assess the increment in knowledge achieved with the presentation, discussion of results and inquiries from the employees. Scores were assigned

without penalty for wrong or blank answers; one point per correct answer. A set of bilingual fact sheets was prepared and delivered to plant managers for future reference to refresh or conduct new employees' trainings (Appendix 9).

Statistical analysis of knowledge assessment. A paired T-test was used to compare pre- and post-training scores for participating employees from the two plants. The analysis was carried out using the PROC TTEST procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC) with $\alpha = 0.05$.

2.4. Results

Environmental sample analysis. Altogether, 1,979 environmental sponge samples from two fresh meat plants were collected over a six-month period. Samples were microbiologically analyzed to detect presence of three major bacterial pathogens, including *L. monocytogenes*, *S. enterica, and E. coli* O157:H7 and non-pathogenic *Listeria* spp. The prevalence of each targeted pathogen is presented in Table 2.1. Participating plants represent different operational styles and locations, thus results are discussed individually for each plant.

Table 2.1. Prevalence of *S. enterica, E. coli* O157:H7, *L. monocytogenes*, and other *Listeria* spp. in a small and very small fresh meat plant by sample type.

Plant	Sample Type[1]	% Positive samples (fraction of tested samples)			
		S. enterica	***E. coli* O157:H7**	***L. monocytogenes***	***Listeria* spp.[2]**
Plant 1	C	0% (0/6)	0% (0/6)	0% (0/6)	33.3% (2/6)
	FC	1.1% (1/90)	0% (0/90)	2.2% (2/90)	8.9% (8/90)
	NFC	9.4% (22/234)	1.7% (4/234)	23.1% (54/234)	35.5% (83/234)
Subtotal		**7% (23/330)**	**1.2% (4/330)**	**17% (56/330)**	**28.2% (93/330)**
Plant 2	C	0% (0/6)	0% (0/6)	0% (0/6)	0% (0/6)
	FC	0% (0/113)	0% (0/114)	0% (0/114)	0% (0/114)
	NFC	2.4% (8/210)	0% (0/210)	1.9% (4/210)	9.5% (20/210)
Subtotal		**2.4% (8/329)**	**0% (0/330)**	**1.2% (4/330)**	**6.1% (20/330)**
TOTAL		**4.7% (31/659)**	**0.6% (4/660)**	**9.1% (60/660)**	**17.1% (113/660)**

[1] C= Beef Carcass; FC=Food-contact surface; NFC= Non-food-contact surface
[2] Indicates samples positive for *Listeria* spp. other than *L. monocytogenes.*

Plant 1. Plant 1 is a very small fresh meat processing plant which operates under custom exempt inspection regime; runs year round with one shift, employs 8 people, and has an annual processing volume of approximately 750,000 lbs. The facility is located in a rural location; its entire physical structure area is 2,500 sq. ft. The building, built 50 years ago, comprises a slaughter area, coolers and a processing area, annexed 19 years ago.

Over the six-month sampling period, a total of 990 environmental samples were collected and analyzed for the pathogens of interest. *S. enterica* was isolated from 7% (23/330) of the samples of which 22 were recovered from non-food-contact (NFC) surfaces and one from a food-contact (FC) surface. The highest prevalence of *S. enterica* was found in

the slaughter area followed by the coolers, where roughly 14% and 13% of samples collected tested positive, respectively (Table 2.2).

Table 2.2. Distribution of *S. enterica, E. coli* O157:H7*, L. monocytogenes* and other *Listeria* spp. positive-samples in Plant 1.

		Organism[2]				
Source[1]	**Samples**	**SE**	**EC**	**LM**	**LI**	**LW**
O	(n=18)	5.6% (1)	5.6% (1)		16.7% (3)	
SA	(n=72)	13.9% (10)	1.4% (1)	15.3% (11)	31.9% (23)	1.4% (1)
C	(n=54)	13.0% (7)		24.1% (13)	38.9% (21)	
M	(n=12)	8.3% (1)	8.3% (1)	16.7% (2)	16.7% (2)	
SMK	(n=54)	7.4% (4)	1.9% (1)	35.2% (19)	46.3% (25)	
PA	(n=114)			9.6% (11)	14.0% (16)	
BC	(n=6)				33.3% (2)	
TOTAL	**(n=330)**	**7.0% (23)**	**1.2% (4)**	**17.0% (56)**	**27.9% (92)**	**0.3% (1)**

[1]O=Outside Area; SA=Slaughter Area; SMK=Smokehouse Area; C= Coolers; M=Maintenance; PA= Processing Area; BC= Beef Carcass
[2]LM=*L. monocytogenes*; LI=*L. innocua*; LW= *L. welshimerii*; LS= *L. seeligeri*; EC= *E. coli* O157:H7; SE= *S. enterica*

Characterization by PFGE using XbaI generated 6 different patterns (Appendix 10). Pulsotype PT6 was predominant among *Salmonella* with 8 isolates, all recovered from NFC surfaces; the second predominant sample was pulsotype 1 (6 isolates) (Table 2.3). Over the course of the study, *S. enterica* was recovered four occasions from a blood catch drain in the slaughter floor; two isolates were further characterized by PFGE typing as pulsotype 1 and the other two corresponding to pulsotypes 3 and 6, respectively. Four additional sampling sites tested positive in two occasions: drains in the smoking area and cooler 2, and the floors at the slaughter area and cooler 2; however different pulsotypes were recovered each time (Appendix 11).

Overall, *E. coli* O157:H7 was rarely found in this study. In Plant 1, only four samples (1.2%) tested positive for *E. coli* O157:H7, but were not recurrently isolated from a single

site. Characterization by PFGE using XbaI generated two different patterns (Appendix 12). Pulsotype 1 was found in two samples collected from the maintenance area floor and offal collection barrels kept outside; whereas isolates recovered from the smokehouse area sink and blood catch drain in the slaughter area were characterized in a different pulsotype PT-2 (Tables 2.3).

Table 2.3. Prevalence of *S. enterica* and *E. coli* O157:H7 subtypes in Plant 1 by sample type.

		% Prevalence	
Species	**PFGE Type**	**NFC[1] (n=234)**	**FC & C [2] (n=96)**
E. coli O157:H	EC PT 1	0.9% (2)	
	EC PT 2	0.9% (2)	
S. enterica	SE PT 1	2.6% (6)	
	SE PT 2	0.9% (2)	
	SE PT 3	1.3% (3)	1.0% (1)
	SE PT 4	0.9% (2)	
	SE PT 5	0.4% (1)	
	SE PT 6	3.4% (8)	

[1] NFC= Non-food-contact surface; [2] C= Beef Carcass; FC=Food-contact surface

L. monocytogenes was isolated from 17% of the samples (56/330) distributed across the facility. *L. monocytogenes* was more frequently isolated from samples taken in the smokehouse and slaughter areas, where roughly 32% and 46% of samples collected were positive, respectively (Table 2.2). Interestingly, 96.4% (54/56) of the *L. monocytogenes* isolates from Plant 1 belonged to the molecular serogroup (1/2b, 3b, 7) further characterized as Ribotype DUP-1042B (Table 2.4).

Table 2.4. Prevalence of *L. monocytogenes* ribotypes in Plant 1.

	Confirmed positive	% Prevalence	
Ribotype	**Total (n=56)**	**NFC[1] (n=234)**	**FC & C [2] (n=96)**
DUP-1042B	96% (54)	22.2% (52)	0.9% (2)
DUP-1057B	4% (2)	0.9% (2)	

[1] NFC= Non-food-contact surface; [2] C= Beef Carcass; FC=Food-contact surface

DUP-1042B was isolated in 22% of samples taken from NFC surfaces and 1% of samples from FC surfaces. The remaining two isolates belong to serotypes 1/2a and 3a and ribotype DUP-1057B, recovered once from two different NFC surfaces. DUP-1042B was isolated at least once from 27% of sampling sites (15/55) and was recurrently recovered, up to five times, from 15 sampling sites; in the smokehouse area, three different sites tested positive at least three times (door, floor and drain) and both a drain located in cooler 2 and the slaughter area floor tested positive five times (Appedix 13). The positive-samples were more frequently detected in NFC surfaces, particularly in drains and floors; our findings are in agreement with previous studies (Thimothe et al., 2004; Williams et al., 2011).

Over the course of the study, 28.2% (93/330) of the samples tested positive for *Listeria* spp. other than *L. monocytogenes*. *Listeria innocua* was the predominant species, recovered from 28% of the samples (92/330) (Appendix 14). *L. innocua* was more frequently found in the smokehouse area and slaughter hall, where 25% and 15% of samples collected tested positive, respectively; moreover, each of those areas had one site that tested positive four times (Appendix 15). *L. innocua* AT-1 was the predominant type, found in roughly 21% (49/234) of samples taken from NFC surfaces and 2.1% (2/96) of samples from FC and beef carcasses (Table 2.5).

Table 2.5. Prevalence of *Listeria spp.* subtypes in Plant 1.

		% Prevalence	
		NFC[1]	FC & C [2]
Species	**Subtype**	**(n=234)**	**(n=96)**
L. innocua	LI AT 1	20.9% (49)	2.1% (2)
	LI AT 2	1.7% (4)	1.0% (1)
	LI AT 3	0.4% (1)	
	LI AT 4	0.9% (2)	1.0% (1)
	LI AT 5	5.6% (13)	1.0% (1)
	LI AT 6	1.3% (3)	2.1% (2)
	LI AT 7	0.4% (1)	1.0% (1)
	LI AT 8	1.7% (4)	1.0% (1)
	LI AT 9	0.4% (1)	
	LI AT 10	1.7% (4)	
	LI AT 11	0.4% (1)	
L. welshimerii	LW AT 1		1.0% (1)

[1] NFC= Non-food-contact surface; [2] C= Beef Carcass; FC=Food-contact surface

The second most prevalent *L. innocua* strain was AT-5 found in 5.6% of samples from NFC surfaces (13/234). *Listeria welshimeri* (LW AT 1) was isolated from one sample collected from a FC surface. Contamination patterns of Plant 1 are presented in color-coded maps to illustrate distribution and frequency of positive-samples and subytpes per sampling collection (Appendix 16-18).

Plant 2. Plant 2 is a small fresh meat processing facility, located in a suburban area. Its 7-year-old building has a total physical area of 5,000 sq. ft. Plant 2 is a federally inspected plant, and also has an annual third-party audit. It operates year-round with one shift, has 15-17 employees, and processes approximately 1,700,000 lbs per year. A total of 989 environmental samples were collected and screened for presence of the target organisms in Plant 2. Overall, the majority of positive samples were collected in the slaughter area, suggesting that contamination is contained in the area (Table 2.6).

Table 2.6. Distribution of *S. enterica, E. coli* O157:H7, *L. monocytogenes* and other *Listeria* spp. positive-samples in Plant 2.

		Organism[2,3]				
Source[1]	**Samples**	**SE**	**LM**	**LI**	**LW**	**LS**
C	(n= 36)				5.6% (2)	
PA	(n= 174)	0.6% (1)	0.6% (1)		1.7% (3)	
SA	(n= 114)	6.1% (7)	2.6% (3)	10.5% (12)	1.8% (2)	0.9% (1)
BC	(n= 6)					
TOTAL	(n=330)	2.4% (8)[4]	1.2% (4)	3.6% (12)	2.1% (7)	0.3% (1)

[1]SA=Slaughter Area; C= Coolers; PA= Processsing Area
[2]LM=*L. monocytogenes*; LI=*L. innocua*; LW= *L. welshimerii*; LS= *L. seeligeri*; EC= *E. coli* O157:H7; SE= *S. enterica*
[3]No samples from Plant 2 were positive for *E. coli* O157:H7
[4]Total samples analyzed for *Salmonella* =329.

S. enterica was detected in eight samples (2.4%) all collected in the same sampling visit. Characterization by PFGE using XbaI generated one single pattern (Appendix 19). All positive samples were collected from slaughter area (drain, floors, walls and doors) except for one sample from the drain located in the processing area. *S. enterica* was not isolated more than once from a single sampling site (Appendix 20). All *Salmonella* PT-1 isolates were collected in a single sample collection (September 2011), suggesting that probably the lot processed during that day brought in contamination to the plant. The absence of *S. enterica* in following sampling visits suggests that the C&S practices were effective in controlling the contamination. *E. coli* O157:H7 was not isolated from any sample collected from Plant 2.

L. monocytogenes was isolated only from four samples (1.2%), all taken from NFC in the slaughter area, except for one sample taken from a drain located in the processing area (Appendix 21). All *L. monocytogenes* isolates correspond to the serotype group 1/2a and 3a and were further classified by ribotyping as DUP-1030A (1 isolate) and DUP-1020B (3 isolates) (Table 2.7).

Table 2.7. Prevalence of *L. monocytogenes* ribotypes in Plant 2.

	% Prevalence[1]
Ribotype	**NFC[2] (n=210)**
DUP-1030A	0.5% (1)
DUP-1030B	1.4% (3)

[1]No carcass or food-contact surfaces from Plant 2 were positive for *L. monocytogenes*
[2] NFC= Non-food-contact surface

Twenty samples (6.1%) tested positive for *Listeria* spp. All *Listeria* spp. positive-samples were collected from NFC surfaces (Appendix 22). The *sigB* gene typing identified the strains as follows: 12 corresponding to *L. innocua*, 7 to *L. welshimeri* and 1 to *L. seeligeri* (Table 2.8). *Listeria innocua* was predominant in the plant, primarily in the slaughter area; different subtype strains observed with every sampling suggesting sporadic contamination. Among the *L. innocua* isolates, type AT-6 was overrepresented with seven isolates recovered from the slaughter area environment. The *Listeria welshimeri* isolates were recovered from the cooler (2), slaughter area (2) and processing area (3); it was isolated in three different occasions from a door in the processing area. Contamination patterns of Plant 2 are presented in color-coded maps to illustrate distribution and frequency of positive-samples and subytpes per sampling collection (Appendix 23-25).

Table 2.8. Prevalence of *Listeria spp.* subtypes in Plant 2.

Species	**Subtype**	**% Prevalence[1]**
		NFC[2] (n=210)
L. innocua	LI AT 6	3.3% (7)
	LI AT 31	0.5% (1)
	LI AT 53	1.0% (2)
	LI AT 109	0.5% (1)
	LI AT 124	0.5% (1)
L. welshimerii	LW AT 129	2.9% (6)
	LW AT NEW	0.5% (1)
L. seeligeri	LS AT 121	0.5% (1)

[1]No carcass or food-contact surfaces from Plant 2 were positive for *Listeria* spp.
[2] NFC= Non-food-contact surface

Manager questionnaire. A questionnaire was delivered to the plant managers to gather data relevant to facilities, general practices in place, production process, etc. A summary of characteristics of each plant is presented in Tables 2.9a, 2.9b, 2.9c and complete plant profiles are presented in Appendix 6. In light of observed differences in the environmental sampling outcome from the participant plants, the data reported by plant managers was compared and contrasted to identify possible risk factors that led Plant 1 to have higher pathogen positive results. Major differences between the participating facilities were: (i) age of buildings, (ii) inspection regime, (iii) existence of a verifiable HACCP plan, including periodic environmental monitoring samplings and carcass interventions. Differences found between procedures and policies in the two plants included: (i) control of contamination, (ii) cleaning and sanitation (C&S), and (iii) employee food safety training.

Contamination control policies and specific measures such as the use of sanitizer foot baths reported by Plant 2 seem to be effective in preventing the spread of pathogens within the plant; whereas, less preventive measures were reported from Plant 1. Sanitizer dip stations at entry points and visitors policy to use protective garments are examples of measures executed in Plant 2. Regarding C&S, significant differences in SSOPs from each plant were identified, including chemical products used in C&S. Concerning C&S procedures, Plant 2 reported the use of foam to clean equipment, and quaternary ammonium compounds (QAC) or chlorine used to sanitize floors and other food contact surfaces, and only QAC for drain sanitation. Plant 2 reported having a designated supervisor or employee responsible of the C&S verification; whereas Plant 1 performs this task informally. Procedures for drain cleaning reported by Plant 1 contained high risk activities, e.g., use of high pressure water for daily cleaning of drains, no sanitation step after cleaning drains, and lack of designated cleaning tools for drains. Plant 1 staff was advised to cease the use of high pressure water and to follow effective drain C&S practices. Lastly, a third-party food safety

and sanitation training is offered once a year to Plant 2 employees, whereas Plant 1 performs the training informally and without written materials. In fact, as reported by manager and employees, the participation of Plant 1 in the current study led to the first in-plant training.

Table 2.9a. Summary of facility-specific information collected from plant managers through questionnaires.

Area	Description	Plant 1	Plant 2
Facility	Location & Area	Rural; 2,500 sq. ft. entire plant; 1,200 sq. ft. processing area	Suburban; 5,000 sq. ft. entire plant; 1,200 sq. ft. processing area
	Pest problems & control	Mice, flies, pets	
	Building construction	1962	2005
	Controlled systems, utilities, other	No air flow control, water from municipal supplier, sewage by public system. Cement floors on raw and finish product areas.	
	Plumbing	No overhead plumbing	Overhead tap water plumbing
Plant activity	Equipment traffic, acquisitions, SSOP	No equipment used or moved from one area of the plant to another. Have acquired both new and used equipment, performing cleaning and sanitation before use.	
	Product storage	Storage of raw and cooked products takes place in coolers, properly separated and identified. No product is stored in processing areas.	
	Daily activities	Employees are not restricted to specific areas. Plant is in operation all year long with one morning shift. Employees take breaks on plant grounds, office or leave the plant.	
	Production volume	~750,000 lbs.	~1,700,000 lbs.
Personnel	Employees	7 permanent + 1 seasonal. Multiple tasks include raw, finished product or C&S.	13 permanent + 2 part time + 2 seasonal. Some have mixed tasks (production/C&S).
	Required garments: Raw areas	Apron, hair cap, gloves (optional), bump caps; Slaughter area: Gloves, boots, helmets	Apron, hats, gloves, boots
	Required garments: Finished products areas	Apron, hair cap, bump caps	Apron, hair net, gloves (cotton & plastic), hats
	Reusable laundry	Outsourced. No color coding per plant area.	In-plant. Color coding per plant area.

Table 2.9b. Summary of facility-specific information collected from plant managers through questionnaires.

Area	Description	Plant 1	Plant 2
Training	Food safety and sanitation training	Provided informally by plant manager. No written materials. No bilingual training option.	**Annual training to all employees** (third-party food safety specialist from industrial firms; Plant manager). Presentations and slides. No bilingual training option.
Control of contamination	Hand washing	Employees assume responsibility for proper hand washing	
	Traffic control	No foot baths or door foamer	Foot baths used (QAC, chlorine), sanitized daily
	Visitors	Visitors are allowed without specific precautions	Visitors must observe: foot baths, hats, hairnets; aprons/coats if handling product
Cleaning & sanitation	C&S procedures	Different C&S procedures applied throughout the plant, same cleaners and sanitizer used. No designated employee for supervision/verification of proper C&S.	Same C&S procedures applied throughout the plant. A supervisor/ employee is responsible for verification of proper C&S
	C&S once a day:	Floors, drains, food contact surfaces, utensils, cleaning devices, all processing equipment used	Floors, drains, food contact surfaces, utensils, cleaning devices, all processing equipment used, walls
	C&S once a week:		Ceilings and coolers
	C&S other frequency:	Walls, ceilings and coolers	Cooler coils, condenser
	Cleaning	No foam**,** hand scrub, equipment always disassembled, high pressure sprayer**,** no compressed air.	Foam is used in all equipment, hand scrub, equipment always disassembled, no high pressure sprayer. Equipment always sanitized after cleaning and air dried before use.
	Sanitizer used	Food contact surfaces: QAC	Quaternary Ammonium (floors, drains, food contact surfaces); Chlorine (floors, food contact surfaces)

Table 2.9c. Summary of facility-specific information collected from plant managers through questionnaires.

Area	Description	Plant 1	Plant 2
Cleaning & sanitation	Drains C&S	Drains cleaned daily with high pressure water, no sanitation after cleaning, tools not designated to drains, tools not sanitized after cleaning	Drains cleaned daily, no high pressure used, always sanitized after cleaning, and tools sanitized after cleaning
Inspection & monitoring	Type of inspection	USDA Custom exempt, inspection through State	USDA inspected and third-party audited
	Environmental monitoring	None	Total Aerobic Plate count, and Generic *E. coli.* Random environmental samples from drains, floors, walls, cold rooms; Processing equipment (4 each/ year); food contact surfaces (100 samples/yr)
Interventions	Carcass interventions	None	Zero Tolerance: no visible feces, milk or ingesta; Organic acid spray (lactic acid, 2.5-5%, spray at room temperature, on beef carcass), or Hot water >160°F, following chilling (surface temp must be below 40F within 24 hrs.
	Ready-to-eat products:	Custom exempt (not for sale or resale) smoked and cured ham and turkey	Custom exempt (not for sale or resale), and retail hams, bacon, sausage & jerky, roasting hogs
			Alternative 3: Sanitation program
HACCP	HACCP plan	None	Beef Harvest

In-plant bilingual training. A one-hour presentation was delivered to employees and management from the participating plants. Given that both facilities lacked a room big enough to fit the group, managers agreed to allow the presentation to be held in the processing area. An advantage of holding the session in the workplace is that it was easier to visualize hot spots of contamination and indicate potential contamination routes. The presentation was performed in an interactive approach, motivating participation of the employees.

Employees from Plant 1 were offered the bilingual training option and all chose the English oral presentation. This training session was the first formal training from an outside source. During the recommendations section, some employees commented about practices used at prior workplaces to control environmental contamination. As a result of the discussion, it was agreed to implement several actions in the short term, namely, foot baths, use of rubber boots in the processing area, and limited access of customers through fabricating areas. In a medium-long term, management shared the intention to invest in relocating the smokehouse to a more suitable area. Options to outsource a periodic C&S crew or a food safety consultant were suggested by staff members in the closing session.

Employees from Plant 2 were offered the bilingual choice where eleven employees chose the English version and five employees chose to receive the training in Spanish. Training sessions were held in separate nearby rooms. Even though food safety trainings have been offered to the employees in the past, this was the first time a bilingual option was available. The employees commented about the dedication of the manager towards food safety and continuous improvement. Recently, the plant had received a third-party audit and the importance of good manufacturing practices and HACCP were well-understood.

Gerenal recommendations were presented to both plants, including: avoid customers entering the facility through the back door and walking across the processing area to the front door, no smoking allowed inside the facilities, prevent cross-contamination in coolers, avoid piling up utensils, tools, brooms, etc., where those items create an obstacle for daily cleaning and sanitation activities, avoid producing aerosol with water at high pressure, and prevent air flow from outdoors into the processing area. A summary of the plant-specific recommendations is presented in Table 2.10

Table 2.10. Recommendations provided to fresh meat plants.

Plant 1
Use separate rubber boots for slaughter and processing areas.
Place a physical barrier to separate slaughter area from smokehouse area. This will aid control air flow and insect infestation migrating into the rest of the plant.
Implement sanitizer dip stations at entry points to processing area.
Organize storage of tools and chair in the smokehouse area and maintenance area, to allow proper wall-to-wall cleaning and sanitation, including corners.
Dissemble all equipment and tables to allow proper cleaning and sanitation. Always keep off the floor and allow to air dry.
Cleaning with water at a maximum temperature of 140°F to dissolve fat.
Do not use high pressure water to clean floor or drains. Only use designated cleaning devices for drains.
Do not use high pressure water to wash offal barrels inside the facility.
Before sanitation step, cleaning crew should change aprons, start sanitizing from the far back and walk their way out of the room.
Use sanitizers at dosage recommended by manufacturer and rotate products (quaternary ammonium componds, chlorine).
Consider the option of using organic acid interventions on carcasses (lactic acid spray).
Ask customers or visitors to use the sanitizer dip stations when walking into the plant; preferably, visitors should not walk into the processing room from back door.
Plant 2
Adjust cleaning and sanitation of area between processing and carcass cooler, including door, floor and near drain.
Implement a preventive measure for contamination of platform in slaughter area, as the material is not easily cleaned.
Prevent drain system clogs and floods.

In both plants, as a result of the discussion of results from environmental sampling, participants took a retrospective approach to analyze (i) potential causes of the contamination observed in positive samples, (ii) room for improvement in daily operational practices to control contamination and (iii) commitment with management to implement positive changes. Following the presentations, glo-germ powder and uv light were used to illustrate how contamination is easily spread by hand shaking between members of the group. Glo-germ lotion was used to illustrate proper hand washing practices; a volunteer spread glo-germ lotion on hands, proceeded to wash hands and UV light was used in dark to inspect for any residues. In both plants, the volunteers had a small residue in fingernails, and the use of the brush was encouraged to improve hand washing technique. Managers made the final remarks, highlighting the value of the environmental contamination results and to raise the awareness of the employees to improve or maintain preventive measures of contamination spread within the premises.

General food safety knowledge assessment. Pre and post-training questionnaires were delivered to employees to assess impact of the session in improving knowledge of general food safety facts. Plant managers handed out and collected the tests prior to the training session. All employees from Plant 1, including the manager, filled out and turned in the pre-training evaluation, whereas only 8/17 employees from Plant 2 returned the pre-training evaluation. Plant 2 manager chose not to participate in the training evaluation. Due to time constraints, after completion of the training session, plant managers agreed to administer the post-training assessment and forward the tests to us by post mail. As of 20 days after the training session, only post-training tests from Plant 1 were available. Summary of scores is presented in Table 2.11.

Table 2.11. Pre and post-training scores of knowledge assessment questionnaires of participating employees.

		Nominal scores		Percentage	
Plant	**Employee ID**	**Pre Score**	**Post Score**	**Pre Score**	**Post Score**
	Max Score	53	53	100%	100%
Plant 1	1	52	53	98%	100%
	2	52	53	98%	100%
	3	50	52	94%	98%
	4	40	51	75%	96%
	5	38	46	72%	87%
	6	25	35	47%	66%
	7	36	44	68%	83%
	Plant 1 Mean	**43.25**	**48.38**	**82%**	**91%**
Plant 2	1	28	28	53%	53%
	2	33	52	62%	98%
	3	30	29	57%	55%
	4	39	44	74%	83%
	5	32	39	60%	74%
	6	36	41	68%	77%
	7	31	28	58%	53%
	8	37	n/a	70%	n/a
	Plant 2 Mean*	**32.83**	**38.83**	**61.7%**	**70.4%**

*Obs 8 was not considered for mean estimate, as the post-test score was not available.

Results from the baseline knowledge evaluation were analyzed. All participating employees, from both plants, selected correctly the definition of food contact surface; 93% (14/15) responded correctly that *Salmonella* is not only a concern to the poultry industry, and identified *E. coli* O157:H7 as the pathogen of concern in raw ground beef; 86% (13/15) selected correctly the definition of a bacterial biofilm, and at least 53% (8/15) identified damp drains, wall cracks, squeegees and hollow table legs as potential harborage sites for *L. monocytogenes*. Before instruction, knowledge was limited for several topics, including the definition of a foodborne outbreak. Overall, 66% (10/15) missed selecting the correct definition of a foodborne outbreak, and only 33% (5/15) identified raw milk as a possible source of *Salmonella*. Participant opinion about the usefulness of safe food handling

recommendations at home was inquired; 93% (14/15) indicated they think these recommendations are useful at home.

Pre and post-training comparison and t-test of significance. Pre-training test mean scores were 82% (max 98%, min 47.2%) for participants from Plant 1 and 61.9% (max 74%, min 53%) for employees from Plant 2. Post-training test mean scores increased to 90% correct (max 100%, min 66%) for participants from Plant 1, whereas Plant 2 mean score was 70% (max 98%, min 53%). The increase in knowledge was significant ($p<0.05$) only for Plant 1, but not for Plant 2. The results indicate: (i) a higher score in theoretical knowledge does not translate into applicable prevention and control measures in Plant 1, and (ii) regardless of a low score in food safety concepts, active supervision and verification of standard operational procedures are the key for a successful control of environment contamination in Plant 2.

2.5. Discussion

A six-month longitudinal study was conducted to estimate the prevalence of *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, and other *Listeria* species in the processing environment of two small fresh meat processing facilities. Testing of 1,979 environmental samples from two fresh meat processing plants allowed us to estimate the prevalence and identify areas that harbor presumed persistent strains. Results from this study indicate (i) the prevalence and molecular ecology of *L. monocytogenes*, and other *Listeria* species varied between the two fresh meat processing plants, (ii) *Salmonella* and *E. coli* O157:H7 have a lower prevalence than *L. monocytogenes* in meat processing premises and (iii) risk factors seem to contribute to a higher prevalence and persistence of *L. monocytogenes* and other *Listeria* spp. in a very small fresh meat processing facility.

The prevalence and molecular ecology of *L. monocytogenes*, and other *Listeria* species varied between the two fresh meat processing plants. Results from this study are in

agreement with previous longitudinal studies where a single or a few predominant *L. monocytogenes* or other *Listeria* spp. strains colonized the environment of various types of food processing plants and each processing facility had a unique contamination pattern and specific ribotypes appeared to persist in the environments over time (Norton et al., 2001; Williams et al., 2011). Drains and other non-food contact surface areas were found to have the highest prevalence, in agreement with previous studies conducted in various food processing environments (Thimothe, et al., 2004; Kabuki et al., 2004; Williams et al., 2010).

Although *Listeria* spp. are not pathogenic, its presence is an indicator of failure in C&S procedures. In Plant 1, predominant *L. innocua* AT-1 was recovered from 15% (51/330) of samples and found widespread across the facility (recovered from 24 sampling sites); these results provide evidence of contamination patterns from slaughter areas to smokehouse and processing areas. With a lower prevalence, *L. innocua* was also the predominant of *Listeria* species in Plant 2; it was recovered mainly in the slaughter area. *L. innocua* AT-6 was the predominant strain with only seven (2%) positive samples and *L. welshimerii* AT-129 was isolated in six occasions in the carcass cooler area, and the door connecting to the processing area; findings which suggest a potential source of contamination in the processing area. *L. monocytogenes* predominant strain was ribotype DUP-1042B, isolated at least once from 27% of sampling sites (15/55) and recovered up to five times from 15 different sampling sites. Ribotype DUP-1042B belongs to a major human outbreak-associated clonal group known as Epidemic Clone I (Kathariou, 2002); it has also been found among sporadic-case isolates, food isolates, various animal and environmental isolates (Chen et al., 2010) and is among specific lineage I strains that represent major epidemic clones with enhanced virulence characteristics (Gray et al., 2004). In light of these results and from a risk assessment standpoint, the high prevalence of ribotype DUP-1042B increases the need for a prompt intervention to control contamination patterns and

harborage sites within Plant 1. On the other hand, in Plant 2, *L. monocytogenes* DUP-1030B was recovered once from three different drains and DUP-1030A from one floor sample. Ribotype DUP-1030A has been isolated from milking farms and dairy facilities (Ho et al., 2007) food and human isolates (Gray et al., 2004); whereas DUP-1030B was recovered in a dairy plant (Ho et al., 2007), ruminant farm environment (Nightingale et al., 2004) and human isolates (Gray et al., 2004).

Overall, results indicate that hurdles in place and C&S practices in Plant 2 prevented contamination spread and persistence of pathogens in the facility. The use of footbaths with sanitizer, and physical barriers (doors, hallways) between the slaughter area and processing area, seem to be key elements to contain the incoming contamination.

***Salmonella* and *E. coli* O157:H7 have a lower prevalence than *L. monocytogenes* in meat processing premises.** Persistence of *E. coli* O157:H7 and *Salmonella* in the plant environment is less well-understood than *L. monocytogenes* persistence. *E. coli* contamination is generally associated with fecal matter cross-contamination; however, a recent study reported the recovery of the same *E. coli* strains from the same sites in the environment of beef packing plants over regular intervals for more than one year, suggesting that *E. coli* can also persist within facility high-risk production areas (Holah et al., 2004). A *Salmonella* Agona strain implicated in two multi-state outbreaks of salmonellosis, that occurred in 1998 and in 2008, was isolated from products manufactured within the same food manufacturing facility (CDC, 1998; CDC, 2008), suggesting the organism persisted in the environment over the 10-year period. Recent studies have illustrated the transmission contamination of *Salmonella* from environment to carcasses in swine production or processing facilities (Swanenburg et al., 2000; Magistrali et al., 2008; Kich et al., 2011); few studies reported *Salmonella* genotypes found to persist within pig farm environments for a period of up to two yrs (Sandvang et al., 2000; Baloda et al., 2001) or

five months (Callaway et al., 2005). Biofilm forming ability of a *Salmonella* strain may be an important factor for persistence in the food processing environment (Vestby et al., 2009).

In our study, although areas visibly contaminated with fecal matter were sampled, we observed a low prevalence of *Salmonella* and almost absence of *E. coli* O157:H7 isolates. These results could be attributed to different factors: (i) incoming lots of cattle and swine had a low prevalence or were not carriers of the pathogen; (ii) the sponge samples were collected in spots where the pathogen was not present but could be present in surroundings, (iii) the pathogen was present but injured cells could not recover during enrichment or were not isolated. Occurrence of certain pathogens at the time of the sample collection may be influenced by level of pathogenic bacteria carried by the lot or species being processed. Additionally, lower positive samples may be caused when injured cells enter a viable but not culturable (VBNC) state. Previous studies in food processing environments have reported the presence of VBNC *E. coli* O157:H7 due to repeated chemical treatments; Marouani-Gadri et al. (2010) found VBNC cells that were able to divide under conditions typical of harborage sites; whereas Xu et al. (2010) observed planktonic and biofilm S. Typhimurium cells can enter the VBNC state under acid stress conditions.

Although *E. coli* O157:H7 or *Salmonella* were recovered from a sampling site, the absence of a repeated isolation from the same site or area may be attributed to (i) cleaning and sanitation (C&S) procedures were effective in prevention of persistence or (ii) resident microflora outcompetes pathogens. Bacterial interactions have been described in biofilms; symbiotic or detrimental interactions between groups of bacteria may take place in multispecies biofilms (Watnick and Kolter, 2000); e.g., resident microbiota of a meat-processing plant was shown to have a favorable effect on *E. coli* O157:H7 colonization of a solid surface (Marouani-Gadri et al., 2000) and, in contrast, nutritional competition was observed between *E. coli* O157:H7 and *Enterobacter absuriae* (Cooley et al., 2006).

Risk factors for environment contamination seem to contribute to a higher prevalence and persistence of *L. monocytogenes* and other *Listeria* spp. in a very small fresh meat processing facility. Major differences between the participating facilities were: (i) age of buildings, (ii) inspection regime, (iii) existence of a verifiable HACCP plan, including periodic environmental monitoring samplings and carcass interventions. Plant 1 is a very small fresh meat processing plant, operates under custom exempt inspection and employs 8 people. The total area of the facility is 2,500 sq. ft. and it is located in a rural location; a portion of the building, which comprises a slaughter area and coolers, was built 50 years ago. In contrast, Plant 2 is a small fresh meat processing facility, operating with 15-17 employees. Its 7-year old building has a total physical area of 5,000 sq. ft. and it is located in a suburban area; Plant 2 is under federal inspection, and also has an annual third-party audit.

The aforementioned differences may play an important role in the general environmental contamination, possibly giving a disadvantage to the plant operating in an older building. However, during the course of this study, differences at the operational level were identified. In light of the bacteriological results from this study, good manufacturing practices for the control of environmental contamination practiced by Plant 2 seem to be effective in the prevention of contamination spread and pathogen persistence. The appropiate adjustments in these procedures may have a significant effect in the reduction of environmental contamination and prevention of *L. monocytogenes* persistence in Plant 1. The procedures and policies found to differ between the two plants were: (i) control of contamination spread within the plant, (ii) cleaning and sanitation (C&S) of drains, and (iii) employee food safety training.

Prevention of contamination spread and pathogen persistence is addressed in Plant 2 by the use of sanitizer dip stations at entry points, use of foam to clean equipment, use of

quaternary ammonium compounds (QAC) or chlorine for sanitation of floors and other food contact surfaces, and QAC for sanitation of drains. Conversely, the lack of these hurdles in Plant 1 seems to be a disadvantage. Furthermore, some high risk procedures practiced by the cleaning crew in Plant 1 may contribute to contamination spread; e.g., use of high pressure water for daily cleaning of drains, no sanitation step after cleaning drains, and lack of designated cleaning tools for drains. As part of the standard sanitation operating procedure, Plant 2 reported having a designated supervisor or employee responsible of the C&S verification; whereas Plant 1 performs this task informally.

Lastly, a third-party training in food safety and sanitation topics is offered once a year to Plant 2 employees, whereas Plant 1 performs the training informally and without written materials. As part of this study, a bilingual English-Spanish training was offered to meat handlers employed at a small and very small meat processing plant in Colorado. Recipients of the training chose the language of oral presentation and received bilingual handouts and food safety knowledge evaluation questionnaires. To assess knowledge of content, a pre and post-training evaluation was developed; participating employees received bilingual questionnaires to fill out voluntarily and anonymously. Food safety knowledge questionnaires have been found to be useful to assess subject matter knowledge before and aftery instruction (Medeiros et al., 2004). Other research studies have reported problems of time restriction, turnover of employees, diversity within the workforce including varying literacy levels and languages, and low literacy of some participants. As a result of such factors, it is recommended that educational materials be developed at the appropriate level for the target audience, taking into account participants may have difficulty with the English language (Fenton et al., 2006). We assessed the effect of the training delivered to meat handlers from participating meat plants. Results showed a contrast between general food safety knowledge and environment contamination in both

plants. The literacy level and rotation of employees were not considered in the analysis and may influence the higher scores in Plant 1 and lower scores in Plant2. In light of our results, active supervision and verification of written procedures, along with manager leadership, are essential for effective control of environmental contamination, regardless of the theoretical knowledge of the meat handlers.

2.6. Conclusion

Although economic resources may be limited, microbiological monitoring of fresh meat processing plant environments paired with molecular subtyping, is useful from a risk assessment standpoint. In this study, the prevalence of *L. monocytogenes* was high and widespread in Plant 1, and a predominant strain belonging to an Epidemic Clone group was identified. This information increases awareness, provides the plant management with valuable information for decision making, and motivates the implementation of new policies and targeted interventions in problematic areas.

Our findings suggest that *L. monocytogenes* has higher prevalence than *S. enterica* and *E. coli* O157:H7 in the fresh meat processing plant environments. While *L. monocytogenes* may persistently contaminate the environment of fresh meat processing plants, *E. coli* O157:H7 and *S. enterica* contamination seem to be mostly sporadic. Targeted sanitation interventions, prevention of cross-contamination and employee training are elements which have been found to be effective in plant-specific strategies to control *L. monocytogenes* contamination (Lappi, et al., 2004). A follow up environmental sampling will probe effectiveness of management to remediate contamination evidenced by results of this study. Further identification of biofilm formation and virulence profiles of bacterial isolates will provide useful information for risk assessment.

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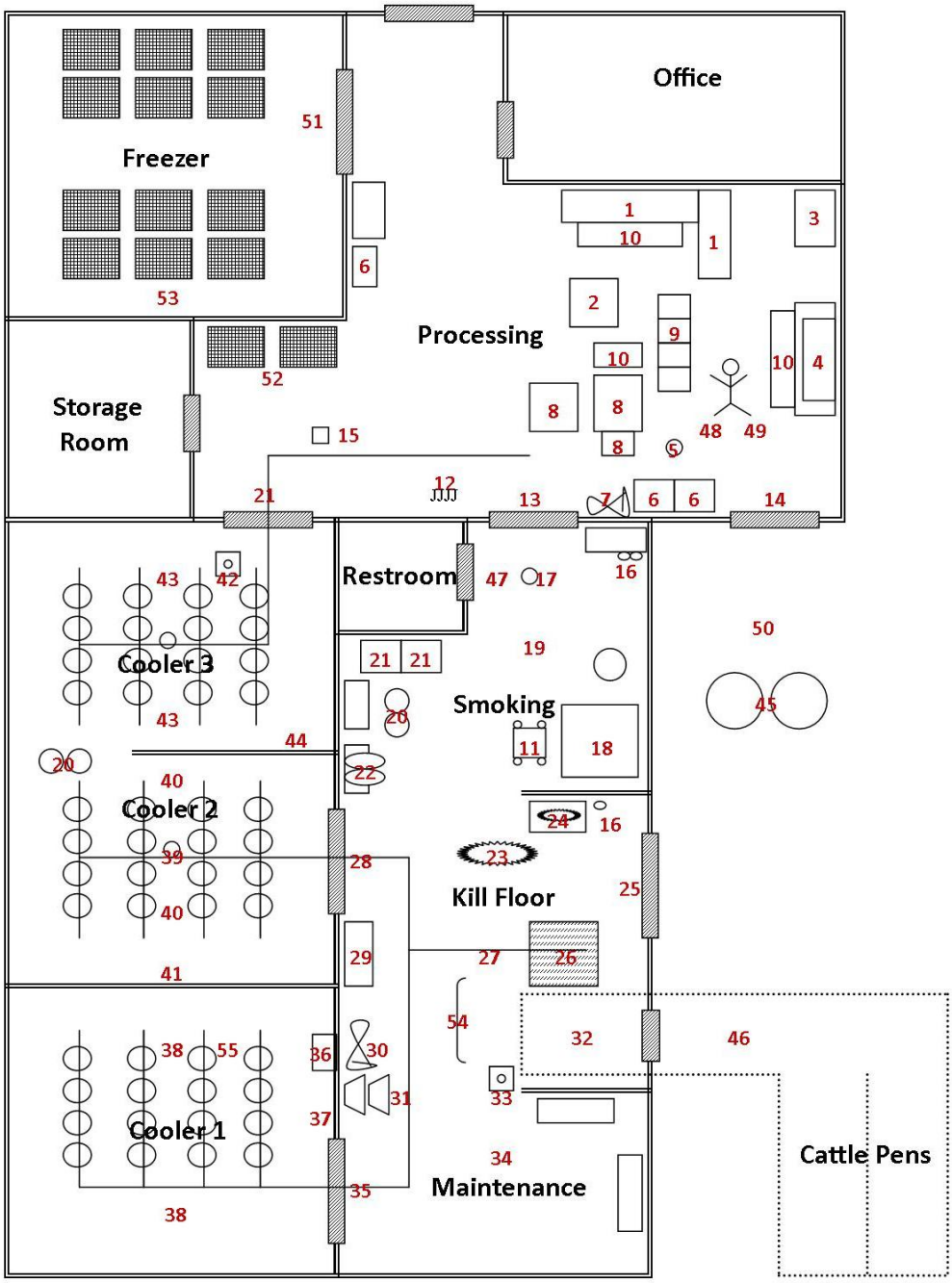
APPENDICES

Appendix 1. Facility layout and sample collection sites in Plant 1.

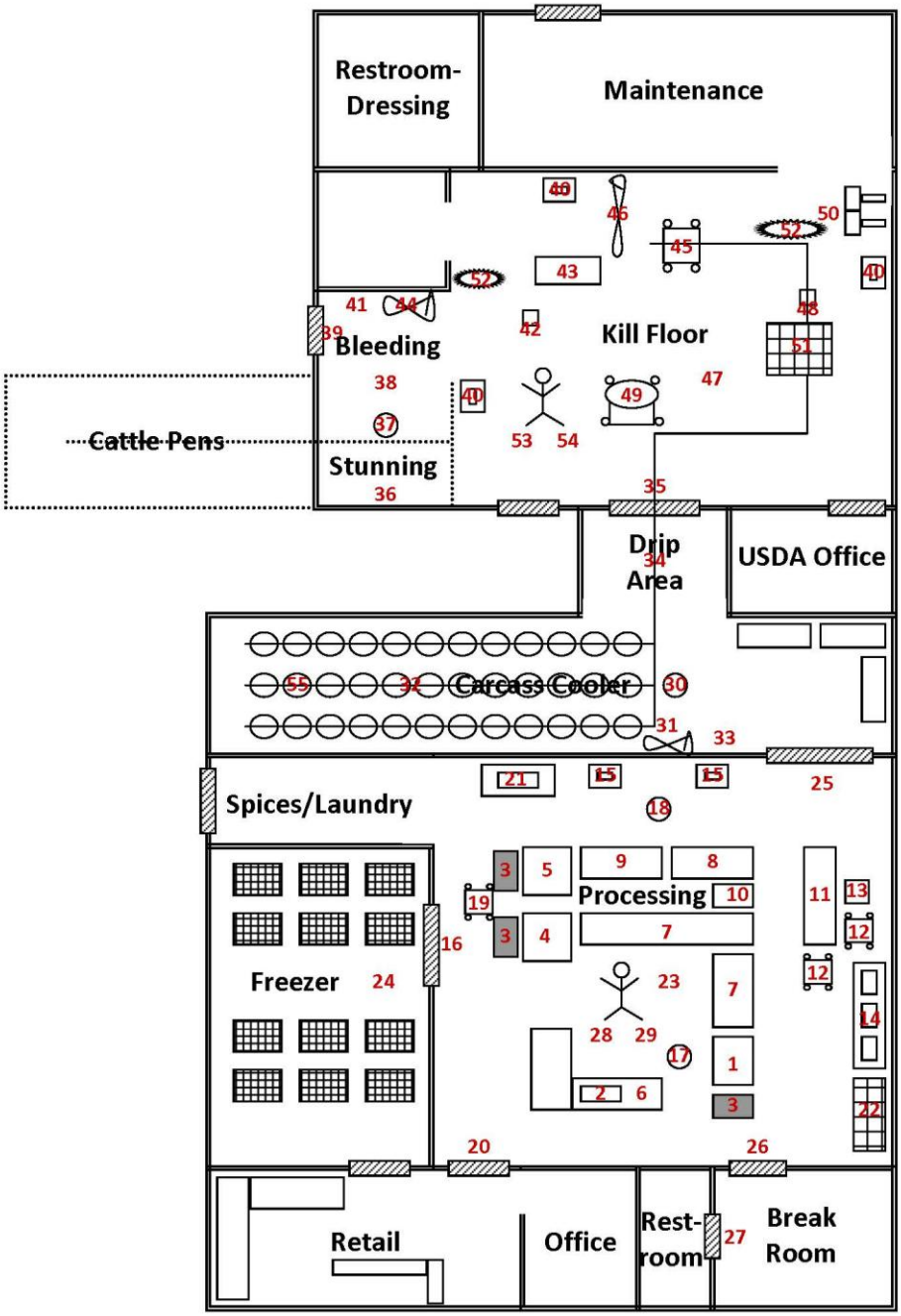


Design by A. Brandt

Plant 1 sample collection sites key:

1. Packaging tables
2. Band saw and table
3. Slicer
4. Grinder with hopper
5. Drain, processing area
6. Sinks, processing area
7. Hose, processing area
8. Fabrication tables and hopper
9. Blue tubs
10. Stress mats, processing area
11. Truck wheels and framework
12. Hooks
13. Door, processing to smokehouse area
14. Door, processing to outside
15. Floor, next to drain
16. Brooms/mops/squeegees in slaughter area
17. Drain, smokehouse area
18. Smokehouse door and handle
19. Smokehouse area floor and floor-wall junction
20. Buckets, smokehouse area and coolers
21. Sink, smokehouse area
22. Employee boots, slaughter area
23. Large circular saw
24. Small hand saw
25. Door, slaughter area to outside
26. Large drain
27. Floor, slaughter area
28. Door, slaughter area to cooler 2
29. Cradle
30. Hose, slaughter area
31. Employee aprons, slaughter area
32. Stunning chute pipework
33. Shackling control button
34. Floor, maintenance area
35. Door, maintenance to cooler 1
36. Draining trap door, cooler 1
37. Walls, cooler 1
38. Floor, cooler 1
39. Drain, cooler 2
40. Floor, cooler 2
41. Walls, cooler 2
42. Control button
43. Floor, cooler 3
44. Walls, cooler 3
45. Offal collection barrels
46. Cattle pens and chutes
47. Door, restroom to smokehouse area
48. Employee boots, processing area
49. Employee apron, processing area
50. Slab area outside
51. Door, freezer
52. Finished product crates, processing area
53. Finished product crates, freezer
54. Cross bar with hooks
55. Beef carcass samples

Appendix 2. Facility layout and sample collection sites in Plant 2.



Designed by A. Brandt

Plant 2 sample collection sites key:

1. Vacuum packager surface
2. Scale #1, processing area
3. Plastic tubs
4. Grinder #1
5. Grinder #2
6. Table
7. Packaging tables
8. Band saw and table
9. Table
10. Basin area
11. Cutting tables
12. Metal carts (interior)
13. Moveable table/pedestal
14. Washing sinks, processing area
15. Hand sinks, processing area
16. Door, freezer to processing
17. Drain #1, processing area
18. Drain #2, processing area
19. Carts (wheels and framework)
20. Door, office to processing
21. Scale #2, processing area
22. Metal racks
23. Floor, processing area
24. Floor, freezer
25. Door, processing to cooler
26. Door, processing to break room
27. Door, restroom to break room
28. Employee apron, processing area
29. Employee shoes, processing area
30. Drain, carcass cooler
31. Hose, carcass cooler
32. Floor, carcass cooler
33. Wall, carcass cooler
34. Floor, drip area
35. Door, drip area to slaughter area
36. Stunning chute area
37. Drain, stunning area
38. Floor, bleeding area
39. Door, bleeding to exterior
40. Hand sinks, slaughter area
41. Bleeding area walls
42. Drain #1, slaughter area
43. Cradle
44. Hose, bleeding area
45. Offal truck #1
46. Hose, slaughter area
47. Floor, slaughter area
48. Drain #2, slaughter area
49. Offal truck #2
50. Plastic shovels
51. Platform
52. Large circular saw and small handsaw
53. Employee apron, slaughter area
54. Employee boots, slaughter area
55. Beef carcass samples

Appendix 3a. PCR Assay targets, primer names, primer sequences, primer concentrations, product sizes, references and thermal cycling conditions.

PCR Assay	Target Gene/ORF	Primer Name	Primer Sequence (5' to 3')	Product Size	Reference
Listeria monocytogenes confirmation	hlyA	LM hly-α (Forward)	CCT AAG ACG CCA ATC GAA AAG AAA	858 bp	Norton et al., 2001
		LM hly-β (Reverse)	TAG TTC TAC ATC ACC TGA GAC AGA		
Other *Listeria* spp. confirmation and speciation-subtyping	sigB	sigB15-F (Forward)	AAT ATA TTA ATG AAA AGC AGG TGG AG	840 bp	Nightingale et al., 2005
		sigB16-R (Reverse)	ATA AAT TAT TTG ATT CAA CTG CCT T		
Salmonella enterica confirmation	invA	CAA1-invAF (Forward)	GAA TCC TCA GTT TTT CAA CGT TTC	678 bp	Kim et al., 2007
		CAA2-invAR (Reverse)	TAG CCG TAA CAA CCA ATA CAA ATG		

Appendix 3b. PCR Assay targets, primer names, primer sequences, primer concentrations, product sizes, references and thermal cycling conditions.

PCR Assay	Target Gene/ORF	Primer Name	Primer Sequence (5' to 3')	Product Size	Reference
Escherichia coli O157:H7 confirmation	hlyE	EC hly-F (Forward)	CCC TGG CAG ACC TTT GAT G	772 bp	Manuel, 2011
		EC hly-R (Reverse)	CCG TGT CTT TTC TGA TAC TCA		
	$fliC_{h7}$	$FLIC_{h7}$-F (Forward)	GCG CTG TCG AGT TCT ATC GAG C	625 bp	Gannon et al., 1997
		$FLIC_{h7}$-R (Reverse)	CAA CGG TGA CTT ATC GCC ATT CC		
	stx2	SLT-IIF (Forward)	GTT TTT CTT CGG TAT CCT ATT CCG	484 bp	Meng et al., 1997
		SLT-IIR (Reverse)	GAT GCA TCT CTG GTC ATT GTA TTA C		
	eaeA	IntF (Forward)	GAC TGT CGA TGC ATC AGG CAA AG	368 bp	Hu et al., 1999
		IntR (Reverse)	TTG GAG TAT TAA CAT TAA CCC CAG G		
	rfbE	RfbF (Forward)	GTG TCC ATT TAT ACG GAC ATC CAT G	292 bp	Hu et al., 1999
		RfbR (Reverse)	CCT ATA ACG TCA TGC CAA TAT TGC C		
	stx1	SLT-IF (Forward)	TGT AAC TGG AAA GGT GGA GTA TAC	210 bp	Meng et al., 1997
		SLT-IR (Reverse)	GCT ATT CTG AGT CAA CGA AAA ATA AC		

Appendix 4. Confidential questionnaire for plant managers.

PLANT PROFILE QUESTIONNAIRE
CONFIDENTIAL

Please print

Plant name or Letter Designation: _____

Location: _____

Phone number: ( ) _____ Fax: ( ) _____

Contact Person(s):_____

Date: _____

The purpose of this questionnaire is to assist in understanding the conditions in the processing plant that can contribute to contamination by foodborne pathogens of interest such as *Listeria monocytogenes, Salmonella and E. coli* O157:H7.

I. Plant Diagram or Floor Plan

Please review the provided plant map. It might not be drawn to scale, but should depict the relative size and location of each of the areas of the plant including:

1. Receiving areas or loading dock(s) for raw materials and/or finished products
2. Storage areas (cooler & freezers) for raw materials and finished products
3. Storage areas for ingredients or packaging materials
4. Raw product handling or work areas
5. Processing areas and equipment location
6. Packing areas or finished product order assembly areas
7. Employee areas and restrooms
8. Hand wash stations and Foot baths (if any)
9. Offices or other non-processing areas of the plant
10. Any other relevant areas located in the same building or physical structure as the plant

II. Product Flow

Please provide a copy of Process Flow Diagrams for the company's products. If possible, provide Process Flow Diagrams for each HACCP plan for products. If available, a processing narrative describing what occurs at each of these steps, would be useful in analyzing the process.

Using the plant map, please identify where each of the activities in the plant's Process Flow Diagram takes place. Use arrows or other designations if appropriate to visually represent how the process flow takes place in the physical structure of the plant.

III. Physical plant characteristics

Unless otherwise indicated, please circle all appropriate answers, fill in answers in the lines provided and include any additional comments as necessary.

EXTERIOR AREA

1. Describe the general area where the plant is located:

a. Rural
b. Urban
c. Suburban
d. None of the above, describe_____

2. Circle any of the following to describe the immediate plant surroundings:

a. Located in an industrial area or park
b. Attached to an adjacent building on one or more sides
c. Detached building surrounded by paved areas, streets or parking lot
d. Detached building surrounded by well maintained grounds
e. Detached building surrounded by unpaved areas, woods, fields, other non-maintained areas

3. Is the plant located near water or on a site that may be exposed to tidal or other flooding, accumulating pools of water or other similar conditions?

a. No
b. Yes. Describe_____
_____
_____

4. Is there a history or evidence of existing or potential problems related to:

a. Gulls or other birds. Describe _____
b. Pets or feral animals on or near premises. Describe_____
c. Rodents. Describe_____
d. Flies or other flying insects. Describe_____

FACILITY INFO

5. Provide the square footage of the:

Entire physical structure where the plant is located?
_____

Processing area?
_____

6. Approximately how old is the building or physical structure of the plant?

_____ Years

7. Please indicate when construction or other upgrades in the physical structure of the entire plant or certain areas of the plant were last made.

Approx time	Description of work done	Plant Location	Plant continue to work (Yes/No)
Past 2 months			
Past 6 months			
Past year			
More than 1 year ago			
More than 3 years ago			
Others			

8. If plant continued to operate during repairs, indicate any special procedures used to segregate production from construction areas.

Please describe _____
_____
_____
_____

9. Do you have an air-flow control system or strategy in your plant?

a. No
b. Yes, finished product room has a positive air pressure.
c. Yes, other. Please specify the control system and airflow _____

10. Is there any overhead plumbing in the plant?

a. No
b. Yes, indicate where it is located: ____ In raw product processing/handling areas
____ In finished product processing/handling areas

11. What type of water is used in your plant? (circle all that apply)

a. Water from a municipal supplier
b. Water from a private supplier
c. Water from a well or other private source.

12. If well water or water from another private source is used:

a. Where is this water used:

____ For processing
____ For cleaning raw product areas
____ For cleaning storage areas
____ For making ice
____ For cleaning finished product areas
____ Other- Describe_____
_____

b. Do you treat the water used from wells or private sources ____ No____ Yes
If yes, what treatments?
_____

c. Do you test or require testing of well water or private suppliers? ____ No____ Yes
If yes, how often?
_____

13. What type of sewage system is used for the plant?

a. Sewage is collected by public system
b. Sewage is collected by a private company
c. Sewage is treated on site
d. Other_____

14. What other waste disposal system is used:

a. Haul garbage to a landfill yourself
b. Dumpster(s) serviced by municipality
c. Dumpster(s) serviced by private firm
d. Other – Describe_____

15. Please describe the type of floors in these two product handling areas?

Raw material handling area(s)	Finished product handling area(s)
a. Cement	a. Cement
b. Cement with epoxy or other coating	b. Cement with epoxy or other coating
c. Tile	c. Tile
d. Other_____	d. Other _____

EQUIPMENT

16. Is equipment used or moved from one area of the plant to another?

a. No
b. If no, is equipment designated for specific areas of the plant marked or color coded or marked or labeled in some way? ____ No____ Yes
c. Yes, describe_____
_____
_____
d. Other_____

17. List any new or used equipment (machines, shelves, tables, etc.) recently acquired

Approx time	Description of Equipment	New / Used	Plant Location
Past 2 months			
Past 4 months			
Past 6 months			
Past year			
More than 1 year ago			
Others			

18. If used equipment was introduced to the facility, please describe any special cleaning or sanitation procedure performed.

_____
_____
_____

19. Please identify locations where products are stored (mark all that apply)

Type of product	Processing Area	Cooler	Freezer	Other
Carcass				
Fresh meat cuts				
Frozen meat				
Brine				
Smoked product				
Cooked product				

20. Are processing areas restricted to employees only?

a. No
b. Yes

21. Are visitors, clients, or groups allowed in the plant?

a. No
b. Yes, with no specific precautions.
c. Yes, but they are required to wear clothing provided by the plant. Please mark what clothing is provided:
   (i) Hats and/or hairnets
   (ii) Boots or special shoes
   (iii) Aprons, coats etc.
   (iv) Other, please specify_____

IV. Production and Process Characteristics

22. When is the plant in operation?
a. All year long
b. Part of the year, please specify season of operation_____

23. What was the total production volume of the product(s) processed in this plant in the year 2011?_____

24. How many shifts are there per day?
a. One, starting at_____
b. Two, starting at_____and_____
c. Three, starting at_____and _____and _____
d. Other _____

V. Processing Personnel

25. How many employees work in the plant?
a. Full-time_____
b. Part-time_____
c. Seasonal workers _____

26. How many employees work in these areas?

	Raw product processing	Finished product processing	Finished product packing	Maintenance and Cleaning	Sales or Office Work	Other
Full time						
Part time						
Seasonal						

27. How many breaks are employees scheduled to have?
a. 1
b. 2
c. 3 or more, specify_____

28. Where do employees take their breaks? (circle all that apply)
a. In processing area(s) of the plant
b. In a designated area used only by employees for lunch or breaks
c. On the grounds of the plant
d. Leave the plant grounds during lunch but not for breaks
e. In the receiving loading area
f. In the office area
g. Other - Describe_____

29. Is there special clothing requirement in the processing area?

a. No (proceed to question # 33)
b. Yes

30. If you have requirements for outer garments, hair restraints etc., circle the required items in the following areas of the plant:

Raw Processing Area	Finished Product Processing Areas
a. Apron	a. Apron
b. hair cap	b. hair cap
c. gloves	c. gloves
d. hat	d. hat
e. boots	e. boots
f. masks	f. masks
g. other	g. other

31. What happens to protective clothes, aprons, hats, gloves, etc., after they get dirty?

Please indicate how frequently garments are changed (more than once a day, once a day, once a week, so on).

Protective gear	Disposable or Reusable	Frequency of change	Provided by company (yes/no)	Responsible of laundry
Aprons				
Hats, helmets				
Gloves				
Wool gloves				
Hairnets				
Footwear				
Other				

32. Do you use color coding for clothing, hair restraints, etc., in various processing areas?

a. No
b. Yes, describe_____
_____

33. Are foot baths used to control contamination from foot traffic?

a. No
b. Yes, please indicate locations of foot baths in the floor plan

34. How is the flow of employees from raw product processing/handling areas to finished product processing/handling areas controlled?

a. Employees are assigned to raw material unit operations and instructed not to go to other finished product processing areas
b. Employees are assigned to finished product processing areas and instructed not to go to raw product processing areas or any other area of the plant
c. Employees are allowed to go to any area as long as they observe sanitation procedures
d. Employees are allowed to go to any area without restriction
e. Other practice_____

35. How do you monitor the use of employee hand wash stations? Please mark all that apply.

a. Supervisors oversee the use of hand wash stations at the beginning of the shift
b. Supervisors oversee the use of hand wash stations throughout the shift
c. Supervisors oversee the use of hand wash stations after breaks and lunch
d. An electronic device or other system is used to ensure hand washing
e. Employees assume responsibility for proper hand washing

TRAINING

36. Do plant employees receive training about food safety, sanitation, hand washing, and other related topics?

Employee	Receives training (Yes/No)	Type of training	Frequency
Supervisors			
Retail areas			
Finished product			
Packaging			
Raw product processing			
Kill floor			
Cleaning crew			
Other			

37. Is a bilingual training option available?

a. No
b. Yes

VI. Cleaning and Sanitizing Procedures

38. Who is assigned to do cleaning and sanitizing procedures in the plant (mark all that apply):

a. Specific employees are assigned to perform only cleaning and sanitizing, and these employees do not have any production responsibilities
b. Employees assigned to cleaning and sanitizing also have production responsibilities
c. All employees are responsible for cleaning and sanitizing their own work areas
d. An outside contractor cleans and sanitizes the plant
e. Other – Describe_____

39. Is a supervisor or other employee responsible for checking to verify that processing areas have been properly cleaned and sanitized?

a. No
b. Yes
c. Occasionally

40. Do you use the same cleaning and sanitizing procedures and products throughout the plant?

a. No
b. Yes

41. If no, which of the following is true?

a. Same procedures are performed in all plant areas but using different cleaners and/or sanitizers
b. Different procedures in some plant areas but the same cleaners and/or sanitizers
c. Same procedures and the same cleaners & sanitizers in all areas of the plant
d. The use of specific cleaners and/or sanitizers is rotated on a regularly scheduled basis

42. How often are the following cleaned and sanitized? (mark one)

	Each time it is used	More than twice/day	Twice/ Day	Once /day	Once/ week	Other
a. Floors						
b. Drains						
c. Product contact surfaces						
d. Conveyors						
e. Walls						
f. Ceilings						
g. Utensils						
h. Cleaning devices (brooms, sponges etc)						
i. Coolers						
i. Cooler coils, condensor						
j. Other Special equipment Specify:						

43. Which sanitizers are used for the following? (mark all that apply)

	a. Floors	b.Drains	c.Conveyers	d. Other product contact surfaces	d. Other, please describe
Quaternary ammonium compounds					
Peracetic acid and peroctanoic acid					
Chlorine					
Acid-anionic					
Peracid					
Iodophors					
Powdered citric acid					
Other					

44. Please indicate the cleaning procedure used for equipment.

	No	Yes	Which equipment?
a. Is foam used to clean equipment?			
b. Is equipment cleaned by hand application?			
c. Is equipment always disassembled before cleaning?			
d. Are high pressure sprayers or power washers used for cleaning?			
e. Is compressed air used to remove debris from equipment?			
f. Is equipment always sanitized after cleaning?			
g. Is equipment always air dried before use?			

50. If yes, mark which indicator organism or foodborne pathogens are tested?

a. Total Aerobic Plate Count
b. Generic *E. coli*
c. *Salmonella*
d. *Listeria* spp.
e. *Listeria monocytogenes*
f. Other_____
_____

51. How often are environmental samples collected in your plant?

a. More than once a week
b. Once a week
c. 1-3 times a month
d. Less than once a month, but more than 4 times a year
e. Less than 4 times a year
f. Other_____

52. Where are samples for environmental testing collected? (mark all that apply):

Location	Sampled (Yes/No)	Frequency	Approx samples collected in 2011
Drains			
Floors			
Walls			
Cold rooms			
Processing equipment Non food-contact surfaces			
Processing equipment food-contact surfaces			
Other food contact surfaces			
Employee hands/gloves			
Other			

53. Please briefly describe how the laboratory test results are used.

_____
_____
_____

INTERVENTIONS

54. Do you use carcass spray interventions?

a. Organic acid spray
b. Steam
c. Other_____
d. Not applicable

55. Do you produce any ready-to-eat products?

a. No (proceed to Section VII SSOP).
b. Yes, please describe_____
_____

56. If you produce ready-to-eat products, please mark which one of the following applies to your process:

a. Alternative 1: Post-lethality treatment **and** antimicrobial agent or process
b. Alternative 2: Post-lethality treatment **or** antimicrobial agent or process **and** sanitation program
c. Alternative 3: Sanitation program

57. If you add any antimicrobial into the formulation of ready-to-eat products or as a post-lethality treatment, please specify_____
_____
_____
_____

VII. SSOP (Standard Sanitation Operation Procedure)

If you have a written SSOP for your plant please provide a copy if possible.

VIII. HACCP Plan

If available, provide a copy of the HACCP Plan.

IX. Observations

Any additional relevant information should be summarized in this space.

Note: The information collected in this questionnaire will be kept confidential, except as necessary to fulfill the objectives of the research plan. Where data are linked to specific plants, the facility will only be identified by the code given to each of the plants participating in this project.

X. Production Volume, Plant Size and Finished Products

Information that provides an estimate of the size of the plants, their production volume, the types of finished products that are produced, and the various raw materials that are used and are present in the plant is needed. Please provide that information below or complete the following two tables on finished products and raw materials as appropriate.

Finished Product Production

Please provide information on the finished products that are produced in your plant. Option: Provide an order form or other existing list of all of your finished products and add the information requested in the chart below.

Finished Product	Packaging	Check Distribution Method		Production volume (lbs/year)	Other information
		Refrigerated	Frozen		

Raw Materials

Please list all incoming raw materials below and indicate their origin, the form in which the product is received (raw, fresh, frozen, gutted, whole, preprocessed, pasteurized, cooked etc.) and briefly describe your purchasing specifications for the raw materials (if any).

Product	Product form (raw, frozen, gutted, whole, fillets, cooked, brined etc.)	Origin – Country State or Region	Purchasing specifications

Appendix 5. Plant profiles as reported by managers

Plant 1 Profile

I. Physical plant characteristics

1. Describe the general area where the plant is located: Rural
2. Circle any of the following to describe the immediate plant surroundings: Detached building surrounded by well maintained grounds
3. Is the plant located near water or on a site that may be exposed to tidal or other flooding, accumulating pools of water or other similar conditions? No
4. Is there a history or evidence of existing or potential problems related to:
   - Pets or feral animals on or near premises: Cats that roam area
   - Rodents: mice, but controlled with exterminator
   - Flies or other flying insects: Controlled with spray and air curtain
5. Provide the square footage of the entire physical structure where the plant is located: 2,500 sq. ft.; processing area: 1,200 sq. ft.
6. Approximately how old is the building or physical structure of the plant? Kill floor built 1962; processing room built 1993
7. Please indicate when construction or other upgrades in the physical structure of the entire plant or certain areas of the plant were last made.

Approx time	Description of work done	Plant Location	Plant continue to work (Yes/No)
Past 2 months	new FRP & dry wall	cooler 2	Yes
Past year	FRP repair	cooler 2	No
More than 1 year ago	FRP repair	processing room	No
More than 3 years ago	new freezer insulation	freezer	Yes
Others	new cooler in 2007	cooler 1	Yes

8. If plant continued to operate during repairs, indicate any special procedures used to segregate production from construction areas. Plastic sheeting and did construction when work was not being performed near construction
9. Do you have an air-flow control system or strategy in your plant? No
10. Is there any overhead plumbing in the plant? No
11. What type of water is used in your plant? Water from a municipal supplier
12. If well water or water from another private source is used: n/a
13. What type of sewage system is used for the plant? Sewage collected by public system
14. What other waste disposal system is used: Dumpster(s) serviced by municipality
15. Please describe the type of floors in these two product handling areas? Raw material and finished product handling areas: Cement
16. Is equipment used or moved from one area of the plant to another? No

    Is equipment designated for specific areas of the plant marked or color coded or marked or labeled in some way? No
17. List any new or used equipment recently acquired

Approx time	Description of Equipment	New / Used	Plant Location
Past 4 months	grinder with hopper	used	processing room
More than 1 year ago	back splitting saw	new	kill floor
Others	Smoker, stun gun	New	Smoking, kill floor

18. If used equipment was introduced to the facility, please describe any special cleaning or sanitation procedure performed. Standard SOP for sanitation

19. Please identify locations where products are stored

Type of product	Cooler	Freezer
Carcass, fresh meat cuts, brine	X	
Frozen meat	X	X
Smoked and cooked product	X	X

20. Are processing areas restricted to employees only? No
21. Are visitors, clients, or groups allowed in the plant? Yes, with no specific precautions.

II. Production and Process Characteristics

22. When is the plant in operation? All year long
23. What was the total production volume of the product(s) processed in this plant in the year 2011? 750,000 lb carcass weight
24. How many shifts are there per day? One, starting at 8:00 am
25. How many employees work in the plant? Full-time: 7; part-time: 0; seasonal workers: 1

III. Processing Personnel

26. How many employees work in these areas?

	Raw product processing	Finished product processing	Finished product packing	Maintenance and Cleaning	Sales or Office Work	Other (slaughter)
Full time	6	6	1-2	5	3	4
Part time	0	0	0	0	0	0
Seasonal	1	1	1	1	0	0

27. How many breaks are employees scheduled to have? 3 breaks: morning (10:00am), lunch (12-1pm), and afternoon (3:00pm)
28. Where do employees take their breaks? On the grounds of the plant; in the office area; other: leave ground for lunch
29. Is there special clothing requirement in the processing area? Yes
30. Requirements for outer garments:

Raw Processing Area	Finished Product Processing Areas
Apron, hair cap, gloves (required on kill floor), boots, other: bump cap (processing) and helmet (kill floor)	Apron, hair cap, other: bump cap

31. How frequently garments are changed?

Protective gear	Disposable or Reusable	Frequency of change	Provided by company	Responsible of laundry
Aprons	Reusable	daily	Yes	private company
Hats, helmets	reusable		Yes	
Gloves	Disposable	frequent	Yes	
Wool gloves	reusable	daily	Yes	Company
Hairnets	reusable	as needed	Yes	
Footwear	Reusable			
Other	cut gloves	daily		Company

32. Do you use color coding for garments in various processing areas? No
33. Are foot baths used to control contamination from foot traffic? No
34. How is the flow of employees from raw product processing/handling areas to finished product processing/handling areas controlled? Employees are allowed to go to any area without restriction
35. How do you monitor the use of employee hand wash stations? Employees assume responsibility for proper hand washing
36. Do plant employees receive training about food safety, sanitation, hand washing, and other related topics? No
37. Is a bilingual training option available? No

IV. Cleaning and Sanitizing

38. Who is assigned to do cleaning and sanitizing procedures in the plant (mark all that apply):
- Employees assigned to cleaning and sanitizing also have production responsibilities
- All employees are responsible for cleaning and sanitizing their own work areas

39. Is a supervisor or other employee responsible for checking to verify that processing areas have been properly cleaned and sanitized? No
40. Do you use the same cleaning and sanitizing procedures and products throughout the plant? No
41. If no, which of the following is true? Different procedures in some plant areas but the same cleaners and/or sanitizers
42. How often are the following cleaned and sanitized?

	Once /day	Other
Floors, drains, food contact surfaces, utensils, cleaning devices, all processing equipment used	X	
Walls, ceilings, coolers		X

43. Which sanitizers are used for the following?

	Other product contact surfaces
Quaternary ammonium compounds	Sanidet

44. Please indicate the cleaning procedure used for equipment.

	No	Yes	Which equipment?
a. Is foam used to clean equipment?	X		
b. Is equipment cleaned by hand application?		X	tables, saw, grinder, back splitting saw, slicer, tenderizer, patty machine
c. Is equipment always disassembled before cleaning?		X	
d. Are high pressure sprayers or power washers used for cleaning?		X	tables, saw, grinder, back splitting saw, tenderizer, floors
e. Is compressed air used to remove debris from equipment?	X		
f. Is equipment always sanitized after cleaning?		X	tables, saw, grinder
g. Is equipment always air dried before use?		X	All

45. Frequency of equipment cleaning and sanitizing.

Frequency	Cleaning	Sanitizing	Which equipment?
At least once per day	X	X	all used
On an "as needed" basis	X		coolers

46. When are the drains cleaned? When processing ends each day
47. Please check the appropriate answer.

	Yes	No
a. Is high pressure used to clean drains?	X	
a. Are drains always sanitized after cleaning?		X
b. Are there cleaning tools assigned to drains only?		X
c. Are cleaning tools sanitized after cleaning?		X

48. What type of inspection applies to the plant? USDA custom exempt- inspection done through state
49. Do you routinely or periodically test for microbial contamination in your plant? No
50. If yes, mark which indicator organism or foodborne pathogens are tested? n/a
51. How often are environmental samples collected in your plant? n/a
52. Where are samples for environmental testing collected? (mark all that apply) n/a
53. Please briefly describe how the laboratory test results are used. n/a
54. Do you use carcass spray interventions? not applicable
55. Do you produce any ready-to-eat products? Ham and turkey, both smoked and cured
56. Alternatives of post-lethality treatment, antimicrobial agent or sanitation program: n/a
57. Antimicrobial into the formulation of ready-to-eat products or as a post-lethality treatment: n/a

V. SSOP

Standard Sanitation Operation Procedure for clean up of cutting room

1. scrape
2. rinse (both water)
3. foam
4. scrub with hot water
5. rinse with hot water
6. scrape
7. rinse after steam cleaning

VI. HACCP Plan: None

VII. Observations

Training for food safety and sanitation is done informally and verbally by owner, no written materials.

Plant 2 Profile

I. Physical plant characteristics

1. Describe the general area where the plant is located: suburban
2. Describe the immediate plant surroundings: Located in an industrial area or park, detached building surrounded by paved areas, streets or parking lot, well maintained grounds.
3. Is the plant located near water or on a site that may be exposed to tidal or other flooding, accumulating pools of water or other similar conditions? No
4. Is there a history or evidence of existing or potential problems related to:
   a. Gulls or other birds: occasional
   b. Pets or feral animals on or near premises: occasional, street dog/fox
   c. Rodents: occasional mouse (some jump off trailers, migrate in)
   d. Flies or other flying insects: serious infestation (summer), migrate in from area feedlots and dairies. Kill them all every day; thousands more arrive or are delivered on cattle and in trailers daily (may to sept, cost is up to $60-$80/day to control).
5. Provide the square footage of the entire physical structure: 5,000 sq. ft./ processing area: 1,200 sq. ft.
6. Approximately how old is the building or physical structure of the plant? 7 years, no construction or other upgrades
7. n/a
8. n/a
9. Do you have an air-flow control system or strategy in your plant? No
10. Is there any overhead plumbing in the plant? Yes , in raw product processing/handling areas, finished product processing/handling areas; tap water high along walls and evaporator coils drain lines high along walls.
11. What type of water is used in your plant? Water from a municipal supplier
12. n/a
13. What type of sewage system is used for the plant? Sewage is collected by public system
14. What other waste disposal system is used: Dumpster(s) serviced by private firm
15. Please describe the type of floors in these two product handling areas? Raw material and finished product handling areas: cement
16. Is equipment used or moved from one area of the plant to another? **No**
    a. If no, is equipment designated for specific areas of the plant marked or color coded or marked or labeled in some way? No equipment except carts, dollies, etc.
17. List any new or used equipment (machines, shelves, tables, etc.) recently acquired

Approx time	Description of Equipment	New / Used	Plant Location
Past 2 months	patty machine, grinder	used	processing
Past 6 months	evaporator coil	new	carcass cooler
More than 1 year ago	sausage stuffer- hydraulic	new	

18. If used equipment was introduced to the facility, please describe any special cleaning or sanitation procedure performed: complete cleaning and sanitizing on both new & used
19. Please identify locations where products are stored (mark all that apply)

Type of product	Cooler	Freezer	Other (walk-inn)
Carcass, fresh meat cuts	X		X
Brine	X		
Frozen meat		X	
Smoked and cooked product	X		

20. Are processing areas restricted to employees only? No
21. Are visitors, clients, or groups allowed in the plant? Yes, but they are required to wear clothing provided by the plant: Hats and/or hairnets, aprons, coats etc.

II. Production and Process Characteristics

22. When is the plant in operation? All year long
23. What was the total production volume of the product(s) processed in this plant in the year 2011? 1.6-1.7MM lb
24. How many shifts are there per day? One, starting at 7:30am

III. Processing Personnel

25. How many employees work in the plant? Full-time: 13; part-time: 2; seasonal: 2
26. How many employees work in these areas?

	Raw product processing	Finished product processing	Finished product packing	Maintenance and Cleaning	Sales or Office Work	Other
Full time	3	4	4	2	2	
Part time		2	1		1	
Seasonal						

27. How many breaks are employees scheduled to have? n/a
28. Where do employees take their breaks?
- In a designated area used only by employees for lunch or breaks
- On the grounds of the plant
- Leave the plant grounds during lunch but not for breaks
- In the office area

29. Is there special clothing requirement in the processing area? Yes
30. Requirements for outer garments:

Raw Processing Area	Finished Product Processing Areas
Apron, hair cap, gloves, hat, boots	Apron, hair cap, gloves, hat

31. How frequently garments are changed?

Protective gear	Disposable or Reusable	Frequency of change	Provided by company	Responsible of laundry
aprons		more than once/day; as soon as they get dirty, daily before works begins	yes	company
hats, helmets				
gloves	disposable			
wool gloves			yes	company
hairnets	disposable			

32. Do you use color coding for clothing, hair restraints, etc., in various processing areas? Yes
33. Are foot baths used to control contamination from foot traffic? Yes
34. How is the flow of employees from raw product processing/handling areas to finished product processing/handling areas controlled? Employees are allowed to go to any area as long as they observe sanitation procedures
35. How do you monitor the use of employee hand wash stations? Employees assume responsibility for proper hand washing.

36. Do plant employees receive training about food safety, sanitation, hand washing, and other related topics? All employees receive training (annually)
37. Is a bilingual training option available? No

IV. Cleaning and Sanitizing Procedures

38. Who is assigned to do cleaning and sanitizing procedures in the plant (mark all that apply):
    - pecific employees are assigned to perform only cleaning and sanitizing, and these employees do not have any production responsibilities
    - Employees assigned to cleaning and sanitizing also have production responsibilities
39. Is a supervisor or other employee responsible for checking to verify that processing areas have been properly cleaned and sanitized? Yes
40. Do you use the same cleaning and sanitizing procedures and products throughout the plant? Yes
41. n/a
42. How often are the following cleaned and sanitized? (mark one)

	Once /day	Once/week	Other
Floors, drains, food contact surfaces, walls, utensils, cleaning devices,	X		
Ceilings, coolers	X	X	
Cooler coils, condensor etc,			X

43. Which sanitizers are used for the following? (mark all that apply)

	Floors	Drains	Conveyers	Other product contact surfaces
Quaternary ammonium compounds	X	X	n/a	X
Chlorine	X			X

44. Please indicate the cleaning procedure used for equipment.

	No	Yes	Equipment
a. Is foam used to clean equipment?		X	all
b. Is equipment cleaned by hand application?		X	
c. Is equipment always disassembled before cleaning?		X	
d. Are high pressure sprayers or power washers used for cleaning?	X		
e. Is compressed air used to remove debris from equipment?			
f. Is equipment always sanitized after cleaning?		X	
g. Is equipment always air dried before use?		X	

45. Frequency of equipment cleaning and sanitizing.

	Cleaning	Sanitizing
a. At least once per day	X	X

46. When are the drains cleaned? when processing ends each day

47. Please check the appropriate answer.

	Yes	No
a. Is high pressure used to clean drains?		X
a. Are drains always sanitized after cleaning?	X	
b. Are there cleaning tools assigned to drains only?		
c. Are cleaning tools sanitized after cleaning?	X	

48. What type of inspection applies to the plant? USDA inspected & third-party auditing
49. Do you routinely or periodically test for microbial contamination in your plant? Yes
50. If yes, mark which indicator organism or foodborne pathogens are tested? Total aerobic plate count and generic *E. coli*
51. How often are environmental samples collected in your plant? Once a week
52. Where are samples for environmental testing collected? (mark all that apply):

Location	Sampled	Samples in 2011
Drains	yes	4
Floors	yes	4
Walls	yes	4
Cold rooms	yes	4
Processing equipment Non food-contact surfaces	yes	4
Processing equipment food-contact surfaces	yes	100
Other food contact surfaces	yes	4

53. Laboratory test results are used to determine bacteria/pathogen presence.
54. Do you use carcass spray interventions? Yes, zero tolerance. Lactic acid on beef carcasses, hot water and chilling.
55. Do you produce any ready-to-eat products? Yes. Custom exempt/not for sale or resale & retail hams, bacon, sausage, jerky, roasting hogs.
56. Alternative 3: Sanitation program
57. Antimicrobial into the formulation of ready-to-eat products or as a post-lethality treatment: n/a

V. SSOP (Standard Sanitation Operation Procedure): Yes

VI. HACCP Plan: Beef harvest

Appendix 6. Generic slides for bilingual training.

Controlling Foodborne Pathogens in Fresh Meat Processing Environment

In-plant training

Bilingual English/Spanish handout

Cornell University · Colorado State University · Texas Tech University

BACTERIA

Living organisms so small, that can't be seen with naked eye.

Classification regarding to humans:

- **Harmless**: skin and gut bacteria
- **Beneficial**: like those found in yogurt, sour cream,probiotics
- **Pathogen**: cause diseases. Often found in animal fecal contamination.

Seres vivos tan pequeños, que no se ven a simple vista

Respecto a los humanos pueden clasificarse en:

- **Inofensivas**: en la piel y nuestro intestino
- **Beneficioso**: ejemplo las que hay en el yogurt, crema ácida y los probióticos
- **Patógeno**: causa enfermedad. Se encuentran a veces en la contaminación fecal de los animales.

2

Foodborne pathogens

1. **A problem of public health**
   - 48 million people get sick per year due to 31 major foodborne pathogens.
   - 17 out of 100 of those are caused by 3 major pathogens:
     - *Listeria monocytogenes*
     - *E. coli* (Shiga-toxin producing)
     - *Salmonella*
2. **Major concern for food industry**
   - Distributed in nature, continuously enter the plant through:
     - Live animals or raw meat
     - Dust
     - Pests (rodents, flies, etc.)
     - Employees shoes
   - Product recalls

Patógenos que transmiten enfermedades a través de alimentos

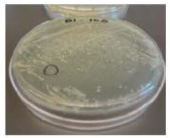
1. **Son un problema de salud pública**
   - 48 millones de personas al año se enferman a causa de los 31 principales patógenos
   - 17 de cada 100 de estos son causados por 3 patógenos importantes:
     - *Listeria monocytogenes*
     - *E. coli* (productores de Shiga-toxina)
     - *Salmonella*
2. **Son una gran preocupación para la industria de alimentos**
   - Al estar distribuidos en la naturaleza, entran continuamente a la planta por:
     - Los animales vivos o la carne cruda
     - Polvo, zapatos de empleados
     - Plagas (roedores, moscas, etc.)
   - Solicitar devolución de productos (Recalls)

3

Listeria monocytogenes

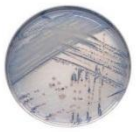
- Widespread in nature
- Persists in the environment
- Higher risk for elderly, infants, pregnant women, HIV patients
- 9 out of 10 sick people need hospitalization
- 2- 3 out of 10 patients die

- Es común en la naturaleza
- Dura largo tiempo en el ambiente
- Mayor riesgo para ancianos, bebes, embarazadas, pacientes con HIV
- 9 de cada 10 enfermos son hospitalizados
- 2 - 3 de cada 10 mueren



4

E. Coli STEC



BBL™ CHROMagar™ O157

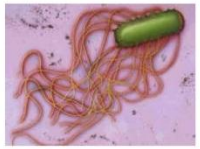
- Generic *E. coli*
  - Fecal contamination indicator
- STEC: Toxin producing *E. coli*
  - May live in animals GI tract without making them sick.
  - Just a few bacteria can cause severe illness
  - Bloody diarrhea and kidney failure

- *E. coli* genérica
  - Indica que hay contaminación fecal
- *E. coli* productora de toxinas (STEC)
  - Pueden vivir en el intestino de algunos animales sin enfermarlos.
  - Tan solo unas pocas bacterias son necesarias para causar enfermedad grave.
  - Diarrea con sangre y falla de riñón

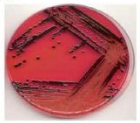
5

Salmonella

- Reside in the GI tract of humans and warm blooded animals
- One of the top causes of foodborne illness
- Survive long time in foods
- Readily killed by proper cooking



- Vive en el intestino de humanos y animales de sangre caliente.
- Una de las mayores causas de enfermedad causada por alimentos
- Sobrevive largo tiempo en alimentos
- Muere al cocinar bien los alimentos



Oxoid XLT-4 AGAR

6

HIGHER RISK

- *Listeria monocytogenes*:
  - RTE: no need to cook before eating
  - i.e., hot dogs, soft cheese, produce
  - Los productos que no necesitan cocinarse antes de comerlos
  - ejemplos: hot dogs, quesos suaves, vegetales

- *E. coli* O157 and toxin producers
  - Raw ground beef, raw milk, produce
  - Any food that may be contaminated
  - Carne molida, leche, vegetales, cualquier comida que se haya contaminado

- *Salmonella*:
  - Wide range! Any contaminated food
  - Raw meat, poultry, eggs, chocolate, raw milk, peanuts
  - Cualquier producto contaminado, carne cruda, pollo
  - huevos, chocolate, leche cruda, cacahuate

PREVENTING CONTAMINATION

PREVENT:

- Cross-contamination
  - Fecal contamination
  - Utensils
  - Raw and cooked
- Foodborne illness outbreaks
  - Outbreak: 2+ people sick from consuming same food

EVITAR:

- La contaminación cruzada
  - Materia fecal
  - Utensilios
  - Crudos con cocidos
- Brotes de enfermedad causadas por alimentos contaminados
  - Brote: 2 o más personas que comieron la misma comida y se enfermaron.

8

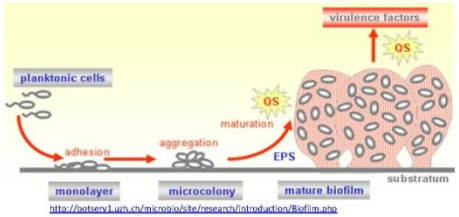
HARBORAGE SITES

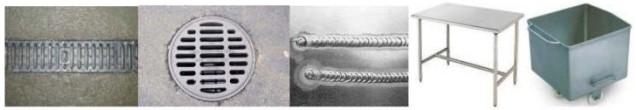
- Damp, cool places
- Hard to reach for cleaning
- Biofilms
- Blood and meat residues accumulate =Ineffective cleaning and sanitizing

SITIOS DE REFUGIO

- Lugares húmedos y difíciles de limpiar
- Biofilm
- Acumulación de residuos como sangre, grasa y carne, bajan efecto de químicos de limpieza y sanitización.

BIOFILM





GMPs

GOOD MANUFACTURING PRACTICES

- Verifiable HACCP plan
- Personal hygiene practices
- Training program provided for all maintenance, production, sanitation, and quality personnel

BUENAS PRACTICAS DE MANUFACTURA

- Plan HACCP verificable
- Normas de higiene personal
- Se le ofrece entrenamiento a todos los empleados de mantenimiento, producción, sanitización y control de calidad.

11

GMPs

- Frock identification for different production areas
- Product container identification: edible and inedible waste bins

- Se siguen reglas de identificación de mandiles en las diferentes área de producción
- Identificación de los botes: material comestible y lo que es desperdicio no comestible

12

CLEANING AND SANITATION

DEFINITIONS

- CLEANING
  - Elimination of soil and food residues
  - A surface that looks clean still has a high microbial load
  - Improper cleaning will impair the efficacy of sanitizers
- SANITATION
  - Reduction of microbial load
  - 99.99% reduction of microorganism of public health importance

- LIMPIEZA
  - Eliminar el sucio y los restos de alimentos
  - Una superficie limpia a simple vista aún tiene una alta carga de microbios.
  - Una limpieza incorrecta afectará la eficacia de los sanitizantes
- SANITIZACIÓN
  - Reducir la carga de microbios
  - Una reducción del 99.99% de los microbios de interés para la salud pública

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CLEANING CHEMICALS

- Alkaline Compounds
  - Dissolve Fats and Protein
- Acid detergents
  - Clear mineral deposits
- Additives
  - Hypochlorite
  - Others
- Enzymes

- Compuestos alcalinos
  - Disuelven grasa y proteína
- Detergentes ácidos
  - Limpiar acumulación de minerales
- Aditivos
  - Hipoclorito
  - Otros
- Enzimas

15

THERMAL SANITIZING

- Steam
  - Steam must heat all surfaces
  - Steam is very effective if used properly
- Hot Water
  - –Immersion of small items into hot water
    - •15 min at 185°F
    - •20 min at 176°F
  - –Hard Water (high calcium & magnesium) may cause scale /residue

- Vapor
  - El vapor debe calentar todas las superficies
  - El vapor es muy efectivo si se usa correctamente
- Agua Caliente
  - Sumergir artículos pequeños en el agua caliente
    - 15 min a 185°F (85°C)
    - 20 min a 176°F (80°C)
  - Aguas Duras (altas en calcio y magnesio) pueden causar sarro

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CHEMICAL SANITIZERS

- Chlorine Sanitizers
- Iodine Compounds
- Quaternary Ammonium Compounds
- Peroxy Acid Compounds

- Sanitizantes clorinados
- Compuestos de Yodo
- Compuestos de Amonio Cuaternario
- Compuestos de Ácido Peroxi

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CHEMICAL EFFECTIVNESS

Sanitizer	Concentration ppm	Exposure Time	Temperature Range (°F)	pH
Chlorine	100-200	2-10 min	70-100	4
Iodophors	25	2-15 min	70-100	<3
Quats	100-200	24 hr	70-120	6-10
Acid-Anionic	200-400	30 min	90-150	1.6-2.3

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STEPS FOR EFFECTIVE C&S

1. Dry Cleanup
   - Entire area; Dissemble equipment (LOTO)
2. First Rinse
   - Hot water 120°F to 140°F
   - Avoid high pressure to prevent aerosols
3. Detergent + scrub
   - Foam detergent is recommended
   - Walls first, then floors and equipment.
   - Follow concentration and contact time recommended by manufacturer
   - Do not let it dry

1. Limpiar en seco
   - Toda el área; Desensamblar el equipo (procedimiento LOTO)
2. Primer enjuage
   - Agua caliente de 120°F a 140°F
   - Evitar alta presión de agua para prevenir aerosol
3. Detergente + restregar
   - Se recomienda detergente en espuma
   - Iniciar con paredes, luego el piso y por último el equipo.
   - Seguir instrucciones del fabricante para concentración y tiempo de contacto
   - No dejar que se seque

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DRAINS C&S

- Clean drains prior to sanitizing floor
- Use designated brushes
- Use a chlorinated alkaline cleaner
- Clean all surfaces of the drain
- Sanitize with QUAT (800 ppm), iodine (75 ppm), or chlorine (800 ppm)
- Minimum: clean drains weekly

- Limpiar los desagues antes de sanitizar el piso
- Usar cepillos asignados
- Usar un limpiador alcalino clorinado
- Limpiar toda la superficie del desague
- Sanitizar con QUAT (800 ppm), Yodo (75 ppm), o Cloro (800 ppm)
- Limpiar desagues al menos una vez por semana.

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STEPS FOR EFFECTIVE C&S

4. Second Rinse
   - First walls, then floor, and last the equipment
   - Inspection of surfaces, use flashlight
5. Remove water
   - Change to clean outer wear and sanitize hands
   - Remove standing water and condensation
6. Assemble equipment
   - Sanitize inaccessible parts prior to assemble
   - Follow LOTO procedures

4. Segundo Enjuage
   - Primero paredes, luego piso y por último el equipo
   - Inspeccionar las superficies, use un foco de mano
5. Eliminar el agua
   - Cambiarse a ropa limpia y sanitizar sus manos
   - Eliminar posas de agua y condensación
6. Ensamble equipment
   - Sanitize inaccessible parts prior to assemble
   - Follow LOTO procedures

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STEPS FOR EFFECTIVE C&S

7. Sanitize
   - Equipment and entire area (walls and floors)
   - Work your way out of the room
   - Eliminate standing sanitizer pools, let air dry
8. Pre-op inspection

7. Sanitización
   - Del equipo y toda el área (paredes y piso)
   - Trabaje del fondo de la habitación hacia la salida
   - Eliminar posas de sanitizante, dejar secar al aire libre
8. Inspección Pre-op

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Appendix 7. Customized presentation for Plant 1.

LONGITUDINAL STUDY PLANT D

RESEARCH PROJECT

23

SAMPLE COLLECTION

- Monthly samplings- 6 month period
- Environmental sponge samples
- 55 sampling sites
- 3 sponge swabs each
- Total 990 sponge swabs

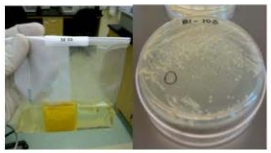
- Muestreos mensual por 6 meses
- Muestras ambientales tomadas con esponjas
- 55 puntos de muestreo
- 3 esponjas cada uno
- 990 muestras en total

24

ANALYSIS

- Microbiology analysis for 3 major foodborne pathogens:
  - *L. moncytogenes*
  - *E. coli* O157:H7
  - *Salmonella*

- Análisis microbiológico de 3 patógenos importantes:
  - *L. moncytogenes*
  - *E. coli* O157:H7
  - *Salmonella*



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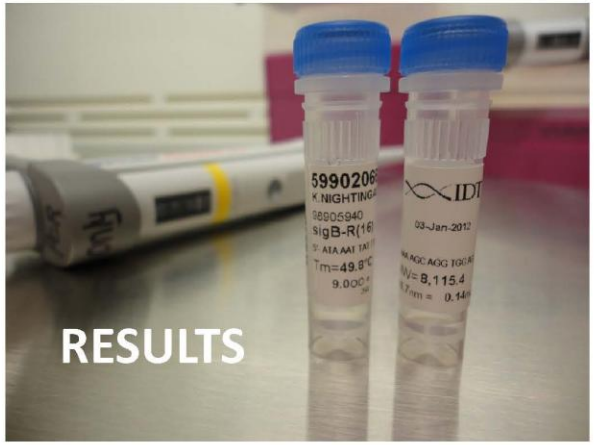
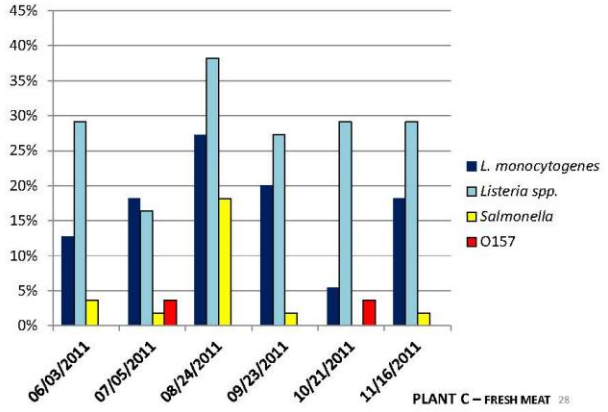


PREVALENCE

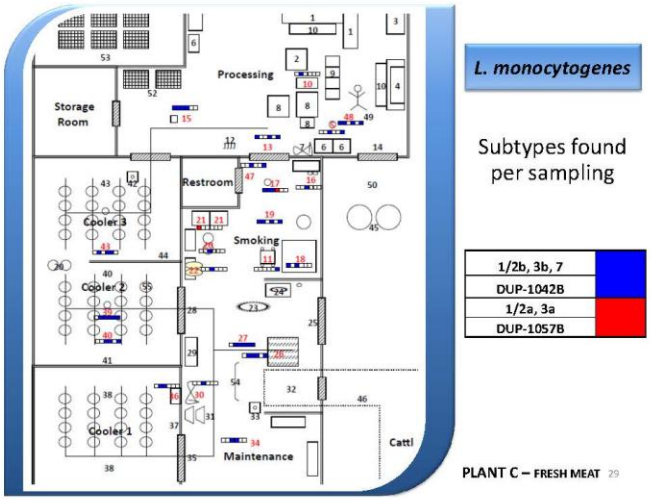
Bacteria	Total Analyzed Samples	Total Positive	%	Sites with repeated isolation
Listeria monocytogenes	330	56	17.0%	15
Listeria spp.	330	93	28.2%	22
Salmonella enterica	330	15	4.5%	2
E. coli O157:H7	330	4	1.2%	0

PLANT C – FRESH MEAT 27

% POSITIVE SAMPLES PER VISIT



PLANT C – FRESH MEAT 28

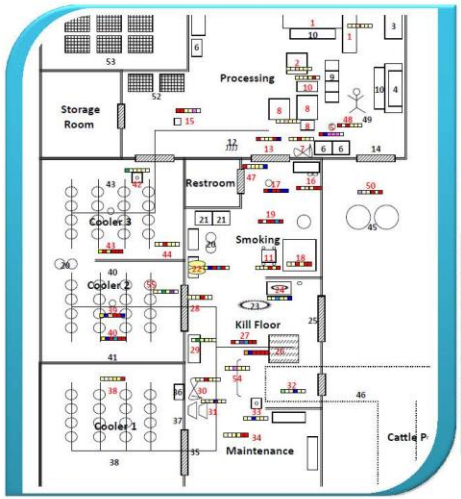


L. monocytogenes

Subtypes found per sampling

1/2b, 3b, 7
DUP-1042B
1/2a, 3a
DUP-1057B

PLANT C – FRESH MEAT 29

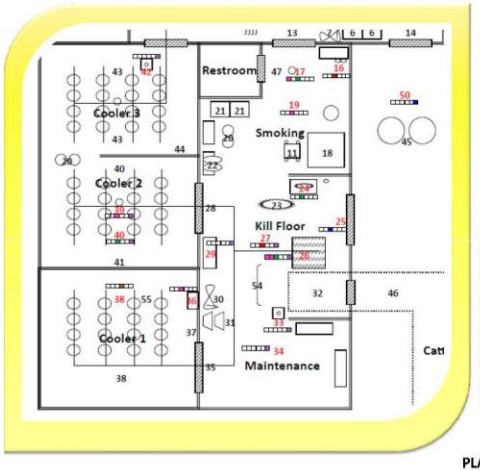


***Listeria* spp.**

Subtypes found per sampling

L. innocua	LI AT 1
L. innocua	LI AT 2
L. innocua	LI AT 3
L. innocua	LI AT 4
L. innocua	LI AT 5
L. innocua	LI AT 6
L. innocua	LI AT 7
L. innocua	LI AT 8
L. innocua	LI AT 9
L. innocua	LI AT 10
L. innocua	LI AT 11
L. welshimeri	LW AT 1

PLANT C – FRESH MEAT 30

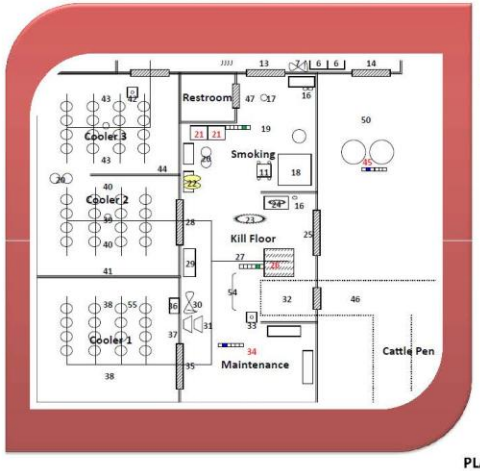


Salmonella

Subtypes found per sampling

S. enterica	SE PT 1
S. enterica	SE PT 2
S. enterica	SE PT 3
S. enterica	SE PT 4
S. enterica	SE PT 5
S. enterica, atypical	SE PT 6

PLANT C – FRESH MEAT 31



***E. coli* O157:H7**

Subtypes found per sampling

EC PT 1	
EC PT 2	

PLANT C – FRESH MEAT 32

CONTROL OF CONTAMINATION

1. Prevention
   - Incoming contamination
   - Avoid spread of contamination within plant
   - Post-cook cross-contamination
2. Plant environment control
   - Temperature control
   - Avoid water ponds
   - Air flow
3. Facilitate sanitation
   - Easy access to clean and sanitize

1. Prevenir
   - Contaminación entrante
   - Evitar regar/dispersar contaminación dentro de la planta.
   - Contaminación cruzada de los productos ya cocidos.
2. Control del ambiente dentro de la planta
   - Control de temperatura
   - Evitar posas de agua
   - Flujo del aire
3. Facilitar sanitización
   - Paso libre para limpiar y sanitizar toda el área.

PLANT C – FRESH MEAT 33

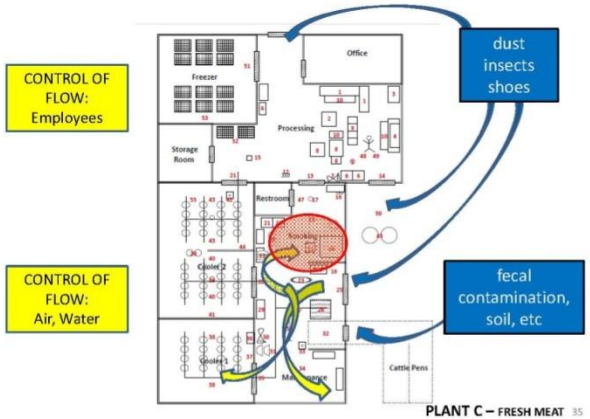
FOCUS AREAS

1. Traffic within plant
2. Plant environment control in the Smoking area
3. Cleaning and sanitizing of drains and floors wall-to-wall
   - Smoking area
   - Maintenance area

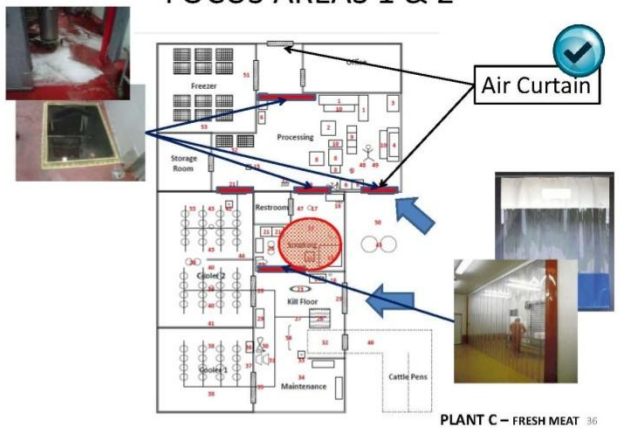
1. Tráfico dentro de la planta
2. Condiciones ambientales del área de Ahumado
3. Limpieza y sanitización de los desagues y los pisos de pared a pared.

PLANT C – FRESH MEAT 34

CONTROL OF CONTAMINATION



FOCUS AREAS 1 & 2



FOCUS AREAS 1 & 2

- Hurdles to prevent incoming contamination or spread within the plant.
  - Footbaths
  - Limited transit of employees
- Increase control of the processing environment:
  - Block incoming dust/flies or other insects
  - Strip curtains: include in daily cleaning and sanitation program

- Barreras para prevenir el ingreso de contaminación o su dispersión dentro de la planta:
  - Alfombras sanitizadoras o lavapiés.
  - Reducir el tránsito de empleados
- Incrementar el control en el ambiente de procesamiento
  - Bloquear ingreso de polvo/moscas u otros insectos
  - Cortinas industriales de plástico: incluirlas en el programa diario de limpieza y sanitización

FOCUS AREAS 3

- Recommended corrective actions in cleaning & sanitation:
  - Clear corners and place utensils on shelves to allow accessibility for c&s
  - Floors (smoking area, kill floor and maintenance area)
  - Drains
  - Intervention targeting "hot spots" of contamination.

- Acciones correctivas recomendadas en la limpieza y sanitización:
  - Dejar las esquinas libres y colocar utensilios en estantes, para dar acceso a limpieza y sanitización
  - Pisos (areas de ahumado, sacrificio y mantenimiento)
  - Desagües
  - Intervención de los sitios más contaminados.

STRONGLY ENCOURAGED TO AVOID:

1. Avoid customers entering the facility through the back door and walking across the processing area to the front door.
2. No smoking allowed inside the facilities.
3. Prevent cross-contamination in coolers. Do not place raw fish, employee food or drinks under carcasses.
4. Avoid piling up utensils, tools, brooms, etc., where those items create an obstacle for daily cleaning and sanitation activities.
5. Avoid producing aerosol with water at high pressure. The fine mist may contain microorganisms and spread them in the environment.
6. Prevent air flow from outdoors into the processing area.

1. Evitar que los clientes entren a la planta por la puerta trasera, caminado através del área de proceso hacia la puerta del frente.
2. Fumar no es permitido dentro de las instalaciones.
3. Prevenir la contaminación cruzada dentro de los cuartos fríos. No colocar pescado crudo, alimentos o bebidas de los empleados debajo de las canales.
4. Evitar acumular utensilios, herramientas, escobas, etc. en lugares donde estorben en las actividades de limpieza y sanitización.
5. Evitar producir aerosoles con el agua a alta presión. Las pequeñas gotas de agua podrían contener microorganismos y dispersarlos en el ambiente de las instalaciones.
6. Prevenir flujo de aire que provenga de afuera hacia el área de proceso.

PLANT C – FRESH MEAT 39

Appendix 8. Customized presentation for Plant 2.

SAMPLE COLLECTION

- Monthly samplings- 6 month period
- Environmental sponge samples
- 55 sampling sites
- 3 sponge swabs each
- Total 990 sponge swabs

- Muestreos mensual por 6 meses
- Muestras ambientales tomadas con esponjas
- 55 puntos de muestreo
- 3 esponjas cada uno
- 990 muestras en total

24

ANALYSIS

- Microbiology analysis for 3 major foodborne pathogens:
  - *L. moncytogenes*
  - *E. coli* O157:H7
  - *Salmonella*

- Análisis microbiológico de 3 patógenos importantes:
  - *L. moncytogenes*
  - *E. coli* O157:H7
  - *Salmonella*

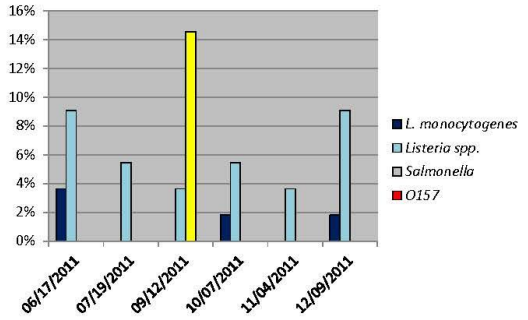
25

PREVALENCE

Bacteria	Total Analyzed Samples	Total Positive	%	Sites with repeated isolation
Listeria monocytogenes	330	4	1.2%	0
Listeria spp.	330	20	6.1%	6
Salmonella enterica	330	8	2.4%	0
E. coli O157:H7	330	0	0%	0

PLANT D – FRESH MEAT 27

% POSITIVE SAMPLES PER VISIT



PLANT D – FRESH MEAT 28

FOCUS AREAS

1. Traffic between carcass cooler-process room
2. Prevention of contamination of metal platform in the kill floor area.

1. Tráfico entre el cuarto frío y el área de proceso
2. Prevención de contaminación de la plataforma metálica en el área de sacrificio.

FOCUS AREAS

- Hurdles to prevent spread of contamination
  - Sanitizer foam or dip station

- Barreras para prevenir dispersión de contaminación dentro de la planta:
  - Espuma sanitizadora o lavapiés.

STRONGLY ENCOURAGED TO AVOID:

1. Avoid customers entering the facility through the back door and walking across the processing area to the front door.
2. No smoking allowed inside the facilities.
3. Prevent cross-contamination in coolers. Do not place raw fish, employee food or drinks under carcasses.
4. Avoid piling up utensils, tools, brooms, etc., where those items create an obstacle for daily cleaning and sanitation activities.
5. Avoid producing aerosol with water at high pressure. The fine mist may contain microorganisms and spread them in the environment.
6. Prevent air flow from outdoors into the processing area.

1. Evitar que los clientes entren a la planta por la puerta trasera, caminado através del área de proceso hacia la puerta del frente.
2. Fumar no es permitido dentro de las instalaciones.
3. Prevenir la contaminación cruzada dentro de los cuartos fríos. No colocar pescado crudo, alimentos o bebidas de los empleados debajo de las canales.
4. Evitar acumular utensilios, herramientas, escobas, etc. en lugares donde estorben en las actividades de limpieza y sanitización.
5. Evitar producir aerosoles con el agua a alta presión. Las pequeñas gotas de agua podrían contener microorganismos y dispersarlos en el ambiente de las instalaciones.
6. Prevenir flujo de aire que provenga de afuera hacia el área de proceso.

Appendix 9. Bilingual questionnaire for food safety knowledge assessment.

Controlling Foodborne Pathogen Persistence in
Small Fresh and Ready-to-Eat Meat Processing Plants
Training Evaluation

Id:_____

1) Not all bacteria can make people sick. Which group of bacteria should we prevent from contaminating a food product?
   a) Beneficial
   b) Spoilage
   c) Pathogen

No todas las bacterias causan enfermedades graves en las personas. ¿Qué grupo de bacterias debemos prevenir que contamine los alimentos?:
   a) Beneficiales
   b) Bacterias que causan deterioro o daño a los alimentos
   c) Patógenos

2) During processing, meat may be contaminated when is touched by _____. (Circle all the correct answers. You may have more than one answer)
   a) Fluids from animal gut, hair or dirt from hide
   b) Utensils (knives, handsaws, containers)
   c) Equipment (grinders, injectors)
   d) Sterile packaging material

Durante el proceso, la carne podría contaminarse al tocar____. (Encierre todas las respuestas correctas. Puede tener más de una respuesta).
   a) Líquidos del estómago del animal, pelos o tierra en el cuero.
   b) Utensilios (cuchillos, sierras, recipientes)
   c) Equipos (molinos, inyectores)
   d) Material de empaque estéril

3) If contaminated, which product is more likely to have a higher bacterial count?
   a) Uncooked large meat cut
   b) Uncooked ground meat
   c) Cooked sausage

Si estuviera contaminado, ¿Cuál producto tendría mayor cantidad de bacterias?:
   a) Un pedazo grande de carne cruda
   b) Carne molida cruda
   c) Chorizo cocido

4) Foodborne disease outbreak means:
   a) There are 2 or more cases of ill people with illness resulting from eating same food.
   b) There is of a lot of ill people with illness resulting from eating same food.

Un brote de enfermedad causada por alimentos contaminados significa:
   a) Que hay 2 o más casos de personas enfermas, con una enfermedad causada por comer un mismo alimento.
   b) Que hay bastante gente enferma por comer un alimento en común.

5) If harmful bacteria are present in the raw meat product, some risks are___. (Circle all the correct answers. You may have more than one answer)
   a) There is no risk. The consumer will cook the meat before eating it.
   b) Improper cooking might not kill the pathogen.
   c) Cross-contamination of other food items.
   d) Foodborne disease outbreak.

Si los productos de carne cruda contienen bacterias dañinas, algunos de los riesgos son___.(Encierre todas las respuestas correctas. Puede tener más de una respuesta).
   a) No hay ningún riesgo, porque el consumidor va a cocinar la carne antes de comerla.
   b) Al no cocinar bien la carne, las bacterias patógenas no se mueren.
   c) Contaminación cruzada a otros alimentos.
   d) Un brote de enfermedad causada por alimento contaminado.

1

6) Ready-to-eat meat products like hot dogs do not require the consumer to heat them before serving them. A biological hazard of major concern in meat processing for ready to eat meat products is:
   a) *E. coli*
   b) *Salmonella*
   c) *Listeria*
   d) BSE or Mad Cow Disease

Los productos listos para el consumo, como los hot dogs, no requieren que el consumidor los caliente antes de comérselos. El peligro biológico más preocupante en los productos de carne listos para el consumo (Ready-to-eat) es:
a) *E. coli*
b) *Salmonella*
c) *Listeria*
d) BSE or Enfermedad de las Vacas Locas

7) The greatest biological hazard of concern in processing for raw ground beef is:
   a) *E. coli O157:H7* and toxin producing *E .coli*
   b) *Salmonella*
   c) *Listeria*
   d) BSE or Mad Cow Disease

El peligro biológico más preocupante en el procesamiento de carne molida de res cruda es:
a) *E. coli O157:H7* and other toxin producing *E. coli*
b) *Salmonella*
c) *Listeria*
d) BSE or Enfermedad de las Vacas Locas

8) What things make *Listeria monocytogenes* different from *E. coli* or *Salmonella*?
   (Circle all the correct answers. You may have more than one answer):
   a) It can be found almost everywhere.
   b) It can grow at cold temperatures.
   c) It can grow in a vacuum package.
   d) Contamination of ready to eat meats with *Listeria monocytogenes* usually occurs in the cooked meat processing and packaging rooms.

Qué es lo que tiene *Listeria monocytogenes* que la hacen diferentes a *E. coli* o *Salmonella?*
(Encierre todas las respuestas correctas. Puede tener más de una respuesta).
a) La podemos encontrar en casi cualquier lugar.
b) Puede multiplicarse al estar en temperaturas frías.
c) Puede multiplicarse dentro de los empaques al vacío.
d) La contaminación de los productos de carne listos para consumir con *Listeria monocytogenes* usualmente ocurren en los cuartos de cocción o empaque.

9) The ready-to-eat meat products that are the greatest risk for food borne illness from *Listeria monocytogenes* include_____ (Circle all the correct answers. You may have more than one answer)
   a) Whole Hams
   b) Hot dogs
   c) Sliced Deli meats
   d) Jerky
   e) Sausages

Los productos de carne listos para el consumo que tienen alto riesgo de provocar una enfermedad transmitida por contaminación con *Listeria monocytogenes* incluyen:
   a) Jamón entero (pierna de cerdo entera)
   b) Hot dogs
   c) Jamones, salamis o mortadelas en rodajas
   d) Jerky
   e) Chorizos

10) Locations in the meat processing plant that are likely to harbor *Listeria* include the following: (Circle all the correct answers. You may have more than one answer)

a) Damp drains
b) Hollow table legs
c) Squeeges
d) Stainless steel table tops
e) Gloved hands
f) Employee aprons and boots
g) Smokehouse cooking screens or sticks
h) Damp cracks in wall and insulation
i) Rubber fatigue mats

En la procesadora de carne, es más probable encontrar *Listeria* refugiada en los siguientes lugares: (Encierre todas las respuestas correctas. Puede tener más de una respuesta).

a) Drenajes de agua (drains, desagües)
b) Patas huecas de las mesas
c) Haladores, gomas o hules para sacar agua del piso (en inglés: squeeges)
d) Superficie de mesas de acero inoxidable
e) Manos con guantes
f) Delantales y botas de los empleados
g) Bandejas y palos del ahumador (smokehouse)
h) Grietas en el piso y las paredes
i) Alfombras de hule

11) What things make *E. coli* O157:H7 different from *Listeria* or *Salmonella*?
(Circle all the correct answers. You may have more than one answer):

a) Once it has infected a human, it can produce a toxin.
b) Can cause bloody diarrhea and kidney failure.
c) Is not a pathogen

Qué características tiene *E. coli* O157:H7 que la hacen diferente a *Listeria* o *Salmonella?* (Encierre todas las respuestas correctas. Puede tener más de una respuesta).

a) Una vez que ha infectado al humano, ésta puede producir una toxina.
b) Puede causar diarrea con sangre y daños a los riñones.
c) No es un patógeno

12) Presence of generic *E. coli* in the meat processing environment can be interpreted as indicative of:

a) The process is under control.
b) There is presence of *E. coli* O157:H7
c) There is presence of fecal contamination.

La presencia de *E. coli* genérica en el ambiente de la planta de procesamiento de carne puede ser interpretada como indicador de_____

a) Que el proceso está bajo control
b) Que hay presencia de *E. coli* O157:H7
c) Que hay presencia de contaminación fecal (de heces fecales).

13) The following are possible sources of *Salmonella*:____
(Circle all the correct answers. You may have more than one answer):

a) Raw meat
b) Poultry and eggs
c) Raw milk
d) Hot dogs

Algunas fuentes probables de *Salmonella* son_____(Encierre todas las respuestas correctas. Puede tener más de una respuesta).

a) Carne cruda
b) Carne de pollo y huevos
c) Leche cruda (sin pasteurizar)
d) Hot dogs

14) *Salmonella* can contaminate read- to-eat meat products in any of the following ways: (Circle all the correct answers. You may have more than one answer):
   a) Handling of cooked product
   b) Using same containers for raw and cooked products
   c) Sterile packaging material

Salmonella puede contaminar productos de carne listos para el consumo, en cualquiera de las siguientes maneras: (Encierre todas las respuestas correctas. Puede tener más de una respuesta).
   a) Manipulación del producto ya cocido
   b) Usando los mismos contenedores para producto crudo y producto cocido
   c) Materia de empaque estéril

15) How could foodborne pathogens find their way into meat processing facility?
   a) Incoming dust
   b) Incoming cattle or raw meat products
   c) Pest (birds, rodents, insects)
   d) Employee shoes

A través de qué medios podrían entrar a la planta de procesamiento los patógenos?
   a) Polvo
   b) Ganado o productos de carne cruda que sean recibidos
   c) Plagas (pájaros, roedores, insectos)
   d) Zapatos de los empleados

16) Which practices are helpful to prevent pathogens from spreading in the processing environment?
   a) Separation of areas (incoming materials, processing, cooking, retail)
   b) Boot baths with disinfectant or foam
   c) Rotating disinfectants
   d) Avoiding water ponds and cracks on the floor

De qué maneras podemos prevenir que los patógenos se distribuyan por toda la planta de procesamiento?
   a) Separación de áreas (entrada de materiales, procesamiento, cocción, venta)
   b) Desinfectando botas en los Lavapiés (alfombras sanitizadoras o espuma).
   c) Rotando los desinfectantes
   d) Evitando acumulaciones de agua o charcos en el suelo

17) The definition of a food contact surface includes:
   a) Only the packaging material of the final product.
   b) Surfaces of equipment or utensils which food normally come into contact with and surfaces from which other foods may drain, drip or splash into food.

La definición de lo que es una superficie de contacto con alimentos incluye:
   a) Solamente el material de empaque del producto final.
   b) Las superficies de los equipos/utensilios que los alimentos tocan y otras superficies desde las que los alimentos pueden escurrir, gotear o salpicar a otros alimentos.

18) A biofilm is a layer of bacteria and organic material on a food contact surface. During cleaning and sanitation, this biofilm:
   a) Is easily removed by warm rinse water during cleaning
   b) Is difficult to remove and requires scrubbing with detergent and high pressure cleaning.
   c) Is prevented by keeping the equipment in cold rooms.

Un biofilm es una capa de bacteria y material orgánico en una superficie de contacto con alimentos. Durante la limpieza y sanitización, éste biofilm:
   a) Se puede quitar fácilmente con un enjuague de agua tibia durante la limpieza
   b) Es difícil de quitar y requiere restregar con detergente y agua a presión.
   c) Se puede prevenir si se guardan los equipos en cuartos con temperatura fría.

19) Which factors contribute to growth of pathogen bacteria in refuges inside the building?
   a) Accumulation of meat residues and blood
   b) Temperature
   c) Sites cannot be easily reached for scrubbing
   d) Pathogen can adapt to survive low concentration of disinfectant

20) Do you think that the recommendations for safe food handling are useful to apply at homes?
   a) Yes
   b) No, those only apply to industry.

Circle the word True or False.

21) True or False. Cross-contamination is a major way for a cooked meat product to be contaminated.

22) True or False. Using aprons and gloves to handle meat products is enough to prevent cross-contamination.

23) True or False. *Listeria* can remain in a food processing plant for a long period of time, even with a good cleaning and sanitation program.

24) True or False. *Salmonella* is only of concern for the poultry industry.

25) True or False. Some kinds of *E. coli* cause disease by producing a toxin called Shiga toxin. These bacteria are called STEC for short.

26) True or False. STEC *E. coli* bacteria are found in the gut of cattle, sheep, deer and elk, but do not make the animals sick.

27) True or False. Chemicals used to sanitize equipment will have the same effectiveness on a clean surface and on a dirty surface.

28) True or False. Most areas need to be cleaned and sanitized on a daily basis; however coolers and freezers can be cleaned and sanitized on a less frequent basis.

The End

Qué factores ayudan a que los patógenos crezcan en refugios dentro del edificio?
   a) Residuos de carne acumulada y sangre
   b) Temperatura
   c) Estos nichos no son fáciles de restregar o cepillar
   d) Los patógenos pueden adaptarse a sobrevivir baja concentración de desinfectantes

Usted piensa que las recomendaciones para manejar los alimentos higiénicamente son útiles para aplicar en los hogares?
   a) Si
   b) No, sólo aplican para la industria.

Encierre la palabra Verdadero o Falso.

Verdadero o Falso. La contaminación cruzada es un peligro de contaminación para los productos de carne cocidos.

Verdadero o Falso. Usar delantal y guantes para manipular productos de carne es suficiente para prevenir la contaminación cruzada.

Verdadero o Falso. Listeria puede permanecer en una planta de procesamiento de alimentos por un largo período de tiempo, aún al tener una buena limpieza y sanitización.

Verdadero o Falso. *Salmonella* solamente es una preocupación para la industria de carne de pollo.

Verdadero o Falso. Algunos tipos de *E. coli* provocan enfermedad produciendo una toxina llamada Shiga toxina. Para abreviar su nombre, son llamadas STEC.

Verdadero o Falso. Las bacterias *E. coli* STEC se encuentran en el aparato digestivo de vacas, toros, ovejas, venados y alces, pero no los enferman.

Verdadero o Falso. Los químicos usados para sanitizar el equipo tendrán la misma efectividad en una superficie limpia y en una superficie sucia.

Verdadero o Falso. La mayoría de áreas se necesitan limpiar y sanitizar a diario; pero los cuartos fríos y congeladores pueden limpiarse y sanitizarse con menos frecuencia.

FIN!

Appendix 10. Bilingual food safety fact sheets.

FOOD SAFETY FACTS

Foodborne Illness

- **PUBLIC HEALTH PROBLEM**
- 48 million people get sick each year in US

- **MAJOR CONCERN FOR FOOD INDUSTRY**
- Pathogens are naturally found in nature and continuously enter food plants
- Contaminated batches of product or batches that may be contaminated, must be recalled from the market

Enfermedades causadas por patógenos en alimentos

- **PROBLEMA DE SALUD PÚBLICA**
- 48 millones de personas se enferman cada año en U.S.A.

- **PREOCUPACIÓN PARA LA INDUSTRIA DE ALIMENTOS**
- Los patógenos se encuentran en la naturaleza e ingresan continuamente a las planta de proceso de alimentos
- Los lotes de productos contaminados o lotes que pudieran estar contaminados, deben ser retirados del mercado

GMP

Effective Cleaning & Sanitation

HACCP

At home:

Clean, Separate, Cook, Chill

How to prevent contamination?

AT THE FOOD PROCESSING PLANT:

1. PREVENT contamination
2. Follow Good Manufacturing Practices (GMP) and Hazard Analysis of Critical Control Points (HACCP) to validate the process
3. Assure effective cleaning and sanitation

AT HOME:

1. Wash produce thoroughly
2. Handle separately the raw and cooked, or meats and produce to avoid cross-contamination
3. Cook food thoroughly
4. Chill leftovers promptly in closed containers

¿Cómo prevenir la contaminación?

EN LA PLANTA DE PROCESO DE ALIMENTOS:

1. PREVENIR la contaminación
2. Seguir Buenas Prácticas de Manufactura (GMP en inglés) y Análisis de Riesgos y Puntos Críticos de Control (HACCP en inglés) para validar el proceso
3. Asegurar una efectiva limpieza y sanitización

EN LA CASA:

1. Lave bien las frutas y verduras
2. Manipule por separado los productos crudos y cocidos, o las carnes y verduras, para evitar la contaminación cruzada
3. Cocine bien los alimentos
4. Refrigere los sobrantes pronto en contenedores cerrados


Three Major Foodborne Pathogens
Tres Principales Patógenos en Alimentos
We can find it in cold places or products
Lo podemos hallar en lugares o productos fríos
L. monocytogenes Key Facts:
Survives and grows at refrigerator temperatures
Most listeriosis patients require hospitalization
Is considered an adulterant in ready-to-eat products
Datos importantes de L. monocytogenes:
Sobrevive y crece a temperaturas de refrigeración
La mayoría de pacientes de listeriosis requieren ser hospitalizados
Se considera un adulterante en los productos listos para el consumo
We can find it in raw or dry products
Lo podemos hallar en productos crudos o secos
Salmonella Key Facts:
Survives dry conditions
One of the top causes of foodborne illness
Its presence at high levels indicates poor hygiene practices are in the food processing plants
Datos importantes de Salmonella:
Sobrevive condiciones secas
Es uno de las principales patógenos en alimentos
Si en una plantas de proceso de alimentos se encuentran altas cantidades de Salmonella, indica que hay pobres prácticas de higiene
Very dangerous toxin producers.
Son productores de una toxina potente.
E. coli Key Facts:
Some E. coli are able to produces a potent toxin known as Shiga Toxin. Among those is E. coli O157:H7
Just a few bacteria are needed to cause severe illness
Is considered an adulterant in raw ground beef
Datos importantes de E. coli STEC:
Algunas E. coli producen una toxina potente conocida como Shiga toxina. E. coli O157:H7 es una de estas
Unas pocas bacterias pueden causar enfermedad grave
Se considera un adulterante en la carne molida de res cruda
Good practices help prevent them from causing illness.
Las buenas prácticas son útiles para prevenir que nos enfermen.
Common Key Facts about these pathogens:
Contaminated food will have a normal smell and taste
Bacteria can get into food through cross-contamination
Are readily killed by cooking temperature
Datos que estos patógenos tienen en común:
Los alimentos contaminados tendrán olor y sabor normal
Las bacterias pueden llegar a los alimentos por contaminación cruzada
Se inactivan fácilmente al cocinar los alimentos a temperaturas adecuadas

L. monocytogenes FACTS

Where is it found?

It is widespread in nature (soil, wood, water), wild animals, agricultural animals, and farm environments. It has been found to persist in food processing facilities.

How could it get into food?

It can be carried by animals, dust, pests or shoes to the processing plants. Then, if it finds a harborage site with favorable conditions, it can survive in harborage sites. Food contact surfaces may be contaminated and transfer the pathogen to food products.

Which are the highest risk foods?

Processed products that are ready-to-eat like hot dogs, deli salads and soft cheese.
Also raw ground meat and raw milk and produce that might have been cross-contaminated.

What's the illness?

Listeriosis. It may cause nausea, diarrhea, and fever. Complications lead to septicemia, meningitis, encephalitis, spontaneous abortions or death.

Who is at higher risk of Listeriois?

Pregnant women, elderly, people who are ill, patients receiving cancer treatment with corticosteroids, anticancer drugs, among others. However, even healthy people can get sick if heavily contaminated food is consumed.

¿Dónde se encuentra?

Se encuentra distribuido en la naturaleza, (en el suelo, la madera, el agua), animales salvajes, animales de producción comercial, y el ambiente de las granjas.

¿Cómo llega a contaminar los alimentos?

Puede ser portado hacia la planta de proceso de alimentos por los animales, el polvo, las plagas o en zapatos. Si encuentra condiciones favorables, podría sobrevivir en un refugio. Las superficies de contacto de alimentos se podrían contaminar y de allí transferir el patógeno a los alimentos.

¿Cuáles son los alimentos de mayor riesgo?

Los alimentos procesados que están listos para comer como los hot dogs, ensaladas preparadas y quesos blandos. También la carne molida de res cruda y la leche cruda. Las legumbres, en caso que hallan sido expuestas a contaminación cruzada.

¿Qué enfermedad causa?

Listeriosis. Puede causar náuseas, diarrea y fiebre. Las complicaciones pueden causar septicemia, meningitis, encefalitis, abortos espontáneos o la muerte.

¿Quiénes tienen mayor riesgo?

Las mujeres embarazadas, ancianos, pacientes en tratamiento con corticosteroides, medicinas anticáncer, entre otros. Sin embargo, incluso las personas sanas puede enfermarse si comen alimentos altamente contaminados.

Salmonella FACTS

Where is it found?

Poultry and swine are often carriers. It can also be found in farm environments, soil, water, insects, pests, etc.

¿Dónde se encuentra?

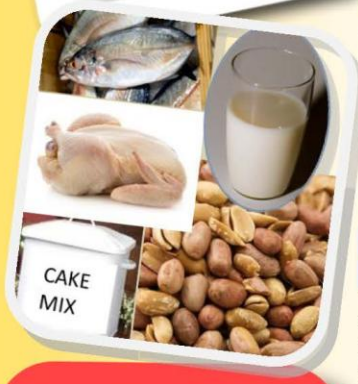
Las aves y cerdos comúnmente la portan. Se halla en el ambiente de las granjas, suelo, agua, insectos, plagas, etc.

How could it get into food?

It could be tracked down to the food processing plant by incoming live animals, raw materials, dust or pests. One major outbreak was associated with a leaking roof and wild birds droppings.

¿Cómo llega a contaminar los alimentos?

Podría ser introducido a la planta procesadora por los animales o materias primas crudas, polvo o plagas. Un brote epidémico grande fue asociado con una gotera de un techo con excremento de aves.



Which are the highest risk foods?

Raw poultry, raw fish and seafood, and raw milk.
Dry products like toasted peanuts or cake mixes and other low moisture like peanut butter or chocolate.

¿Cuáles son los alimentos de mayor riesgo?

Carne cruda de pollo, pescado, mariscos crudos o leche cruda. Productos secos como maní tostado o harinas para pasteles. Otros productos bajos en agua como la mantequilla de maní o el chocolate.

What's the illness?

Salmonellosis. Symptoms are nausea, vomiting, abdominal cramps, diarrhea, fever, and headache. Arthritic symptoms may follow 3-4 weeks after onset.

¿Qué enfermedad causa?

Salmonelosis. Los síntomas son nausea, vómito, dolor de estómago, diarrea, fiebre y dolor de cabeza. Síntomas de artritis pueden aparecer unas 3-4 semanas después.

All group ages are susceptible!

More severe on elderly, infants and infirm.

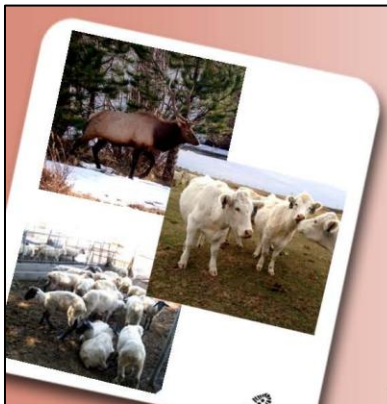
More frequently reported from AIDS patients.

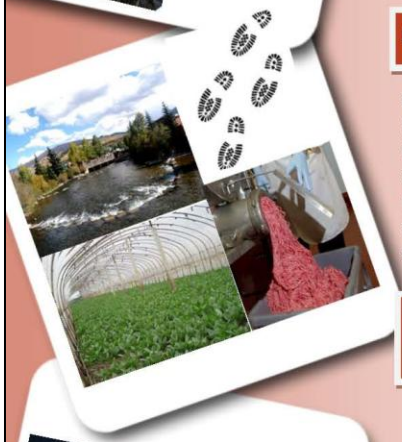
Who is at higher risk of Salmonellosis?

All group ages are at risk! But the elderly, infants and infirm suffer more severe symptoms. More frequently reported from AIDS patients.

¿Quiénes tienen mayor riesgo?

La gente de toda las edades tiene riesgo. Los ancianos, niños y enfermos sufren síntomas más severos. Es frecuentemente reportado en pacientes con SIDA.





Also sprouts, and leafy greens

All group ages are susceptible!

More severe on elderly, and the very young.

E. Coli STEC FACTS

Where is it found?

Ruminants are a natural reservoir. Even healthy animals may shed the pathogen in feces. Infected people will shed it also, and may pass the bacteria to other people, surfaces or food, if hygiene practices are inadequate.

How could it get into food?

Runoff water contaminated with fecal matter from farms could make its way to crops. It can be carried by animals, dust, pests or shoes to the meat processing plant. If meat gets contaminated, the bacteria will be distributed in the grinding process.

Which are the highest risk foods?

Major outbreaks have been linked to undercooked ground beef patties. Other products include alfalfa sprouts, lettuce, spinach, cheese, and unpasteurized fruit juices. Any food product that may have been cross-contaminated is of high risk.

What's the illness?

Hemorrhagic colitis. Symptoms are severe cramping and watery or bloody diarrhea. Complications, can lead to kidney function failure and anemia, a condition called Hemolytic Uremic Syndrome.

Who is at higher risk?

Anybody that consumes a contaminated food product is likely to become ill. Children and elderly have severe complications more frequently.

¿Dónde se encuentra?

Los rumiantes son una reserva natural. Incluso animales sanos eliminan la bacteria en las heces fecales. Personas infectadas la eliminan en las heces y podrían pasarla a otras personas, superficies o alimentos, si tienen malas prácticas de higiene.

¿Cómo llega a contaminar los alimentos?

La escorrentía de agua contaminada con materia fecal de las granjas puede llegar a contaminar cultivos. La bacteria puede se llevada por animales, polvo, plagas o zapatos a las procesadoras de alimentos. Si la carne es contaminada, la bacteria será distribuida en el molino.

¿Cuáles son los alimentos de mayor riesgo?

Importantes brotes epidémicos han sido asociados con carne molida semi-cruda. Otros productos como brotes de alfalfa, lechuga, quesos y jugos no pasteurizados de frutas. Cualquier alimento que hallan sido expuestas a contaminación cruzada es riesgoso.

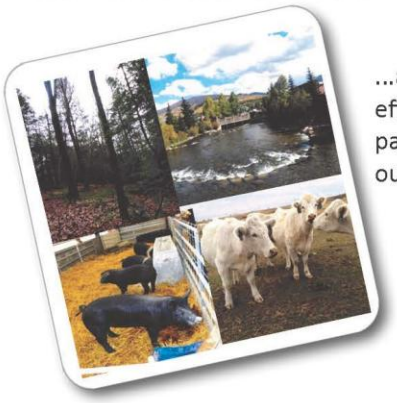
¿Qué enfermedad causa?

Colitis hemorrágica. Los síntomas son dolor fuerte de estómago y diarrea rala o con sangre. Las complicaciones puede causar fallas de riñón y anemia, lo que se llama Síndrome Urémico Hemolítico.

¿Quiénes tienen mayor riesgo?

Cualquier persona puede enfermar si come alimentos contaminados. Pero es más frecuente que los niños y ancianos sufran complicaciones severas.

Remember that pathogens are found in nature...



...and we need to make efforts to prevent the pathogens from reaching our food.

Listeria monocytogenes:

- Survives and grows at refrigerator temperature
- Most listeriosis patients require hospitalization
- Is considered an adulterant in ready-to-eat products

Salmonella:

- Survives dry conditions
- One of the top causes of foodborne illness
- Its presence at high levels indicates poor hygiene practices are in the food processing plants

***E. coli* O157:H7:**

- Some *E. coli* are able to produce a potent toxin known as Shiga Toxin. *E. coli* O157:H7 is a shiga toxin producer
- Just a few bacteria are needed to cause severe illness
- Is considered an adulterant in raw ground beef

ONLINE RESOURCES:

A gateway to food safety information provided by US government agencies is available at:

http://www.foodsafety.gov/

FSIS Food Safety Education web page available at:

http://www.fsis.usda.gov/Food_Safety_Education/index.asp

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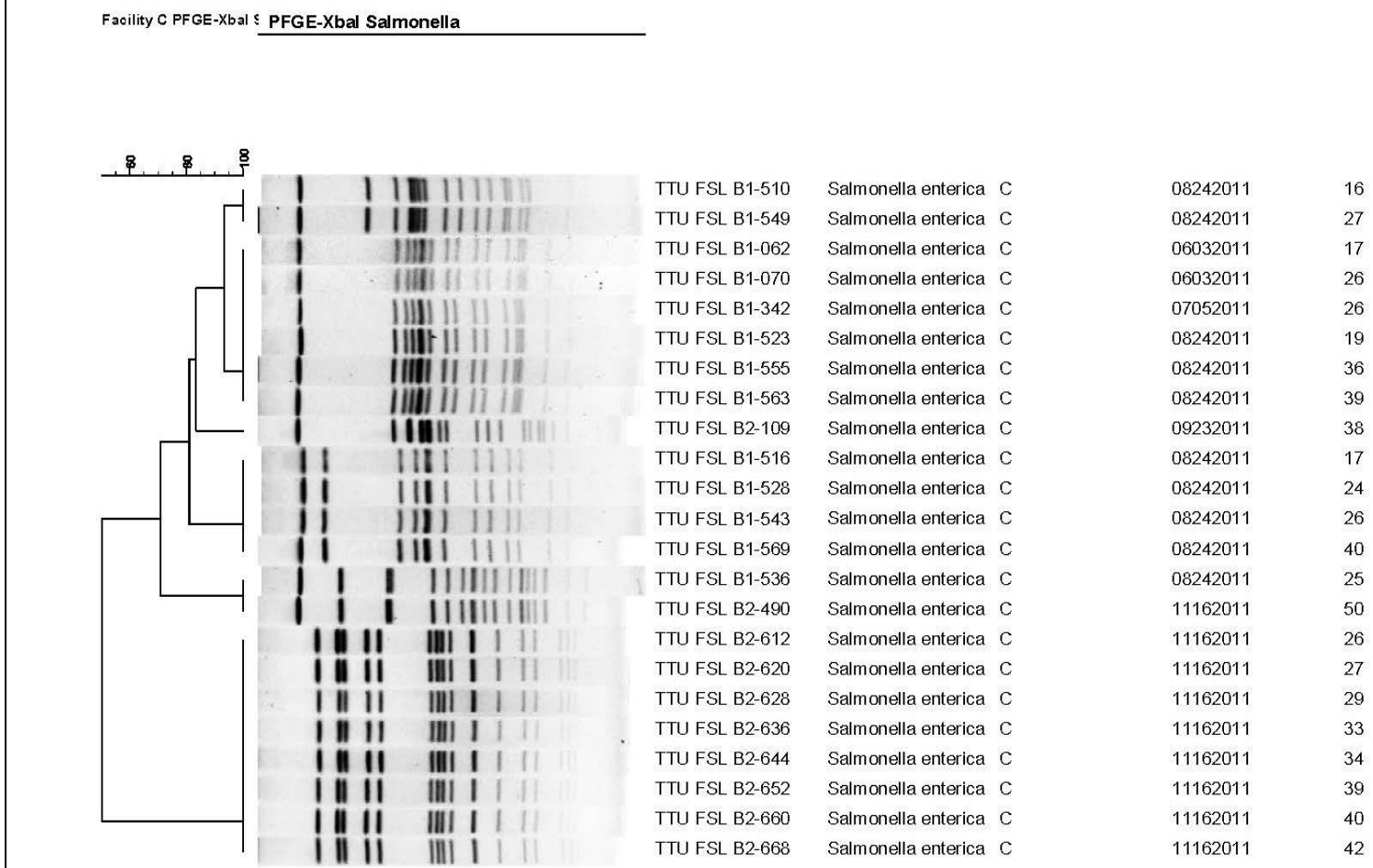
Appendix 11. Dendogram of *S. enterica* isolates collected in Plant 1.



Appendix 12. *S. enterica* and *E. coli* O157:H7 and contamination patterns in Plant 1.

Sampling	Isolate ID	Source	Sample type	Sampling Site	PFGE type
2	B1-327	M	NFC	Floor	EC PT 1
2	B1-329	E	NFC	Offal Barrels	EC PT 1
5	B2-283	SMK	NFC	Sinks	EC PT 2
5	B2-291	SA	NFC	Blood Catch Drain	EC PT 2
1	B1-062	SMK	NFC	Drain	SE PT 1
1	B1-070	SA	NFC	Blood Catch Drain	SE PT 1
2	B1-342	SA	NFC	Blood Catch Drain	SE PT 1
3	B1-510	SMK	NFC	Brooms/Mops/Squeege	SE PT 2
3	B1-516	SMK	NFC	Drain	SE PT 3
3	B1-523	SMK	NFC	Floor & Floor-Wall Junction	SE PT 1
3	B1-528	SA	FC	Small Hand Saw	SE PT 3
3	B1-536	SA	NFC	Door to Slab area outside	SE PT 4
3	B1-543	SA	NFC	Blood Catch Drain	SE PT 3
3	B1-549	SA	NFC	Floor	SE PT 2
3	B1-555	C	NFC	Draining Trap, Cooler 1	SE PT 1
3	B1-563	C	NFC	Drain	SE PT 1
3	B1-569	C	NFC	Floor	SE PT 3
4	B2-109	C	NFC	Floor	SE PT 5
6	B2-490	E	NFC	Floor, Slab Area	SE PT 4
6	B2-612	SA	NFC	Blood Catch Drain	SE PT 6
6	B2-620	SA	NFC	Floor	SE PT 6
6	B2-628	SA	NFC	Cradle	SE PT 6
6	B2-636	SA	NFC	Shackling Control Button	SE PT 6
6	B2-644	M	NFC	Floor	SE PT 6
6	B2-652	C	NFC	Drain	SE PT 6
6	B2-660	C	NFC	Floor	SE PT 6
6	B2-668	C	NFC	Control Button	SE PT 6

Appendix 13. Dendogram of *E. coli* O157:H7 isolates collected in Plant 1.

Appendix 14. Contamination patterns of *L. monocytogenes* in Plant 1.

Sampling	Isolate ID	Source	Sample type	Sampling Site	Molecular Serogroup	EcoRI Ribotype
1	B1-078	SMK	NFC	Door, to Smoking	1/2b, 3b, 7	DUP-1042B
1	B1-079	PA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
1	B1-080	SMK	NFC	Drain	1/2b, 3b, 7	DUP-1042B
1	B1-081	SMK	NFC	Smokehouse Door & Handle	1/2b, 3b, 7	DUP-1042B
1	B1-085	SMK	NFC	Floor & Floor-Wall Junction	1/2b, 3b, 7	DUP-1042B
1	B1-087	SMK	NFC	Sinks	1/2a, 3a	DUP-1057B
1	B1-091	PA	NFC	Boots	1/2b, 3b, 7	DUP-1042B
2	B1-264	PA	NFC	Stress Mats	1/2b, 3b, 7	DUP-1042B
2	B1-268	PA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
2	B1-272	SMK	NFC	Drain	1/2b, 3b, 7	DUP-1042B
2	B1-276	SMK	NFC	Floor & Floor-Wall Junction	1/2b, 3b, 7	DUP-1042B
2	B1-280	SA	NFC	Blood Catch Drain	1/2b, 3b, 7	DUP-1042B
2	B1-283	SA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
2	B1-287	C	NFC	Draining Trap, Cooler 1	1/2b, 3b, 7	DUP-1042B
2	B1-291	C	NFC	Drain	1/2b, 3b, 7	DUP-1042B
2	B1-293	C	NFC	Floor	1/2b, 3b, 7	DUP-1042B
2	B1-296	PA	NFC	Boots	1/2b, 3b, 7	DUP-1042B
3	B1-574	PA	NFC	Drain	1/2b, 3b, 7	DUP-1042B
3	B1-578	PA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
3	B1-582	SMK	NFC	Brooms/Mops/Squeegees	1/2b, 3b, 7	DUP-1042B
3	B1-586	SMK	NFC	Drain	1/2b, 3b, 7	DUP-1042B
3	B1-587	SMK	NFC	Smokehouse Door & Handle	1/2b, 3b, 7	DUP-1042B
3	B1-588	SMK	FC	Buckets	1/2b, 3b, 7	DUP-1042B
3	B1-592	SA	NFC	Boots	1/2b, 3b, 7	DUP-1042B
3	B1-593	SA	NFC	Blood Catch Drain	1/2b, 3b, 7	DUP-1042B
3	B1-594	SA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
3	B1-597	SA	NFC	Hose	1/2b, 3b, 7	DUP-1042B
3	B1-598	M	NFC	Floor	1/2b, 3b, 7	DUP-1042B
3	B1-602	C	NFC	Draining Trap, Cooler 1	1/2b, 3b, 7	DUP-1042B

Sampling	Isolate ID	Source	Sample type	Sampling Site	Molecular Serogroup	EcoRI Ribotype
3	B1-606	C	NFC	Drain	1/2b, 3b, 7	DUP-1042B
3	B1-610	C	NFC	Floor	1/2b, 3b, 7	DUP-1042B
3	B1-614	SMK	NFC	Door to Restroom	1/2b, 3b, 7	DUP-1042B
4	B1-909	SMK	NFC	Door, to Smoking	1/2b, 3b, 7	DUP-1042B
4	B1-913	PA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
4	B1-917	SMK	NFC	Drain	1/2a, 3a	DUP-1057B
4	B1-919	SMK	NFC	Floor & Floor-Wall Junction	1/2b, 3b, 7	DUP-1042B
4	B1-920	SA	NFC	Blood Catch Drain	1/2b, 3b, 7	DUP-1042B
4	B1-922	SA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
4	B1-924	M	NFC	Floor	1/2b, 3b, 7	DUP-1042B
4	B1-928	C	NFC	Drain	1/2b, 3b, 7	DUP-1042B
4	B1-929	C	NFC	Floor	1/2b, 3b, 7	DUP-1042B
4	B1-932	C	NFC	Floor	1/2b, 3b, 7	DUP-1042B
4	B1-936	PA	NFC	Boots	1/2b, 3b, 7	DUP-1042B
5	B2-373	SA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
5	B2-376	C	NFC	Drain	1/2b, 3b, 7	DUP-1042B
5	B2-380	C	NFC	Floor	1/2b, 3b, 7	DUP-1042B
6	B2-512	PA	NFC	Drain	1/2b, 3b, 7	DUP-1042B
6	B2-516	SMK	FC	Smokehouse Truck	1/2b, 3b, 7	DUP-1042B
6	B2-520	SMK	NFC	Door, to Smoking	1/2b, 3b, 7	DUP-1042B
6	B2-521	SMK	NFC	Floor & Floor-Wall Junction	1/2b, 3b, 7	DUP-1042B
6	B2-523	SA	NFC	Blood Catch Drain	1/2b, 3b, 7	DUP-1042B
6	B2-524	SA	NFC	Floor	1/2b, 3b, 7	DUP-1042B
6	B2-525	C	NFC	Drain	1/2b, 3b, 7	DUP-1042B
6	B2-528	C	NFC	Floor	1/2b, 3b, 7	DUP-1042B
6	B2-529	SMK	NFC	Door to Restroom	1/2b, 3b, 7	DUP-1042B
6	B2-533	PA	NFC	Boots	1/2b, 3b, 7	DUP-1042B

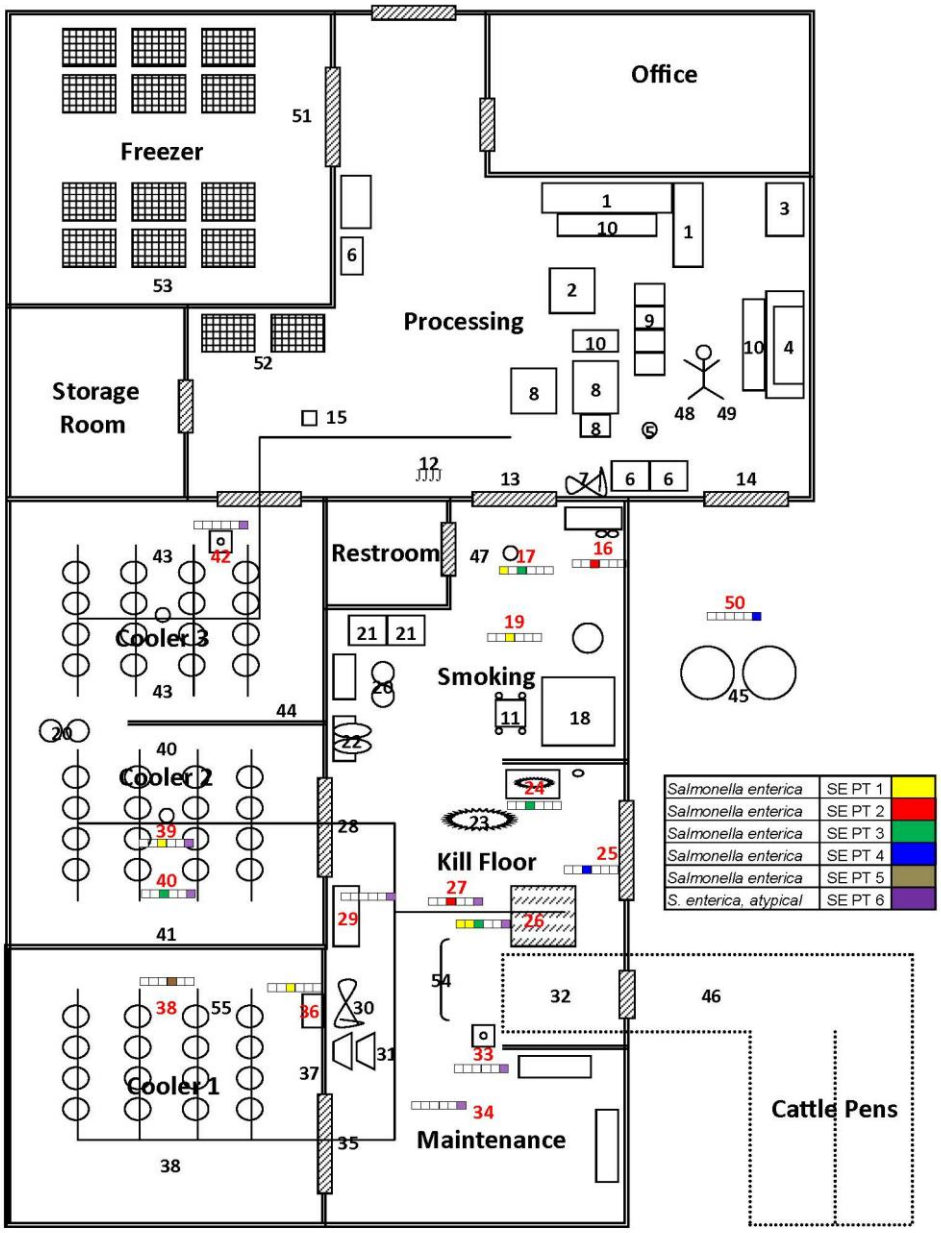
Appendix 15. Contamination patterns of *Listeria* spp. other than *L. monocytogenes* in Plant 1.

Sampling	Isolate ID	Source	Sample type	Sampling Site	sigB allele
1	B1-092	PA	NFC	Drain	LI AT 1
1	B1-094	SMK	NFC	Door, to Smoking	LI AT 1
1	B1-096	PA	NFC	Floor	LI AT 1
1	B1-098	SMK	NFC	Brooms/Mops/Squeegees	LI AT 1
1	B1-100	SMK	NFC	Drain	LI AT 1
1	B1-102	SMK	NFC	Floor & Floor-Wall Junction	LI AT 1
1	B1-104	SA	NFC	Blood Catch Drain	LI AT 11
1	B1-106	SA	NFC	Floor	LI AT 1
1	B1-108	SA	NFC	Stunning Area Pipework	LI AT 9
1	B1-110	C	NFC	Drain	LI AT 10
1	B1-112	C	NFC	Floor	LI AT 10
1	B1-114	C	NFC	Control Button	LI AT 4
1	B1-116	C	NFC	Floor	LI AT 10
1	B1-118	SMK	NFC	Door to Restroom	LI AT 1
1	B1-120	PA	NFC	Boots	LI AT 1
1	B1-122	E	NFC	Floor, Slab Area	LI AT 3
2	B1-300	PA	NFC	Drain	LI AT 5
2	B1-302	PA	NFC	Floor	LI AT 1
2	B1-304	SMK	NFC	Drain	LI AT 5
2	B1-306	SA	NFC	Boots	LI AT 5
2	B1-308	SA	NFC	Blood Catch Drain	LI AT 5
2	B1-310	SA	NFC	Cradle	LI AT 4
2	B1-312	C	NFC	Drain	LI AT 5
2	B1-314	C	NFC	Floor	LI AT 5
2	B1-316	C	NFC	Floor	LI AT 1
3	B1-616	PA	FC	Packaging Tables	LI AT 6
3	B1-618	PA	FC	Band Saw and Table	LI AT 6
3	B1-619	PA	NFC	Drain	LI AT 8
3	B1-621	PA	NFC	Hose	LI AT 1
3	B1-623	PA	NFC	Tables and Hopper	LI AT 6
3	B1-625	SMK	NFC	Brooms/Mops/Squeegees	LI AT 6
3	B1-626	SMK	NFC	Drain	LI AT 1
3	B1-628	SMK	NFC	Smokehouse Door & Handle	LI AT 1
3	B1-630	SMK	NFC	Floor & Floor-Wall Junction	LI AT 1
3	B1-632	SA	NFC	Boots	LI AT 1
3	B1-634	SA	FC	Small Hand Saw	LI AT 2
3	B1-636	SA	NFC	Blood Catch Drain	LI AT 1
3	B1-638	SA	NFC	Floor	LI AT 7
3	B1-640	SA	NFC	Hose	LI AT 1
3	B1-642	SA	NFC	Shackling Control Button	LI AT 5
3	B1-644	C	NFC	Drain	LI AT 6
3	B1-646	C	NFC	Floor	LI AT 1
3	B1-648	C	NFC	Walls	LI AT 1

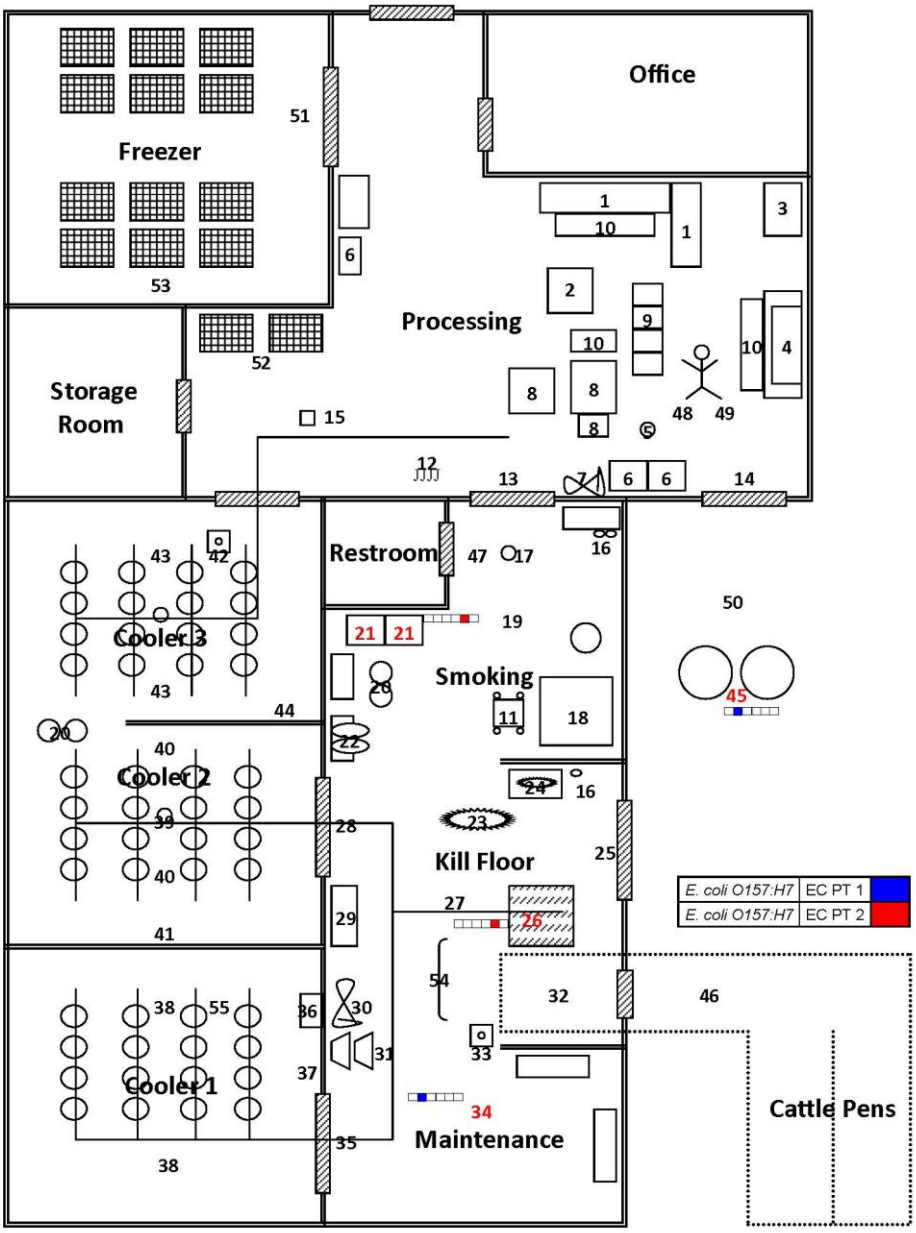
Sampling	Isolate ID	Source	Sample type	Sampling Site	sigB allele
3	B1-650	PA	NFC	Boots	LI AT 1
3	B1-652	PA	FC	Cross Bar and Hooks	LI AT 8
3	B1-654	BC	Carcass	Surface	LI AT 4
4	B1-940	PA	NFC	Drain	LI AT 8
4	B1-942	SMK	FC	Smokehouse Truck	LI AT 1
4	B1-944	SMK	NFC	Drain	LI AT 1
4	B1-946	SMK	NFC	Floor & Floor-Wall Junction	LI AT 1
4	B1-948	SA	NFC	Boots	LI AT 2
4	B1-950	SA	NFC	Blood Catch Drain	LI AT 1
4	B1-952	SA	NFC	Floor	LI AT 2
4	B1-954	SA	NFC	Door to Cooler 2	LI AT 1
4	B1-958	SA	NFC	Stunning Area Pipework	LI AT 5
4	B1-960	C	NFC	Floor	LI AT 10
4	B1-962	C	NFC	Drain	LI AT 1
4	B1-964	C	NFC	Floor	LI AT 2
4	B1-966	C	NFC	Floor	LI AT 1
4	B1-968	E	NFC	Floor, Slab Area	LI AT 1
5	B2-382	PA	NFC	Drain	LI AT 8
5	B2-384	PA	NFC	Stress Mats	LI AT 1
5	B2-386	SMK	NFC	Door, to Smoking	LI AT 1
5	B2-388	PA	NFC	Floor	LI AT 8
5	B2-390	SMK	NFC	Brooms/Mops/Squeegees	LI AT 1
5	B2-392	SMK	NFC	Drain	LI AT 5
5	B2-394	SMK	NFC	Smokehouse Door & Handle	LI AT 1
5	B2-396	SMK	NFC	Floor & Floor-Wall Junction	LI AT 2
5	B2-398	SA	NFC	Boots	LI AT 1
5	B2-400	SA	NFC	Blood Catch Drain	LI AT 1
5	B2-402	SA	NFC	Floor	LI AT 1
5	B2-404	M	NFC	Floor	LI AT 1
5	B2-406	C	NFC	Drain	LI AT 1
5	B2-408	C	NFC	Floor	LI AT 1
5	B2-410	C	NFC	Floor	LI AT 1
5	B2-412	BC	Carcass	Surface	LI AT 7
6	B2-537	SMK	FC	Smokehouse Truck	LI AT 1
6	B2-539	SMK	NFC	Door, to Smoking	LI AT 5
6	B2-541	SMK	NFC	Brooms/Mops/Squeegees	LI AT 1
6	B2-543	SMK	NFC	Drain	LI AT 5
6	B2-545	SMK	NFC	Smokehouse Door & Handle	LI AT 1
6	B2-547	SMK	NFC	Floor & Floor-Wall Junction	LI AT 1
6	B2-549	SA	FC	Small Hand Saw	LI AT 5
6	B2-551	SA	NFC	Blood Catch Drain	LI AT 1
6	B2-553	SA	NFC	Floor	LI AT 1
6	B2-555	M	NFC	Floor	LI AT 1
6	B2-558	C	NFC	Floor	LI AT 1
6	B2-560	C	NFC	Drain	LI AT 5

Sampling	Isolate ID	Source	Sample type	Sampling Site	sigB allele
6	B2-562	C	NFC	Floor	LI AT 1
6	B2-564	C	NFC	Floor	LI AT 1
6	B2-566	SMK	NFC	Door to Restroom	LI AT 5
6	B2-568	E	NFC	Floor, Slab Area	LI AT 1
4	B1-956	SA	FC	Aprons	LW AT 1

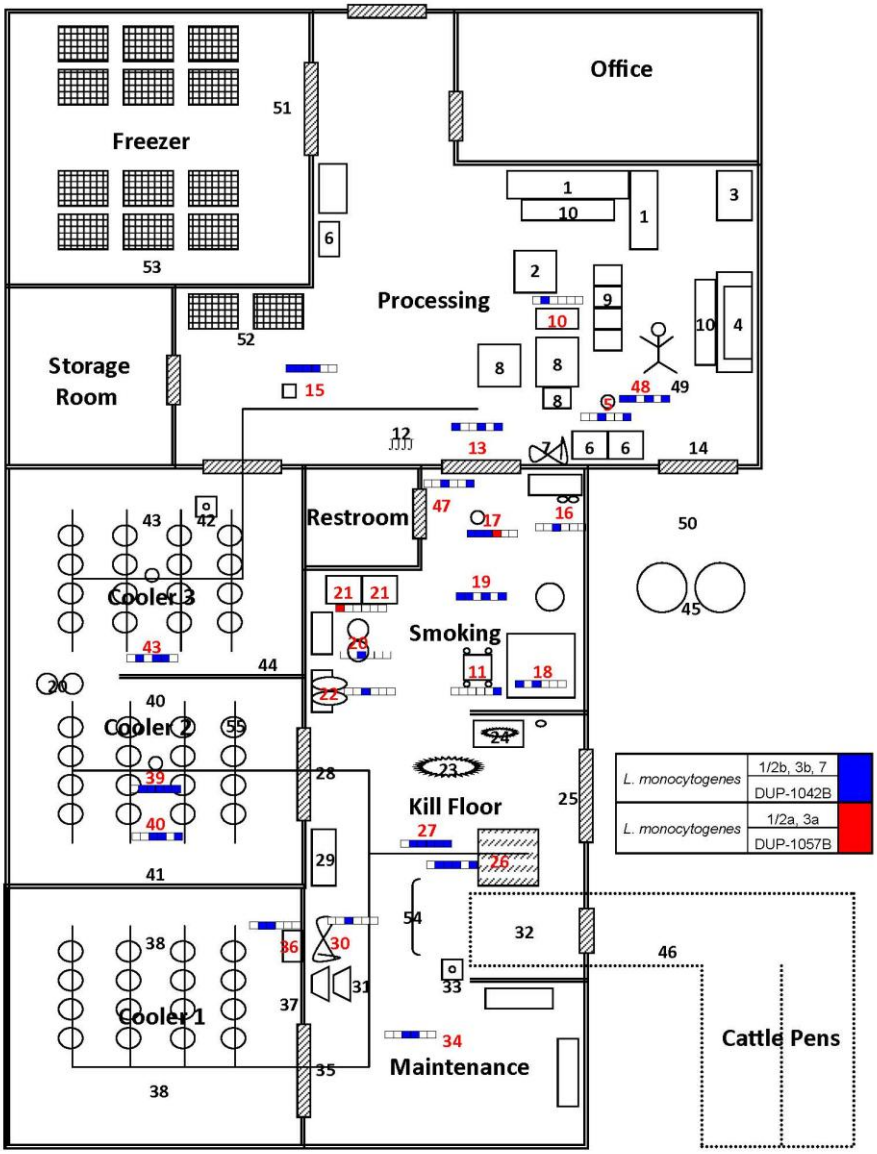
Appendix 16. Map of *S. enterica* contamination patterns in Plant 1.



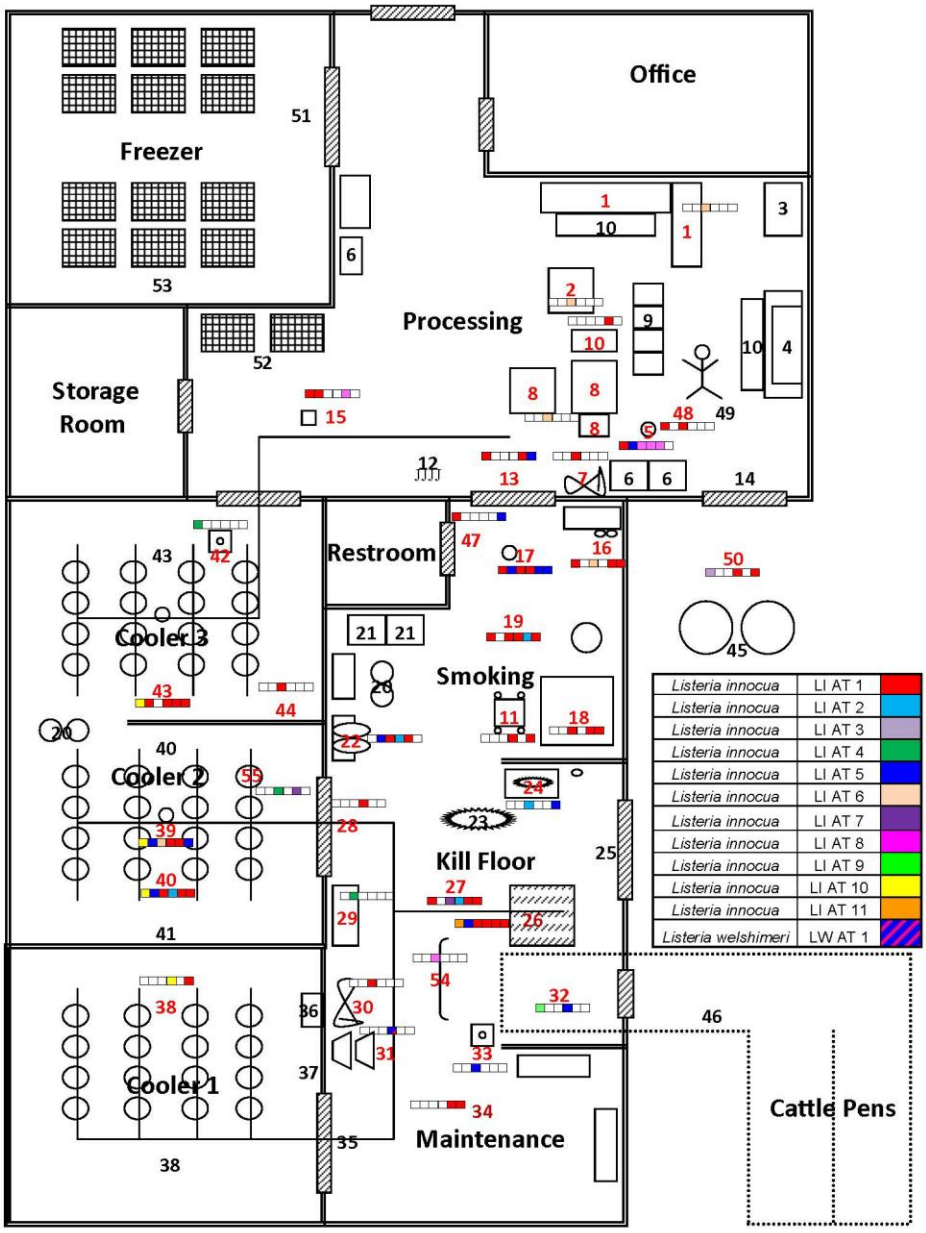
Appendix 17. Map of *E. coli* O157:H7contamination patterns in Plant 1.



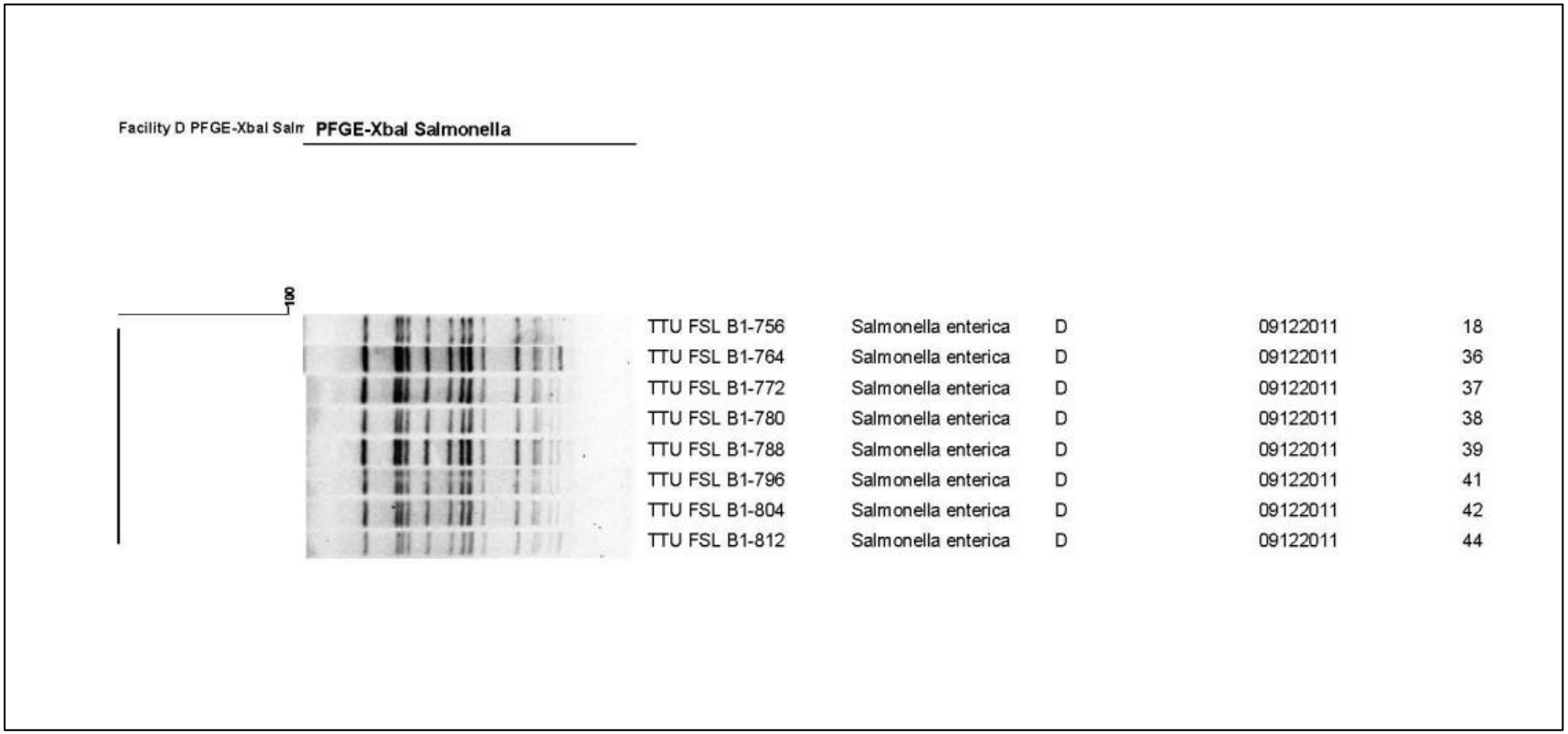
Appendix 18. Map of *L. monocytogenes* contamination patterns in Plant 1.



Appendix 19. Map of *Listeria* spp. other than *L. monocytogenes* contamination patterns in Plant 1.



Appendix 20. Dendogram of *S. enterica* isolatates collected in Plant 2.



Appendix 21.Contamination patterns *S. enterica* in Plant 2.

Sampling	Isolate ID	Source	Sample type	Sampling Site	PFGE type
3	B1-756	PA	NFC	Drain #2	SE PT 1
3	B1-764	SA	NFC	Stunning Area	SE PT 1
3	B1-772	SA	NFC	Drain, Stunning Area	SE PT 1
3	B1-780	SA	NFC	Floor	SE PT 1
3	B1-788	SA	NFC	Door to Exterior	SE PT 1
3	B1-796	SA	NFC	Walls	SE PT 1
3	B1-804	SA	NFC	Drain #1	SE PT 1
3	B1-812	SA	NFC	Hose	SE PT 1

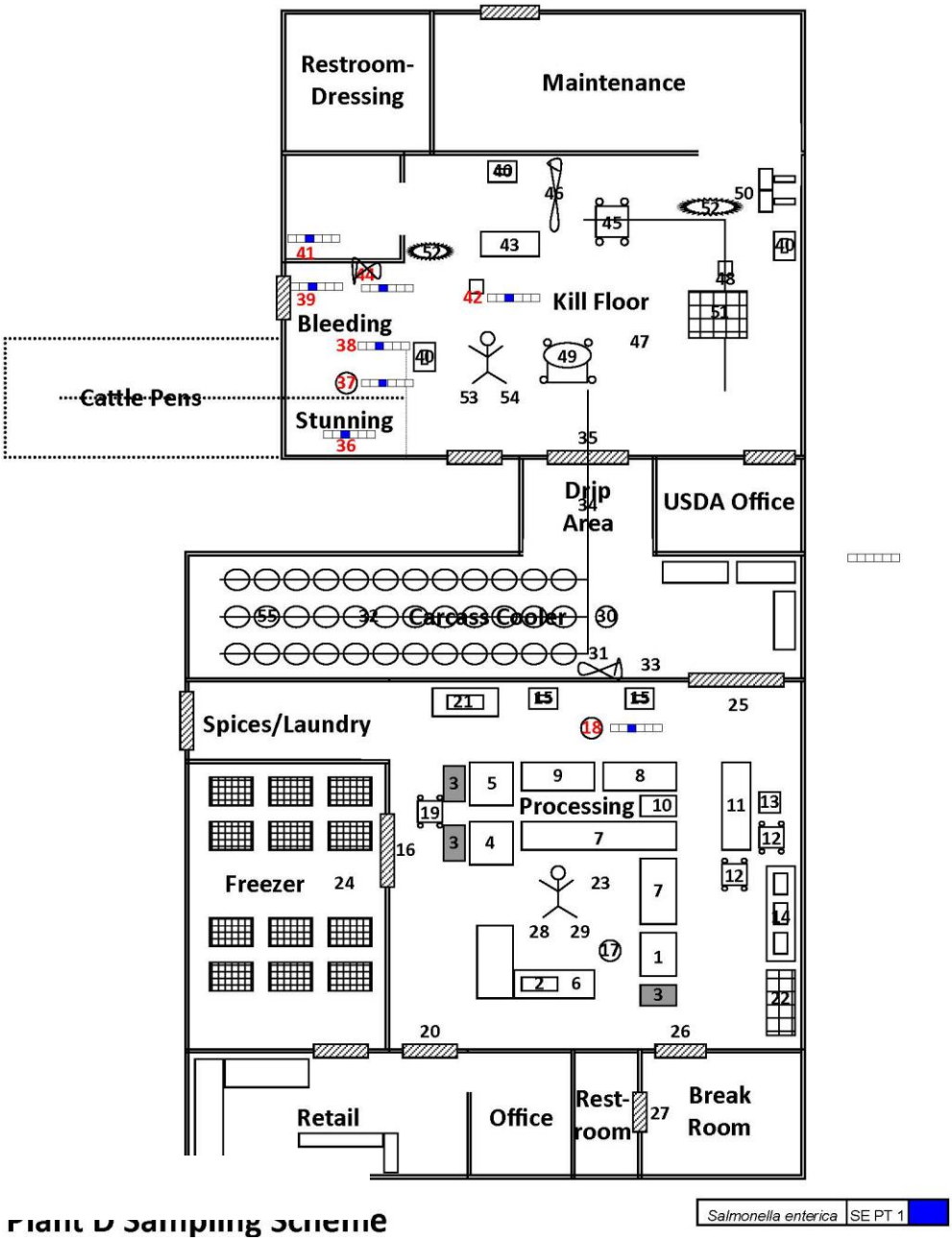
Appendix 22. Contamination patterns of *L. monocytogenes* in Plant 2.

Sampling	Isolate ID	Source	Sample type	Sampling Site	Molecular Serogroup	EcoRI Ribotype
1	B1-197	SA	NFC	Drain, Stunning Area	1/2a, 3a	DUP-1030B
1	B1-198	SA	NFC	Floor	1/2a, 3a	DUP-1030A
4	B2-218	PA	NFC	Drain #2	1/2a, 3a	DUP-1030B
6	B2-601	SA	NFC	Drain #1	1/2a, 3a	DUP-1030B

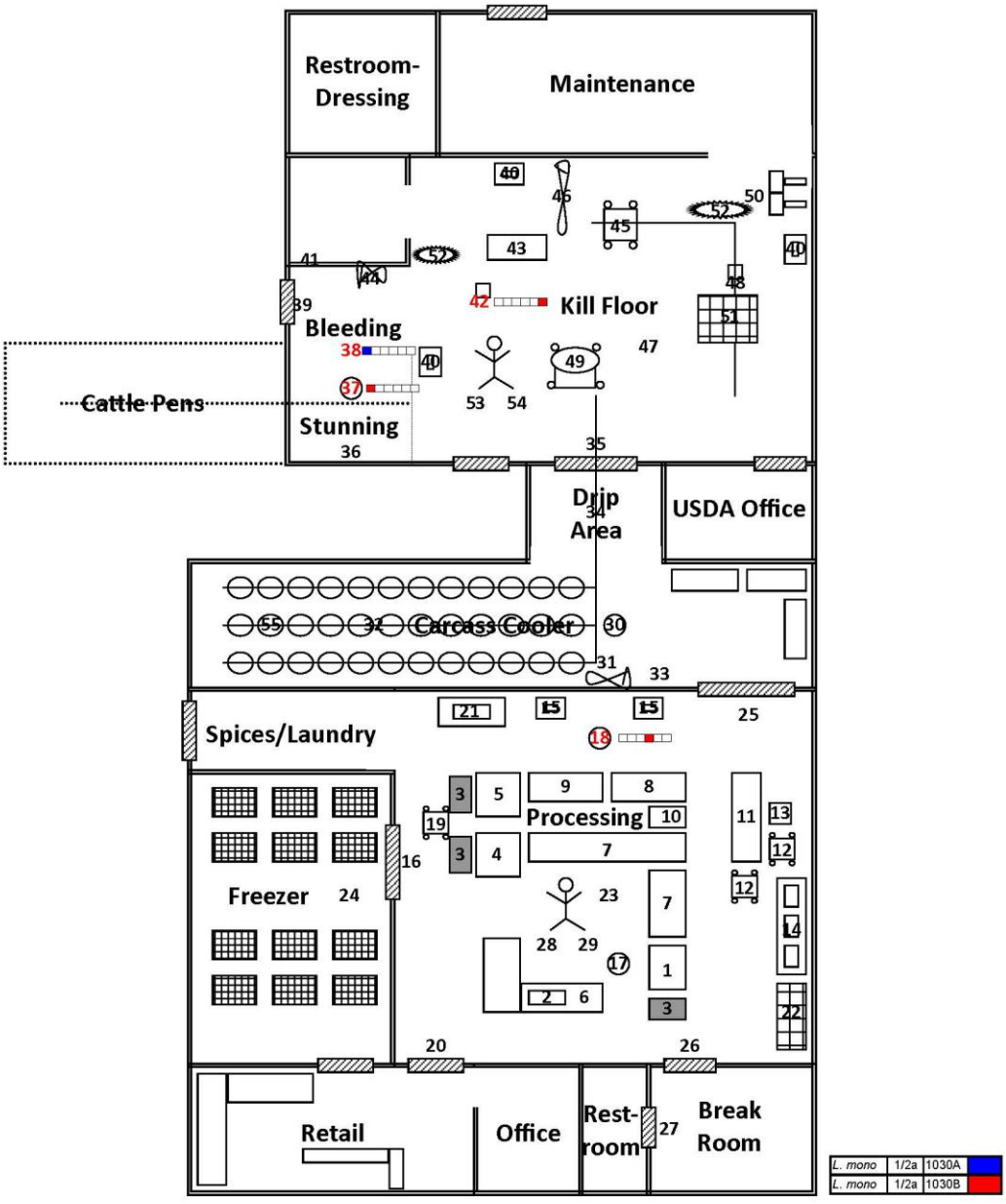
Appendix 23. Contamination patterns of *Listeria* spp. other than *L. monocytogenes* in Plant 2.

Sampling	Isolate ID	Source	Sample type	Sampling Site	sigB allele
1	B1-199	SA	NFC	Drain, Stunning Area	LI AT 109
1	B1-201	SA	NFC	Floor	LI AT 6
1	B1-203	SA	NFC	Hose	LI AT 124
1	B1-205	SA	NFC	Floor	LI AT 6
1	B1-207	SA	NFC	Platform	LI AT 6
2	B1-473	SA	NFC	Stunning Area	LI AT 6
2	B1-475	SA	NFC	Drain, Stunning Area	LI AT 6
3	B1-740	SA	NFC	Boots	LI AT 53
5	B2-449	SA	NFC	Hose	LI AT 53
6	B2-606	SA	NFC	Stunning Area	LI AT 31
6	B2-608	SA	NFC	Drain #1	LI AT 6
6	B2-610	SA	NFC	Floor	LI AT 6
3	B1-738	PA	NFC	Door to Cooler	LW AT 129
4	B2-222	PA	NFC	Door to Cooler	LW AT 129
4	B2-224	SA	NFC	Drain, Stunning Area	LW AT NEW
4	B2-226	SA	NFC	Floor	LW AT 129
5	B2-447	PA	NFC	Door to Cooler	LW AT 129
6	B2-602	C	NFC	Drain	LW AT 129
6	B2-604	C	NFC	Hose	LW AT 129
2	B1-477	SA	NFC	Floor	LS AT 121

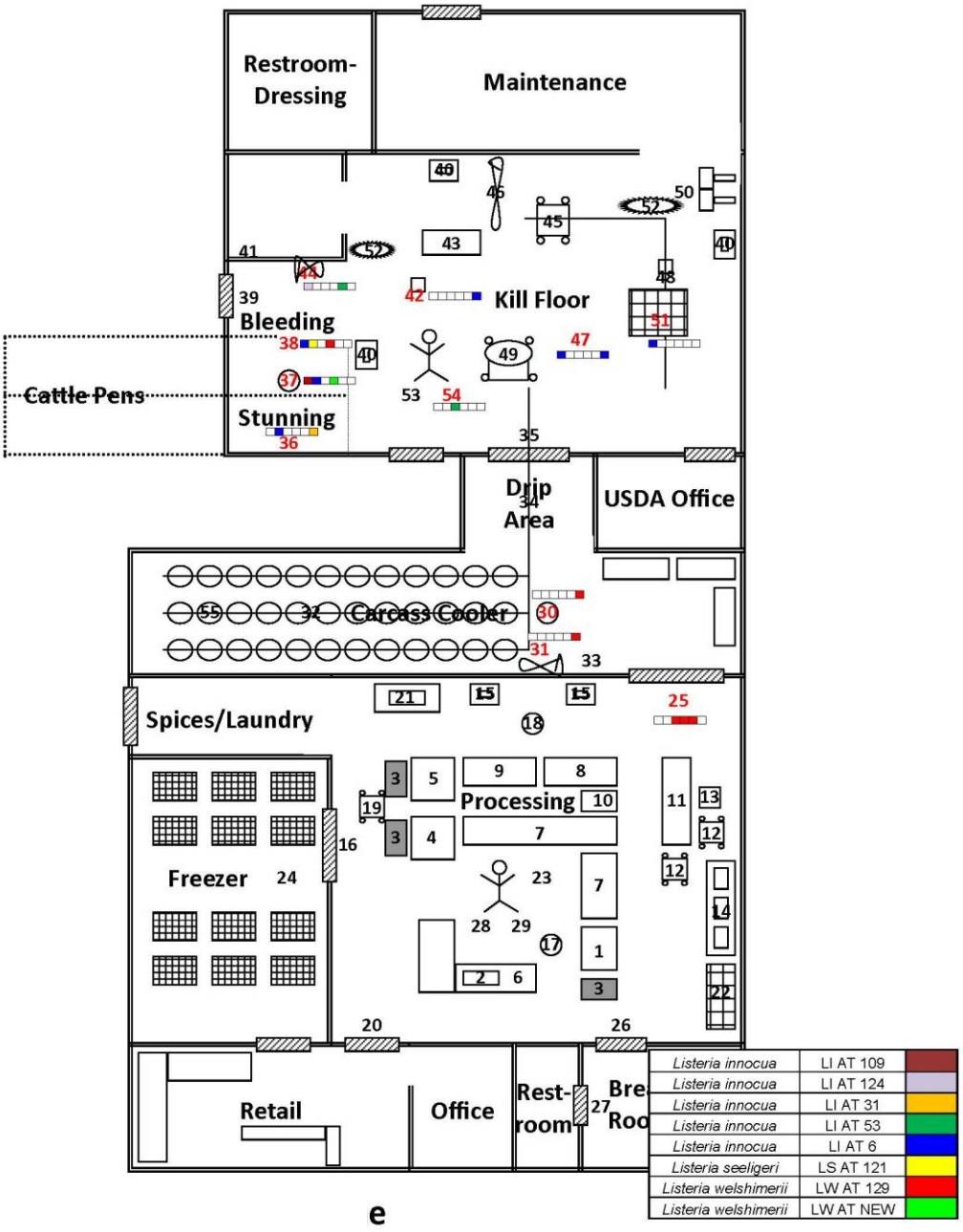
Appendix 24. Map of *S. enterica* contamination patterns in Plant 2.



Appendix 25. Map of *L. monocytogenes* contamination patterns in Plant 2.



Appendix 26. Map of *Listeria* spp. other than *L. monocytogenes* contamination patterns in Plant 2.



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