INCIDENCE AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* IN THE PROCESSING ENVIRONMENT OF FULLY COOKED, READY-TO-EAT BEEF PRODUCTS

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FORMAT OF DISSERTATION

This Dissertation is presented in Journal of Animal Science style format, as outlined by the Oklahoma State University Graduate College Style Manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER I

INTRODUCTION

An estimated 76 million people contract foodborne illnesses each year in the United States **(**Mead et al., 1999). *L. monocytogenes* is in a group of pathogens, including *Salmonella, Toxoplasma*, Norwalk-like viruses, *Campylobacter* and *E. coli* O157:H7, that account for more than 90% of estimated food-related deaths in the United States (Henning and Cutter, 2001). Individual contribution to food related deaths are: *Salmonella* (31%), *L. monocytogenes* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *E. coli* O157:H7 (3%) (Mead et al., 1999). Serious infections with *L. monocytogenes* are often characterized by abortion, septicemia, meningitis and/or encephalitis (Bille and Rocourt, 1996). The infections generally occur only in the immunocompromised, elderly and very young populations and pregnant women (Hof et al., 1997). Infections during pregnancy can result in miscarriage and stillbirth due to *L. monocytogenes* crossing the placental barrier (Guidelines for Developing Good Manufacturing Practices, Standard Operating Procedures and Environmental Sampling/Testing Recommendations. Ready-to-Eat Products, April 1999**).** As a growing proportion of our population falls into a more elderly, and consequently higher risk group, improved methods for reducing levels of *L. monocytogenes* in foods becomes more and more essential (Norton et al., 2001).

L. monocytogenes continues to be of worldwide interest to the food industry, regulatory agencies, scientists, and consumers. Such interest is prompted by the occasional appearance of *L. monocytogenes* in ready-to-eat foods leading to the removal of such products from the marketplace. It's estimated that 99% of listeriosis infections are caused by eating food contaminated with the bacterium *L. monocytogenes* (Nadon et al., 2001; Ryser and Marth, 1999). Twenty percent of sporadic cases of listeriosis in the U.S. were due to either eating hot dogs not thoroughly reheated before serving or eating under cooked chicken (Tompkin et al., 1991).

Accordingly, it is one of the responsibilities of the food industry, and more specifically in reference to this project, the processed meat industry, to develop standard sanitation operating procedures, processing procedures, processing aids, and pathogen reduction and tracking strategies to minimize the risk of contamination of ready-to-eat meat products and consequent foodborne illness. The overall objectives of this project were to establish *L. monocytogenes* and *L*. spp. as environmental contaminants within defined zones in a ready-to-eat meat processing plant, evaluate ribotypes of *L. monocytogenes* confirmed samples and identify the source to facilitate improved environmental control and to utilize information gained during the project to establish corrective actions to minimize the risk of contamination of ready-to-eat beef products.

CHAPTER II

LITERATURE REVIEW

The name of the organism, *Listeria*, a gram positive, rod-shaped bacterium, and its associated disease, listeriosis, gets its name from Baron Joseph Lister (1827 – 1912), an Englishman considered the father of modern antiseptic surgery. *Listeria monocytogenes* is a pathogenic bacterium often found in the intestines of healthy animals (including humans) and in the environments in which food-producing animals are raised and processed. The first report of human listeriosis was in 1929. It was shown that there was direct transmission of cutaneous listeriosis to veterinarians delivering infected calves. Thought to be a zoonotic disease, it was considered an "occupational disease" of animal care takers and abattoir workers. "Circling disease" was used to describe a condition in sheep in Wales in 1933 and the term is still applied to listeric encephalitis in ruminants. Foodborne transmission of *Listeria* was established in 1981 with the consumption of coleslaw in Nova Scotia, Canada. There were subsequent reports in 1983 (pasteurized milk) and 1985 (Mexican style cheese) of foodborne related outbreaks (Kaferstein, 1999).

Despite the decreasing incidence, human listeriosis continues to be a serious public health threat in the United States (CDC, December 1999b). It's annual incidence decreased by 44% between 1989 and 1993 (CDC, December 1999a; Mead et al., 1999; Chen et al., 2003). An analysis of the incidence trend from 1996 to 2002 shows a 38% decline (CDC. 2003). In recent years, (2000-2001), based on FoodNet active surveillance

program, CDC reported listeriosis frequency of 3 cases per million population (Chen et al., 2003). Each year, *L. monocytogenes* causes an estimated 2,493 cases of listeriosis compared to salmonellosis at 1.4 million cases per year (Chen et al., 2003). Of these, 2,298 (92%) persons were hospitalized and 499 (20%) persons died (Ryser and Marth, 1999; Mead et al., 1999). Although this represents a small percentage of the total foodborne illness cases in the United States, listeriosis is characterized by having a high mortality rate within cases among the very young, elderly and immunocompromised victims. Siegman-Ingra et al. (2002) reported as high as 45% mortality within perinatal cases in Israel from 1995 to 1999. Of all the bacterial foodborne pathogens tracked by CDC, *L. monocytogenes* had the second highest case fatality rate (20%) after *Vibrio vulnificus*, and the highest hospitalization rate (92.2%) (Mead et al., 1999).

Due to the ubiquitous nature of this organism, and its ability to grow at refrigeration temperatures and resist common food preservative agents, it is extremely difficult to eliminate all *L. monocytogenes* from the food processing environment (Bille and Racourt, 1996; AMI, 1998; Guidelines for developing good manufacturing practices, standard operating procedures and environmental sampling/testing recommendations. April 1999). Additionally, Saunders et al. (2004) concluded that *Listeria* control measures at retail establishments which process and handle ready-to-eat meat products are a critical component in the farm-to-table *L. monocytogenes* control program. Given current technology, it is not possible to eradicate *L. monocytogenes* from the processing environment or totally eliminate the risk of finished product contamination (Tompkin et al., 1991, 1999). Vacuum packaging, cold storage and high salt concentrations may favor growth of pathogenic *L. monocytogenes* compared to competing microflora (Miettinen et al., 2001).

Controlling growth of *L. monocytogenes* in food processing plants is an important aspect of hygienic food production (Blackman et al., 1996). The available evidence on the presence of *L. monocytogenes* in food processing environments demonstrates the potential for the contamination of products after a food has been processed to destroy pathogenic microorganisms**, (**CDC. December 1999b; Tompkin et al., 1999) and is evidenced by *L. monocytogenes* in finished ready-to-eat products (Norton et al., 2001).

Consistent with the proposed ruling, efforts to control *L.* spp. have already been made by food processors. A survey in March 2000 by seven industry organizations (National Food Processors Assn., American Meat Institute, National Turkey Federation, National Chicken Council, National Meat Association, National Association of Meat Processors, and American Association of Meat Processors) showed that more than 90% of processors who responded to the survey use microbiological testing to verify effective control of *L. monocytogenes*.

Contamination more likely will occur after the organism has become established in a niche, as in biofilms, (Blackman et al., 1996) after which routine cleaning and sanitizing become ineffective (Tompkin et al., 1999; Autio et al., 1998; Lawrence and Gilmour, 1995; Nesbakken et al., 1996). All reported that endemic strains in plants may persist for several years.

The processing environment can serve as indirect sources of *L. monocytogenes* (Norton et al., 2002; Tompkin et al., 1999). The non-contact areas of the facility may harbor the organism and under certain conditions lead to contamination of contact surfaces and finished product. Arimi et al. (1997) reaffirmed the importance of *L. innocua* as a potential indicator of *L. monocytogenes* contamination. Therefore,

controlling *L.* spp. in the environment should reduce the risk of contamination. The significance of the area will vary depending upon the facility, the process(es), the temperature and humidity of the room, and the food product (Norton et al., 2002).

It is possible that the pattern of strain distribution in the environment is dynamic, varying both by location in the plant and time of production. Because strains differ in their response to various control strategies, understanding the pattern of strain distribution in foods and food processing environments and ways in which this distribution changes with time and location within the environment is important. Data suggest the overall population of *L. monocytogenes* consists of a number of persistent, widely distributed strains from diverse incoming sources frequently coexist in the environment. (Gendel and Uldasek, 2000; Arimi et al., 1997). Data from Autio et al. (2002) suggest a wide geographical and temporal distribution of *L. monocytogenes* strains and the existence of similar strains in various food products. Ten of the 66 pulsetypes in their study, were detected in more than one product type. Lawrence and Gilmour (1995) reported *L. monocytogenes* strains (typed by RAPD) were identified in cooked poultry processing environments up to one year after initial identification demonstrating the potential for environmental strains to persist for long periods, survive the cleaning procedures in place and ultimately contaminate the final product. Kathariou (2002) reported strains of *L. monocytogenes* may persist in a facility for more than 10 years. Lunden et al. (2002) showed that it is possible to transfer *L. monocytogenes* from one plant to another via equipment transfer.

Consideration should be given to the potential for bringing *L. monocytogenes* back into the clean environment. This may occur because of traffic in the processing/packaging areas such as people and equipment entering from contaminated areas in the operation, or

unscheduled equipment maintenance (Norton et al., 2001; Tompkin et al., 2002). Berrang et al. (2005) reported that drains may have been seeded during the shift by personnel, product, or equipment transfer from the raw side to the cooked (RTE) side of the plant. In all but two cases in their study, the subtype detected in drains on the RTE side was also detected somewhere on the raw side of the plant on the same day. Their data suggest "Type D" entered the plant on raw material, became a "resident" in the plant and eventually was detected in association with RTE product.

Gombas et al. (2002) reported 1.82% prevalence in 31,705 samples taken with a range in percent positive samples of 0.17% (soft cheeses) to 4.70% (seafood salads). [Note: In this study 402 of the 577 positive samples (69.7%) the number of cells were below enumeration levels (< 0.3MPN/g)].

Henning and Cutter (2001) stated that *L. monocytogenes* contamination of cooked meat products most frequently occurs when a product or food contact surface is contaminated between the cooking step and packaging (e.g. during slicing or peeling operations). Examples of potential sources of contamination (in plant) of *L. monocytogenes* might include:

- Raw product and ingredients
- Solutions to chill foods (e.g. brine solution)
- Loose product
- Rework
- Returned product

It's crucial for implementation of control measures to understand were points in the food chain contamination can occur (Bruhn et al., 2005). Arimi et al. (1997) recorded

data supporting a link between on-farm sources of *Listeria* contamination (dairy cattle, raw milk, silage) and subsequent contamination of dairy processing environments. Data from Nightingale et al. (2004) showed the beef production system maintains a high prevalence of *L. monocytogenes* including subtypes linked to human illness.

The processing environment can serve as indirect sources of *L. monocytogenes* (Tompkin et al., 1999). The non-contact areas of the facility may harbor the organism and under certain conditions lead to contamination of product contact surfaces and eventually finished product. Thimothe et al. (2004) showed *L. monocytogenes* prevalence in the plant environment had a positive relation ($P < 0.0001$) with the prevalence in finished product.

Additionally, they reported *L. monocytogenes* environmentally in 23.7% of samples from drains, 4.8% of samples from food contact surfaces, 10.4% of samples from employee contact surfaces and 12.3% of samples from non-food contact surfaces. However, in one plant a specific *L. monocytogenes* strain (identified by ribotyping) persisted in a raw fish handling area and that ribotype was never found in the RTE area. This would indicate that the plant had very good controls separating the raw handling areas from RTE production areas. Henning and Cutter (2001) pointed out that conducting environmental monitoring can identify areas in the processing environment that may harbor *Listeria* and can provide focus areas for intensified sanitation. It may give notice to problems in airflow, people traffic patterns, personal hygiene (hand washing, frock cleaning) improper movement of equipment/material from raw handling areas to RTE areas of the plant.

Newer techniques (PFGE and ribotyping) provide much greater insight into the sustained microflora of food operations and should provide guidance for improving the control of *L. monocytogenes* (Tompkin, 2002). Lappi et al. (2004) used subtyping techniques to study the impact of plant-specific *Listeria* control strategies in crawfish processing plants. Some were more general in nature, such as employee training and improved sanitation practices. However, some were more specific, including:

- Control measures implemented in receiving, storage and washing areas.
- Color code clothing dedicated to personnel to handle live crawfish only.
- Removal and management of crawfish wash water to prevent splashing.
- Proper disposal of crawfish wash water and of sacks used to transport live crawfish.
- Sanitize colanders and trays (3 to 4 times per day) used for holding peeled crawfish .
- Controls were put in place to ensure that peeled crawfish tail meat was packaged at the same rate it was being received into packaging to minimize the exposure of finished product to ambient temperatures.

Understanding the sources of potential contamination is very important in producing a safe RTE product. In general, once proper cooking has occurred, the burden for producing a safe product depends on proper sanitation, operational hygiene to prevent contamination and minimizing adverse time/temperature effects in all phases of handling and transportation (Henning and Cutter, 2001).

Health Risk

Occasional incidence of *L. monocytogenes* in ready-to-eat (RTE) foods, which leads to the removal of such products from the marketplace, keeps the pathogen top-of-mind among industry and regulatory personnel.

As was previously stated, the annual incidence of listeriosis decreased by 44% from 1989 to 1993 and 38% from 1996 to 2002. However, in 1998, a large outbreak that resulted in 101 illnesses and 21 deaths and over one million pounds recalled in 22 states was traced to consumption of contaminated hot dogs (CDC, December 1999a; Henning and Cutter, 2001). In 2002, an outbreak that resulted in 53 illnesses, 8 deaths, and 3 fetal deaths in 9 states was traced to consumption of contaminated turkey meat (CDC. 2002).

Listeria monocytogenes can grow in most ready-to-eat meat products at refrigeration temperatures. Contamination of cooked meat products most frequently occurs when a product or food contact surface is contaminated between the cooking step and packaging (e.g. during slicing or peeling operations). USDA, FSIS Microbiological Testing Program shows prevalence of *L. monocytogenes* in RTE meat and poultry products (Table 1). Additionally, their data show a continual decline in the incidence of *L. monocytogenes* in the last several years (Table 2).

Regulatory

Various approaches have been proposed to minimize the risk of foodborne listeriosis. The World Health Organization (WHO) recommended action in 1988 based on the type of food involved categorized into four groups (Tompkin et al., 1991):

1. Raw foods (i.e., raw meat)

2. Transformed raw foods (i.e. fermented sausages)
3. Processed foods given a listericidal process and then subjected to recontamination during subsequent handling (i.e. sliced luncheon meat)
4. Processed foods which are packaged and then given listericidal treatment in the process (i.e. canned ham)

Levine et al. (2001) reported that, "during the 1990s, the FSIS established eight microbiological testing programs for RTE meat and poultry products produced and collected at federally inspected establishments (Table 2). These testing programs did not include sample collection at retail outlets. These ongoing sampling programs are strictly regulatory in nature and, as such, are not statistically designed".

According to the report of Levine et al. (2001), establishments to be sampled are randomly selected each month from the database of all establishments known to be producing the particular class of product. Sampling probability is not proportional to plant size or production volume. Inspection personnel notify establishment management at the time of sampling offering management the option of voluntarily holding the sampled product pending FSIS laboratory test results. If samples test positive for a microbial hazard, regulatory action will be taken on the product represented by the samples. Such products are subject to retention, seizure, or voluntary recall.

In January 2001, USDA, FSIS issued the Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* among Selected Categories of Ready-to-Eat Foods. FDA's Center for Food Safety and Applied Nutrition (CFSAN) and USDA/FSIS wrote the assessment, in consultation with the Centers for Disease Control and Prevention (CDC). The draft risk assessment and the joint release (by Health and

Human Services and USDA) of "Risk Management Action Plan" to address *L. monocytogenes* were in response to the May 5, 2000, Presidential directive to reduce *L. monocytogenes* related disease by 50% by the year 2005. In the risk assessment, FSIS defined factors that are relevant in determining whether *L. monocytogenes* contamination is a food safety hazard reasonably likely to occur in the production process. Factors include exposure to contamination after lethality treatment and the evidence of *L. monocytogenes* in finished ready-to-eat products (USDA, Federal Register, May 1999).

In Washington, D.C. on Jan. 19, 2001, the following statement was given in a FSIS press release. "In an effort to further reduce the risk of human illness from ready-to-eat meat and poultry products, Agriculture Secretary Dan Glickman announced a proposed regulation that would require meat and poultry processors to conduct environmental testing for generic *Listeria* and establish food safety performance standards for illness-causing bacteria in all ready-to-eat and partially heat-treated meat and poultry products".

Subsequently, the USDA, FSIS published a proposed ruling to set forth requirements for food processors to control *L.* spp. on food contact surfaces and eliminate *L. monocytogenes* in "ready-to-eat" (RTE) meat and poultry products (FSIS, USDA. 2000. Fed. Reg.).

National Surveillance of Foodborne Illness

The Foodborne Diseases Active Surveillance Network (FoodNet) is the principle foodborne disease component of CDC's Emerging Infections Program. FoodNet is a

collaborative project of the CDC, USDA, the Food and Drug Administration (FDA) and the ten sites involved in the Emerging Infections Program.

In 1996, active surveillance began for laboratory-diagnosed cases of infection with *L. monocytogenes*, and other microbiological food contaminants in Minnesota, Oregon, and selected counties in California, Connecticut, and Georgia. From 1996 to 2001, the FoodNet surveillance population increased from five sites and a population of 14.2 million to nine sites and 37.8 million persons (13% of the U.S. population). During 2001, a total of 13,705 laboratory-diagnosed cases of ten foodborne diseases under surveillance were identified: 5,198 of *Salmonella* infection, 4,740 of *Campylobacter*, 2,201 of *Shigella*, 574 of *Cryptosporidium*, 565 of *E. coli* O157, 145 of *Yersinia*, 94 of *L. monocytogenes*, 80 of *Vibrio*, 32 of *Cyclospora,* and 76 of HUS. *Listeria* cases ranged from 0.1 infections per 100,000 persons in Minnesota to 0.5 infections per 100,000 persons in California. Infants and young children had the highest case rate of most foodborne infections. For *Listeria,* it was 1.9 cases per 100,000 persons. The incidence of *Listeria* cases in persons aged $\geq$ 75 years (1.7) approached the incidence in infants (CDC. 2002).

Substantial variations in incidence of specific diseases, defined as laboratory-diagnosed infections per 100,000 persons, were reported among the sites. These surveillance data show a decrease in the major bacterial foodborne illnesses, indicating progress toward meeting the national health objectives of reducing the incidence of foodborne diseases by 2010 (U.S. Department of Health and Human Services, 2000).

Microbiological Typing Methods

Subtyping microorganisms is using phenotypic or genotypic characteristics for segregation into like groups. Most bacteria species have sufficient differences among these phenotypic and/or genotypic characteristics to allow for identification of different subtypes (Ryser and Marth, 1999).

The development and application of molecular subtyping methods for *L. monocytogenes* has greatly improved our ability to study and understand the transmission and ecology of *L. monocytogenes* (Saunders et. al., 2004). Current methodologies are classified as either phenotypic (biotyping, antimicrobial susceptibility testing, serotyping, and bacteriophage typing) or genotypic (plasmid profile analysis, multilocus enzyme electrophoresis, restriction endonuclease analysis, ribotyping, pulsed-field gel electrophoresis, polymerase chain reaction, PCR restriction digestion, random amplified polymorphic DNA, and nucleotide sequence analysis).

For the purpose of this study, ribotype identification was determined to enhance the overall knowledge of type of *L. monocytogenes* in the processing environment and raw materials. Ribosomal DNA fingerprinting (ribotyping) is a raw form of DNA typing so it represents more of the genotypic material responsible for different serotypes. It is genetically more specific because there can be several ribotypes under the same serotype. It has been shown to be a sensitive method for subtyping pathogenic bacteria to aid in epidemiologic investigations. Automated *Eco*RI (a restriction enzyme obtained from *Escherichia coli*) ribotyping provides a highly standardized subtyping method commercially accessible to the food industry (Lappi et al., 2004). It has been shown to

be a sensitive, reproducible method for subtyping pathogenic bacteria to aid in epidemiologic investigations (Swaminathan et al., 1996; Saunders et al., 2004).

Most bacteria contain multiple ribosomal operons, therefore ribotyping typically provides an adequate number of bands in a profile to make it sufficiently discriminating for subtyping (Swaminathan et al., 1996). Saunders et al. (2004) reported that automated *Eco*RI ribotyping provided good discrimination of *L. monocytogenes* isolates from foods (D=94.3%)[1] and environmental isolates (D=80.8%), as well as of human isolates (D=93.0%), supporting the *Eco*RI ribotyping provides appropriate discriminatory power.

Serotyping is a grouping of intimately related organisms based on antigen-antibody (antigen – a protein or carbohydrate capable of stimulating an immune response) reactions to cell wall and flagellar proteins (Riser and Marth, 1999). It is a phenotypic distinction.

In the food industry, microbiological typing methods are sometimes used along with epidemiologic information to identify sources of foodborne illness outbreaks. Molecular subtyping (Pulsed Field Gel Electrophoresis (PFGE)), Ribotyping, Random Amplification of Polymorphic DNA (RAPD)), have not linked specific foods and disease (Gilot et al., 1996), however, information from typing can contribute to identification of a major food vehicle as well as exclusion of foods that did not contribute to a listeriosis outbreak (Jacquet et al., 1995; Bille and Racourt, 1996; Saunders et al., 2004; Wiedmann, 2002).

A review of the literature pertaining to typing *L. monocytogenes* revealed a variety of methods used and some of the advantages and disadvantages of each. Caugant

[1] Simpson's diversity index: A mathematical measure that characterizes species diversity in a community. The proportion of species I relative to the total number of species (pi) is calculated and squared. The squared proportions for all the species are summed and the reciprocal is taken.

et al. (1996) reported multilocus enzyme electrophoresis (MEE) is a typing method used to detail the epidemiology of a variety of bacterial species. The discriminatory power of MEE was relatively low as a consequence of somewhat low genetic diversity of *L. monocytogenes* compared to other bacterial species. Wernars et al. (1996) reported on the use of RAPD as a method of subtyping *L. monocytogenes.* The overall correlation between the results from different participating labs ranged from 32% to 85% showing difficulty in reproducible results among the labs (Bille and Rocourt, 1996). Zhang et al., (2004), reported that multi-virulence-locus sequence typing (MVLST) was able to differentiate strains that were undistinguishable by *Eco*RI ribotyping and its discriminatory power was similar to PFGE. Multi-virulence-locus sequence typing also provided more discriminatory power for serotype 1/2a and 4b compared to multilocus sequence typing. Gendel and Ulaszek (2000) reported that 75% of *Eco*RI ribotypes were distributed nationally. This suggests that multiple strains of *L. monocytogenes* may occur frequently in the environment. Ribotyping provides a useful level of strain resolution and allows recognition of the relation between strains obtained over a long period of time.

Newer techniques (PFGE and ribotyping) provide much greater insight into the ecology of food operations and should provide guidance for improving the control of *L. monocytogenes* (Tompkin, 2002) and are the most common methods used in listeriosis outbreak investigations **(**Pagotto et al., 2004).

As mentioned before, advantages of ribotyping include 1) it is a completely automated system (thus standardization of experimental procedure) and 2) it is commercially available. Swaminathan et al. (1996) concluded that ribotyping satisfies two of the three requirements to qualify as a good subtyping method; typeability and

reproducibility. Pulsed field gel electrophoresis typing is more discriminatory but is more technically demanding and time consuming (Gray et al., 2004).

Different researchers have classified different serotypes into Lineage I or Lineage II, and sometimes Lineage III. For the most part they are consistent, however there is some disagreement on which lineage certain serotypes should fall.

Mereghetti et al. (2002) reported segregation of 130 epidemiologically unrelated strains using ribotyping and random multiprimer DNA analysis. Lineage I had 48, 20, and 48 strains in 1/2a, 1/2b and 4b, respectively. Lineage II had 6, 1, 5, and 2 strains in 1/2c, 3a, 3b, and 4a, respectively. In their work, there was a significant relationship between Lineage I and human strains, suggesting that strains from Lineage I are better adapted to human hosts.

Nadon et al. (2001) reported on 235 isolates (human isolates – 161; animal isolates – 72; and food isolates – 2), the majority being identified as serotype 1/2a (33%), 1/2b (17%) or 4b (40%). Ribotyping differentiated the isolates into 24 distinct ribotypes (8 in Lineage I; 10 in Lineage II; and 6 in Lineage III) (Table 4).

With ribotyping and PCR-restriction fragment length polymorphism typing, Norton et al. (2001) showed a much higher proportion of human isolates (69.1%) than industrial isolates (36.8%) classified in Lineage I. All other industrial isolates (63.2%) were classified as Lineage II.

Throughout the world, of the thirteen known serotypes of *L. monocytogenes*, three serotypes (4b, 1/2a, 1/2b) account for 89-96% of cases of human listeriosis (Tompkin, 2002; McLauchlin, 1990; Mereghetti et al., 2002;) providing additional evidence that certain strains are more likely to cause illness. Variability in virulence within the species

L. monocytogenes is slowly becoming recognized. It's important that differences in virulence among different subtypes be understood (Bruhn et al., 2005). Regarding *L. monocytogenes* it has been stated that "most strains are pathogenic, some strains may be pathogenic, and some strains are non-pathogenic" (Tompkin, 2002). More interesting is the realization that a small number of clonal lineages have been responsible for large documented outbreaks in different regions of the world. (Tompkin, 2002; Mereghetti et al., 2002). Gray et al. (2004) reported that lineages I and III were significantly associated ($P<0.0001$ and $P<0.01$, respectively) with isolation from humans and lineage II was significantly associated ($P<0.0001$) with isolations from foods.

Economic Impact to the Industry

The economic impact to the industry for product recalls because of confirmed or potential contamination of *L. monocytogenes* is tremendous. Product may be recalled because of a *L. monocytogenes* positive finding in a meat sample in a RTE facility or in other sectors of the food production chain or because of an outbreak of listeriosis and the confirmed or suspected association with a RTE meat product (Table 5).

Many facilities voluntarily hold product that is sampled (whether for internal or regulatory needs) until a negative result is confirmed; therefore, the actual amount of product being retained and reworked/destroyed and the associated cost to the industry is significantly higher.

CHAPTER III

INCIDENCE AND CHARACTERIZATION OF LISTERIA MONOCYTOGENES IN THE PROCESSING ENVIRONMENT OF FULLY COOKED, READY-TO-EAT BEEF PRODUCTS PHASE I

Abstract

Five commercial ready-to-eat (RTE) facilities were divided into four zones based on the contamination risk to the RTE product being produced. These zones include: Zone 1 (final slicing/packaging of RTE products); Zone 2 (common areas including hallways and cooling areas); Zone 3 (raw material receiving and storage); and Zone 4 (cafeteria and dry storage). In this investigation, environmental samples (n = 6,030) were collected from pre-selected, non-product contact sites in RTE facilities located in diverse geographical regions of the U.S. Test facilities produced a variety product types (i.e. hot dogs, roast beef, sliced luncheon meats, pepperoni, tacos, taco meat, pastrami and pizza toppings). Samples were sent to an outside laboratory for *Listeria* analysis and ribotyping. *Listeria monocytogenes* was present in 13% and *L.* spp. in 27% in environmental samples. There were 19 different ribotypes identified from the 600 selected isolates. The predominant ribotypes in this study were DUP-1039, DUP-1052, DUP-1059 and DUP-1062. In general, based on data from these five facilities, the industry must continue to focus on floors, drains and traffic patterns to maintain control of *Listeria* and reduce the potential for cross contamination. *L.* spp. control must be

implemented, monitored and maintained in adjoining areas to the processing facility to maintain adequate control in the RTE area.

Key Words: *Listeria monocytogenes*, Zones, Ribotypes

Introduction

In the processed meat industry, whether it be roast beef, corned beef, pizza toppings, wieners, sausages, tacos, meat loaf, meat balls, etc., personnel must be constantly mindful of the risk of *L. monocytogenes* contamination of the product between cooking and packaging. It is industry's responsibility to produce products that meet the customers' demand (quality and value), but most importantly, are safe to consume. When a story reveals a situation where there has been sickness or loss of life, industry professionals are personally saddened for the families affected and are aware that trust in the industry has been compromised. Somehow, the protective systems put in place have failed. The processed meat industry must be ever vigilant to discover ways to make food production systems more resistant to the risk of producing contaminated product.

In continued search of ways to understand and control *L. monocytogenes* in the processing environment, this study was designed to give added understanding of the subtypes of *L. monocytogenes* present in five processing locations that would encompass many types of meat products produced. The team of investigators also wanted to observe incidence at different times of production during the day. In addition, the team looked at the effectiveness of gathering information, formulating corrective actions based on that information, and then followed up to quantitatively evaluate the impact of those corrective actions.

The overall objectives of Phase I of this study were to:

- Identify critical entry points of *L. monocytogenes* in ready-to-eat (RTE) facilities.
- Establish *L. monocytogenes* as an environmental contaminant by zones of the facility.
- Compare the *L.* spp. present in different zones of the facility.
- Evaluate and compare the ribotypes of the *L. monocytogenes* confirmed samples and identify source to enable control of the environment based on the results.

Materials and Methods

Sample Collection

Environmental samples were collected from five commercial RTE facilities located in diverse geographical regions. The test facilities were selected based on the variety of products produced to maximize product types and thereby represent a cross section of the products produced industry-wide. Two of the five facilities were in the process of extensive expansion projects (construction) during the sampling visits. To maintain confidentiality, the facilities were coded with the following names: Uniform, Victor, X-ray, Yankee and Zulu. The RTE beef items produced at the facilities included hot dogs, roast beef, sliced luncheon meats, pepperoni, tacos, taco meat, pastrami and pizza toppings.

Samples, taken at 134 sites, were collected from three separate sample dates from each facility at approximately thirty-day intervals between each sampling date. Additionally, on each of the fifteen sampling dates (5 plants x 3 sampling dates), samples were collected at pre-operational (prior to start of operations), operational (at the end of

first shift) and end-of-production (latter part of second shift) times. This sampling plan enabled data collection and review from a cross section of the environment over a sixteen hour production span.

Each facility was divided into four zones based on the contamination risk to the RTE product. The sample sites (n = 134) were selected prior to each facility visit. The selected sites were sampled for *L. monocytogenes* and *L.* spp. at each of the three sample times (pre-operational, operational and end) resulting in 402 samples collected per plant visit for a total of 6,030 (5 plants x 3 sample dates x 3 sampling times each day x 134 sites).

The zones were identified as follows:

- Zone 1: Final slice/packaging areas and coolers storing RTE products
- Zone 2: Common areas (RTE and raw may cross), hallways and cook areas
- Zone 3: Raw material storage and processing areas and coolers
- Zone 4: Cafeteria, welfare facilities, dry storage and maintenance

The corresponding percentages of samples collected from each zone were as follows: Zone 1 – 40%, Zone 2 – 30%, Zone 3 – 20% and Zone 4 – 10%.

Due to regulatory implications, only non-product contact surfaces were selected for sampling. The sites included areas such as drains, floors, refrigeration units, curbing, framework of conveyors, forklifts, pallet jacks, etc. Areas that had compromised surface integrity thereby making the sites "difficult to clean" were selected and sampled.

Samples were collected aseptically using a Hydra-Sponge^{TM} (Biotrace International, Inc., Bothell, Washington), with sterile glove. The sample size for each environmental sample was a minimum area of 4.7 cm by 4.7 cm. The sampling protocol

was established to be aggressive in finding the sites for potential positives, if there was *Listeria* present. Therefore, areas considered to be harborage sites were selected and sampled as determined by the sampling team and plant management.

Samples were shipped via overnight express carrier in pre-chilled insulated coolers packed with commercial ice substitutes and sent to Food Safety Net Services, Ltd. in San Antonio, TX, for microbiological analyses. Upon receipt, all containers were examined for any evidence of handling or temperature abuse (samples > 4ºC) during shipping. While procedures were in place to discard samples that had been temperature abused, all samples were maintained at < 4ºC and were used in the analyses described below.

Microbiological Analyses - Listeria monocytogenes

Detection of *L. monocytogenes* from foods and food processing environments can be difficult because the bacterium is normally found in very low numbers in a heterogeneous population of microbes (Bruhn et al., 2005). Therefore, an enrichment procedure is necessary. For this project, 200 ml of University of Vermont Medium (UVM) enrichment broth was added to the environmental sponge samples and stomached at medium speed for 2 min. This primary enrichment was incubated 24 ± 2 hours at 30°C. Post incubation, 0.1 ml of the UVM primary enrichment was transferred to 10 ml of Fraser broth with Ferric Ammonium Citrate (Sigma Chemical Company, St. Louis, MO) additive. The Fraser tubes were incubated at 35° ± 2°C for 24 ± 2 hours or 48 ± 2 hours (if the Fraser tube was negative at 24 hours, it was re-incubated for an additional 24 hours and interpreted at 48 hours before reporting as a negative). Positive Fraser broth

tubes were streaked to MOX plates, incubated at 35°C for 24 or 48 hours, and examined for typical growth. Typical colonies were analyzed for hemolysis reaction on blood agar, Beta-lysin reaction, motility, gram stain characteristics, catalase reaction and carbohydrate fermentation. A Modified Oxford (MOX) plate was also inoculated directly from the UVM primary enrichment broth and observed as above, and run concurrently with the MOX plated from positive Fraser broth reactions. *Listeria monocytogenes* incidence data were compiled and expressed as the percentage positive for specific environmental surfaces.

Ribotyping

Molecular subtyping methods can help identify plant specific *L. monocytogenes* contamination routes and provide information for corrective actions and control strategies (Thimothe et al., 2004). For the purpose of this baseline study, ribotype identification was determined to enhance the overall knowledge of type of *L. monocytogenes* in the processing environment.

Six hundred isolates confirmed as *L. monocytogenes* positive were selected for ribotype identification. Ribotype patterns were obtained with the Qualicon Riboprinter® Microbial Characterization System (DuPont Qualicon; Wilmington, DE), following manufacturer's instructions (Qualicon, 1998). Cells were grown overnight on tryptic soy agar plates at 37°C, and isolates were transferred from the plates to a lysis buffer. Cells were inactivated by incubation at 90°C for 10 min. and placed in the Riboprinter. The Riboprinter then lysed the cells, carried out an *Eco*RI restriction digestion (*Eco*RI was used as the restriction enzyme) of the chromosomal DNA, separated the restriction

fragments by agarose gel electrophoresis, transferred the fragments to a nylon membrane, probed the membrane with a chemiluminescent ribosomal probe, and recorded the riboprint image produced.

Comparison and classification of the riboprint patterns were carried out using pattern-matching software included in the Riboprinter system. Each *Eco*RI pattern was compared to a library of ribotype patterns supplied by Qualicon (the DUP-ID library) and identified by the most similar library pattern. To obtain an identification, the similarity between the test isolate and the database isolate must be > 0.85[1]. Independently, each isolate's ribotype pattern was also compared to all of the other isolate patterns produced using the same restriction enzyme. These isolate-to-isolate comparisons were used to define ribogroups, each consisting of those patterns that were $\geq$ 0.90[1] similarity to the group average pattern.

Results and Discussion

For purpose of analyses and discussion, the incidence (% positive) rates of *L. monocytogenes* and *L.* spp. were evaluated. These data for *L.* spp. included the samples that tested positive for *L. monocytogenes. L. monocytogenes* was present in 13% and *L.* spp. in 26% of the 6,030 environmental samples analyzed.

An aggregate of *Listeria* specie (*L.* spp.), and more specifically *L. innocua*, is an important indicator of increased risk of *L. monocytogenes* (Miettinen et al., 2001). Thimothe et al. (2004) demonstrated a positive relationship between *L.* spp. in the environment to *L. monocytogenes* in the environment ($P < 0.0005$) and *L. monocytogenes*

[1] Simpson's diversity index: A mathematical measure that characterizes species diversity in a community. The proportion of species I relative to the total number of species (pi) is calculated and squared. The squared proportions for all the species are summed and the reciprocal is taken.

in finished product (P = 0.031). It is crucial for industry to be responsive to all *L.* spp. positives and develop control plans to reduce the incidence of environmental positives, thereby reducing the likelihood of contamination in RTE products.

Figure 1 shows the incidence of *L. monocytogenes* and *L.* spp. for all plants segmented by visit. The range in percent positive among the five plants was 10 to 18% for *L. monocytogenes* and 25 to 27% for *L.* spp. It should be mentioned that this information represents all zones included in the study. Overall, there was a reduction in incidence of *L. monocytogenes* from visit 1 to visits 2 and 3. There was 18% incidence on visit 1 and it was reduced to 10% and 11% on visits 2 and 3, respectively**.** Although changes were made in cleaning and sanitizing, due to the limited time period between sampling visits 2 and 3, the facilities did not have an adequate opportunity to review results and develop formal corrective action plans and implementations.

Figures 2 through 6 show the incidence of *L. monocytogenes* and *L*. spp. for each plant segmented by visit. There appeared to be a relationship between control of *Listeria* and the simplicity of the operation. The facilities with less product complexity, and therefore fewer HACCP plans had lower incidence of *L. monocytogenes* and *L.* spp. on all three sampling visits (i.e. X-ray and Yankee). Additionally, Yankee was undergoing an expansion (construction) project during each sampling visit. These data confirm the need for individual facilities to develop specific baseline data and control programs. Upon completing the baseline study, the facility can establish a history and incidence rate trends unique to the operation and corrective action plans to address increased incidence levels.

Figure 7 shows the incidence of *L. monocytogenes* and *L.* spp. for all plants combined segmented by sampling time. As expected, there was an increase in the incidence of *L. monocytogenes* and *L.* spp. from the pre-operational samples proceeding throughout operational samples to samples taken at the end-of-operations. Operational samples were those collected at least six h after start of operations for first shift and end samples were collected at least six h after the second shift operation began. These data suggested the level of *Listeria* detected during first and second shift operations reached a plateau during operations and remained relatively constant through production at the end of the day. This observation may be the result of excessive water usage on between-shift wash downs or sanitizing, which should be further explored.

Figures 8 through 12 show the incidence of *L. monocytogenes* and *L.* spp. for each individual plant segmented by sampling time. The association between the complexity of the facility and the incidence of *L.* spp. appeared to be consistent with previous discussions when evaluating data by sampling time. The more complex plants with a higher number of HACCP plans had higher levels of *L.* spp. in the environment. In general, regardless of the level of *L.* spp. during pre-operational sampling, the incidence of *L. monocytogenes* and *L.* spp. increased approximately 1.5 times in the subsequent sampling times (operational and end).

Figure 13 shows the incidence of *L. monocytogenes* and *L.* spp. for all plants stratified by zone. As expected, the highest incidence levels were from Zone 3, which were areas associated with raw material cooler storage and raw processing areas. Typically, facilities concentrate and sample only in Zone 1 (RTE areas). However, it became evident that facilities need to further concentrate sampling efforts and control

Listeria in the adjoining areas of the facility to improve control in the RTE zones. These data suggested this pathogen was being tracked into Zone 1 from the surrounding areas of the facility and/or the *L.* spp. present in Zone 1 was being spread and increased within the RTE areas throughout the production day. Although most plants had "barriers" in place to prevent the spread of *Listeria* into the RTE areas, cross contamination seems to be occurring. This was consistent with Lunden et al. (2003) that observed "the separation of raw and post-heat treatment areas seemed especially important in contamination status of post-heat treated lines.

In a study conducted by Thimothe et al. (2004), a *L. monocytogenes* subtype was found on an employee's apron in the raw area and was also found on a door handle leading into the finished product area. Subtyping was able to show a specific example where control of traffic between raw and finished product areas may have been compromised. In addition to describing *L. monocytogenes* transmission pathways, that study showed ribotyping could be used as a valuable tool to depict contamination pathways and implement improved *Listeria* control measures.

Figures 14 through 18 show the incidence of *L. monocytogenes* and *L.* spp. for each plant segmented by zone. On a plant-by-plant basis, there were substantial differences in the level of *L. monocytogenes* versus *L.* spp. For example, the level of *L. monocytogenes* and *L.* spp. reported at facility X-ray (Figure 16) were similar at each Zone versus facility Zulu (Figure 18), which had a much higher proportion of *L.* spp. incidence versus that of *L. monocytogenes*. This may be an indication of better overall *Listeria* control (X-ray) because the *L. monocytogenes* incidence comparing the two is similar, but X-Ray had much incidence of *L*. spp. (fewer competitive organisms).

Therefore, perhaps as *Listeria* is controlled in the environment, the incidence of *L. monocytogenes* and *L.* spp. equilibrate to the same level.

Figure 19 illustrates the incidence of *L. monocytogenes* and *L.* spp. for all plants evaluating sampling time segmented by zone. Overall, pre-operational, Zone 1 samples had the lowest incidence rate for *L. monocytogenes* and *L.* spp. However, over the sampling time, Zones 1, 2 and 4 appeared to equilibrate to similar levels. As expected, Zone 3 (raw product areas) had the highest incident rate of *L. monocytogenes* and *L.* spp. at all three sampling times.

Figures 20 through 24 illustrate the incidence of *L. monocytogenes* and *L.* spp. for each individual plant stratified by sampling time and zone. The general trend for all tested facilities was Zone 3 remained the highest at all three sampling times. Additionally, there was a trend pre-operational sampling had the lowest incidence rates with the incidence increasing with the operational and end sampling times. These data reinforce the idea of facilities with fewer HACCP plans, thereby less product mix and changeovers, seem to maintain better control of environmental *Listeria* consistently throughout all zones of the facility (Figure 22, Yankee and Figure 24, X-Ray). Furthermore, these data suggest that over time there was not much differentiation between Zones 1, 2 and 4 as the incidence rates came together as the day progressed. Therefore, although the facilities maintain Zone 1 as a "protected" RTE area, there was not a substantial difference in the levels of *Listeria* in Zone 1, 2 and 4 during operational and end-of-day sampling.

Figure 25 illustrates data compiled for the number of positive *L. monocytogenes* and the distribution of the positives by sample site category. In general, based on these

data from the five facilities, continued emphasis should be focused on floors, drains and traffic patterns to maintain control of *Listeria* and reduce the potential for cross contamination. There were some notable sampling sites that were repetitively positive throughout many facilities (i.e. trench drains, restrooms, laundry areas, floors in and around hand wash facilities, ladders, forklifts/pallet jacks, and QA carts). An investigator may be interested in sites with such repetitive positives in that there may have been a niche microorganism colony established there. There were no positives found from Zone 1 on condensate or drip pans from air handling units.

Figure 26 illustrates the ribotypes for all plants by DUP identification number. There were 19 different ribotypes identified from the 600 selected isolates. The isolates were selected with the same percentage weighting by zone as the sample site selection. The diverse numbers of ribotype groups were dominated by DUP-1039, DUP-1052, DUP-1059 and DUP-1062. Three facilities (Uniform, Victor and Zulu) had DUP-1039 and DUP-1052 as the dominant ribotypes. These three facilities were the most complex based on product mix and number of product types. Although the two remaining facilities, X-ray and Yankee, had notable levels of DUP-1039 and DUP-1052, they were dominated by DUP-1059 and DUP-1062, respectively.

Figures 27 through 31 show the number of each ribotype for individual plants segmented by sampling time. The predominant ribotypes at pre-operational sampling remained predominant and increased throughout the production day.

Figures 32 through 37 illustrate the number of each ribotype for all plants (total) and each plant individually by zone. In the compilation of all plants, Zone 1, 2 and 3 had similar numbers of ribotypes identified (i.e. DUP-1039, DUP-1052, DUP-1059 and

DUP-1062). Zone 4 had the lowest number of different ribotypes identified at each individual facility. The dominant ribotype for each facility was noted in all four zones.

Figure 38 shows a comparison of percentage ribotypes from this study to others reported in the literature. The bars designated as "Environmental" represent the percentage ribotypes from the 600 *L. monocytogenes* positive isolates typed in this study. The "Industrial Fish" bars represent 117 ribotypes reported by Norton et al., 2001. The "Smoked Salmon" bars represent 72 ribotypes reported by Gendel and Ulaszek, 2000. The bars depicting 275 human isolates were also reported by Norton et al., 2001. These human isolates include the World Health Organization's *L. monocytogenes* strain collection and other isolates from patients with listeriosis symptoms. The predominant ribotype (DUP-1039) in this study was comparable to the industrial and smoked fish isolates. DUP-1039 was shown to be persistent in fish processing facilities for at least 6 months (Norton et al., 2001). That study also indicated that the ribotypes that persisted in the processing facility were significantly more prevalent among industrial isolates than among human isolates. Norton et al. (2001), showed a predominance of DUP 1039c (21.4%) and DUP 1045 (13.7%) out of 117 isolates from the fish processing industry.

The plant ribotypes in this study, DUP-1039, DUP-1052, DUP-1059 and DUP-1062 were more prevalent than among those predominantly found in human isolates. The predominate ribotypes from human isolates are DUP-1038 and DUP-1042 (almost 40%) (Norton et al., 2001). Only one DUP-1038 isolate and three DUP-1042 isolates were identified in this study. These four isolates were from 3 different processing plants, underscoring the fact that the human illness 4b serotypes were very rare in this study.

Ribotyping and virulence gene allelic analyses have been shown to subdivide *L. monocytogenes* into three lineages that may differ in pathogenicity (Nadon et al., 2001). Referring to Table 4, Nadon et al. (2001) reported Lineage I contains all the 4b and 1/2b serotypes, whereas Lineage II is composed primarily of 1/2a and 1/2c serotypes. Lineages I and II also correspond to the primary divisions of *L. monocytogenes* by multilocus enzyme electrophoresis (MEE) and pulsed-field gel electrophoresis (PFGE). Lineage III (the predominant Lineage for one plant) is a distinct taxonomic unit. No human isolates were classified into Lineage III. Based on data published by Nadon et al. (2001), 59.6% of the ribotypes in their study fell into Lineage I, 36.2% into Lineage II, 11.5% Lineage III. Using the riboprints/genetic lineage correlation from that same study and comparing to the riboprints reported in this study, we found the distribution was 28.7%, 56.9% and 11.3% among Lineage I, Lineage II and Lineage III, respectively (Figure 39). There were 3.0% of the *L. monocytogenes* positive samples that fell into a ribotype of unknown lineage. DUP-1039, the most prevalent riboprint, accounted for 75.4% of the Lineage II isolates.

Conclusions

The aggressive nature of the sampling plan utilized in this study combined with sampling from all areas of the plant led to notable conclusions for processing facilities. The current industry practice is to establish *L.* spp. control programs for the RTE Zones of the facility. Based on the information from this study, strict control in the RTE area alone was not sufficient. *L.* spp. control must be implemented, monitored and maintained in adjoining areas of the processing facility to maintain adequate control in the RTE area.

This study confirms the need for individual facilities to develop specific baseline data and control programs. Upon completing the baseline study, the facility can establish a history and incident rate trends unique to the operation and corrective action plans to address increased levels. Furthermore, there was a substantial correlation between complexity of the operation and control of *L. monocytogenes.* These data suggest the more complex operations, with multiple HACCP plans (multiple product types), have higher incidence rates of *L.* spp. throughout all zones in the facility. These data from this study support the previous hypothesis of Dr. Wiedmann's group from Cornell University that at least some *L. monocytogenes* subtypes from meat processing plants may have limited human-pathogenic potential (Saunders et al., 2004).

CHAPTER IV

INCIDENCE AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* IN THE PROCESSING ENVIRONMENT OF FULLY COOKED, READY-TO-EAT BEEF PRODUCTS PHASE II

Abstract

Three commercial ready-to-eat (RTE) facilities were divided into four zones based on the contamination risk to the RTE product being produced. These zones include: Zone 1 (final slicing/packaging of RTE products); Zone 2 (common areas including hallways and cooling areas); Zone 3 (raw material receiving and storage); and Zone 4 (cafeteria and dry storage). In this investigation, environmental samples (n = 3,000) were collected from pre-selected, non-product contact sites and analyzed for *L. monocytogenes* and *L.* spp. All *L. monocytogenes* positive samples were evaluated for ribotype identification (DUP number). Raw material meat samples and air samples were collected at each facility and evaluated for *L. monocytogenes. Listeria monocytogenes* was present in 9% and *L.* spp. in 17% of the environmental samples. From the raw material samples (n = 321) collected, 25% and 41% were positive for *L. monocytogenes* and *L.* spp., respectively. Seventeen different ribotypes were identified among the 545 environmental isolates tested. Two, designated DUP-1039 and DUP-1052, dominated the isolate pool. Thirteen different ribotypes were identified from the 80 raw meat isolates evaluated, with DUP-1039 and DUP-1052 comprising 45% and 21% of the

isolates, respectively. *Listeria monocytogenes* was not recovered from any of the air samples collected.

Key Words: *Listeria monocytogenes*, Zones, Ribotypes

Introduction

In the processed meat industry, whether it be roast beef, corned beef, pizza toppings, wieners, sausages, tacos, meat loaf, meat balls, etc., personnel are constantly mindful of the risk of *L. monocytogenes* contamination of the product between cooking and packaging. It is industry's responsibility to produce products that meet the customers' demand (quality and value), but most importantly are safe to consume. When a story reveals a situation where there has been sickness or loss of life, industry professionals are personally saddened for the families affected and are aware trust in the industry has been compromised. Somehow, all the protective systems put in place have failed. The processed meat industry must be ever vigilant to discover ways to make food production systems more fool proof and more resistant to the risk of producing contaminated product.

Based on the critical entry points for *L. monocytogenes* identified in Phase I, plant specific corrective actions were established and implemented to improve its control in ready-to-eat products. Additionally, a common perception (or misperception) in the industry is that a source of contamination is the air. The investigation team wanted to evaluate whether air flow was a particular threat to safe food production. In addition, the team wanted to evaluate the effect of different ribotypes found in the raw material might have on the population of subtypes found in the other "zones" of the plant.

The overall objectives of Phase II of this study were to:

- Based on the critical entry points for *L. monocytogenes* identified in Phase I, establish and implement plant specific corrective actions to improve the control of *L. monocytogenes* in ready-to-eat beef products.
- Evaluate air samples in ready-to-eat facilities for the incidence of *L. monocytogenes.*
- Identify the incidence rates and ribotypes of *L. monocytogenes* in the raw materials utilized in ready-to-eat products.

Materials and Methods

Sample Collection

In the second phase, processing plants Victor, Yankee, and X-Ray were selected primarily because it appeared that we could adequately satisfy the objectives of Phase II and they were close in geographical proximity to each other. There were additional factors involved in the decision that will be discussed in association with each plant.

Environmental samples were collected from three commercial processing facilities producing ready-to-eat (RTE) beef products. Test facilities were selected based on the variety of products produced to maximize product types and represent a cross section of the products produced industry-wide. The beef, ready-to-eat products produced at these facilities included roast beef, sliced deli meats, taco meat, tacos, meatballs, taquitos, pizza toppings and pepperoni.

Phase I was instrumental in confirming *L. monocytogenes* as an environmental contaminant in the plants sampled. From the samples collected, the predominant areas of opportunity were classified in three general categories: Sanitation, Traffic Patterns, and

Segregation of Raw and RTE areas. Samples were collected during two sample visits to each facility. The time period between the first and second sampling dates was approximately sixty days. The first sampling visit was conducted to re-establish baselines for the facilities due to facility and product modifications in the past twelve months. After the first sampling visit (approximately 15 days), data were presented and corrective action plans were discussed with key management personnel. Each establishment was given approximately forty-five days to implement the corrective actions prior to the second sampling visit, which consisted of collecting samples on two consecutive production dates. The time of day that samples were taken (pre-operational, operational and end-of- production) were identical to those of Phase I.

As in Phase I, each facility was divided into four zones based on the contamination risk to the ready-to-eat product by the environment and employees. A total of 195 sample sites were selected prior to each facility visit. The selected sites were sampled at each of the three sample times (pre-operational, operational and end) for a total of 585 samples collected on each of three sampling days.

The definition of zones and sampling protocol were identical to that of Phase I. Sample sites that were repetitively positive for *L. monocytogenes* in Phase I were intensified and additional sites selected in those areas to further identify and quantify the incidence of this pathogen in those areas. Employee boots were added as a site in Phase II.

Air samples were collected at each of the three sample times (pre-operational, operational and end) and evaluated for *L. monocytogenes*. The sample sites were selected based on employee traffic patterns and product flow as well as air movement patterns

throughout the facility to isolate the potential of contamination from different areas of the plant into the ready-to-eat zones. For each site tested, an air sample volume of 200 liters was collected using the MAS™ 100 Eco Air Sampler (EM Science, Gibbstown, NJ). The microbial air sampler was loaded with 90 mm petri plates containing selective agar media.

Raw meat samples were collected at each of the three participating plants to quantify the incidence rate of *L. monocytogenes* upon receipt at the establishments. Approximately 320 samples were analyzed for *L. monocytogenes.* Excised tissue samples were collected aseptically from the raw meat and deposited in a 10 X 20 cm Whirl-Pak® bag (Nasco, Fort Atkinson, WI).

Samples were shipped via overnight express carrier in pre-chilled insulated coolers. The coolers were packed with commercial ice substitutes and sent to Food Safety Net Services, Ltd. in San Antonio, TX, for microbiological analyses. Upon receipt, all containers were examined for any evidence of handling or temperature abuse (samples > 4ºC) during shipping. While procedures were in place to discard any samples that had been temperature abused, all samples were < 4ºC and were used in the analyses described below.

Microbiological Analyses – *L. monocytogenes*

Procedures for microbial analysis of environmental samples were identical to those of Phase I. For the air plates, the selective solid media was processed using FSIS-USDA recommended procedures (FDA, BAM) for the evaluation of *L.* spp. *Listeria*

monocytogenes incidence data were compiled and expressed as the percentage positive for specific environmental surfaces and raw meat samples.

Ribotyping

Nine hundred isolates that confirmed as *L. monocytogenes* were selected for ribotype identification. Ribotype patterns were obtained with the Qualicon Riboprinter Microbial Characterization System (Qualicon, Wilmington, DE), as in Phase 1.

Comparison and classification of the riboprint patterns were carried out using pattern-matching software included in the Riboprinter system. Each *Eco*RI pattern was compared to a library of ribotype patterns supplied by Qualicon (the DUP-ID library) and identified by the most similar library pattern. To obtain an identification, the similarity between the test isolate and the database isolate must be > 0.85[1]. Independently, each isolate's ribotype pattern was also compared to all of the other isolate patterns produced using the same restriction enzyme. These isolate-to-isolate comparisons were used to define ribogroups, each consisting of those patterns that had $\geq$0.90[1] similarity to the group average pattern (Qualicon. 1998).

Results and Discussion

Environmental

For purposes of analyses and discussion, the incidence (% positive) rates of *L. monocytogenes* and *L.* spp. were evaluated. *L.* spp. included all samples that tested

[1] Simpson's diversity index: A mathematical measure that characterizes species diversity in a community. The proportion of species I relative to the total munber of species (pi) is calculated and squared. The squared proportions for all the species are summed and the reciprocal is taken.

positive for any species of *Listeria,* including *L. monocytogenes, L. innocua, L. welshimeri,* and *L. grayi.* The overall impact of this study on the three test plants from Phase I to Phase II was a 50% reduction in incidence of *L. monocytogenes* from 18% to 9% (Figure 40 and Figure 41). Figure 40 details both *L. monocytogenes* and *L.* spp. for the total and for each facility. It is evident that progress was made as a result of the corrective actions implemented during and after this study. *L.* spp. was reduced from 27% to 17% when combining all plants. The most drastic reduction occurred in facility X-Ray where incidence levels dropped from 18% (Phase I) to 4% (Phase II) for *L. monocytogenes* and from 23% (Phase I) to 9% (Phase II) for *L.* spp. Figure 41 shows the percent reduction in *L. monocytogenes* overall and for each facility. Significant reductions were achieved in all three facilities when comparing baseline data to data collected after corrective actions were implemented. The reductions for *L. monocytogenes* were 22%, 45%, and 74% for facility Victor, Yankee, and X-Ray, respectively.

Corrective Actions

Establishing corrective actions was the primary objective for this study. Autio et. al. (1998) reported from their study that after an eradication program (corrective actions) samples taken from critical contamination points determined by sampling during contamination analysis were all negative for *L. monocytogenes*.

The management team, with input from the sampling team, developed the corrective actions after close review of data and trends that were evident. The facility

implemented the corrective actions prior to the second sampling visit. The specific corrective actions developed for each facility are detailed below.

Facility Victor

Facility Victor was the facility selected to focus on sanitation issues and procedures due to the high incidence of *L.* spp. during pre-operational sampling. There were five specific corrective actions that were implemented in this facility to improve the control of *L.* spp. at pre-operational inspection and during the entire production day. In general, corrective actions were:

- Cleaning procedures for drains and floors were implemented and several areas that were damaged were repaired to facilitate proper cleaning.
- A boot policy was implemented for all persons entering the facility.
- Sanitizer floor foamers were installed in the main traffic hallway.
- Personnel entry was evaluated and modified to ensure that employees pass through a sanitizer foamer as they enter the production areas.
- Cleaning procedures for the spiral freezers were revised and implemented.

As listed above, the first area of concentration was floor and drain cleaning procedures. Additionally, there were several problem areas that due to their surface conditions, were not cleanable. Therefore, the problem areas were repaired to facilitate proper cleaning and then extensive cleaning procedures and training were implemented.

The next target areas were incorporated to minimize the organism entering the facility with employees. The initial step was implementing a rubber boot policy for all persons entering the facility. Secondly, floor foamers were incorporated and the

procedures for employee traffic at the start of the shift were modified. The combination of these items insured that personnel wore footwear capable of being cleaned and sanitized, and employees had to proceed through sanitizer foam as they entered the production area. Furthermore, the addition of the hallway foamers insured that the forklift and pallet jack traffic would pass through a sanitizer solution to minimize the contamination potential created by these vehicles.

The final focal point identified in facility Victor was the repetitive positive findings around the outside of the spiral freezers. The areas were typically in poor condition due to wear and tear of the concrete curbing and peripheral areas of the spirals. Therefore, intensive and targeted procedures were implemented to prevent and control harborage sites around the spiral freezer areas.

Facility Yankee

Facility Yankee was selected to study and better understand traffic patterns of personnel, equipment and product, therefore the corrective actions were:

- Re-implement a captive boot policy
- Utilize granular quaternary ammonia on the production floors
- Increase the number and volume distributed from floor sanitizer foamers
- Eliminate a personnel door between a raw area and a RTE room
- Implement segregation of raw and RTE personnel by adding separate welfare facilities for each department

A corrective action taken that was broad in scope was re-implementation of a captive boot policy. The captive boot policy was eliminated for a brief time, during

which plant personnel collected samples to evaluate the impact on the operational incidence of *L.* spp. as a result of the policy change. Figure 42 details the increase from 34% to 66% (in-plant data) in environmental percentage positives of *L.* spp. during operational sampling when the captive boot policy was eliminated. Upon noting the increase in percentage positive, the policy was re-instituted prior to the second sampling visit with a consequent drop to 51% incidence of *L.* spp.

Other "broad scope" corrective actions were the introduction of a granular quaternary ammonia product spread on the floors in production areas. Furthermore, the number of sanitizer floor foamers and the volume of sanitizer from each foamer throughout the plant were increased. It was evident that when operating properly, the floor foamers that personnel and equipment had to pass through were effective barriers against the spread of microorganisms throughout a processing facility. It is difficult to "pinpoint" the efficacy of these good manufacturing practices other than the overall reduction in percent incidence of *L. monocytogenes* and/or *L.* spp. when comparing Phase I and Phase II.

A corrective action that was more targeted in nature was the removal of a doorway between an area where raw product was present and a RTE area. The door removal was part of the construction and renovation between Phases I and II meant to eliminate foot traffic between the two areas which gave further distinction between raw and RTE areas of the plant. This action resulted in a 32% reduction of positive samples taken in that particular RTE area.

With the expansion project at facility Yankee, it allowed for separate welfare facilities for the RTE employees and employees in the raw storage/processing areas. This

separation reduced the potential for cross-contamination of personnel to influence the environment and ultimately the finished product.

Facility X-Ray was selected to explore separation of raw and RTE because this facility was initially designed to maintain separation of raw and RTE areas. In evaluating data from Phase I, this facility maintained more consistent control of the environment in all zones throughout the processing day. Due to the design and age of the facility, corrective actions were basically fine-tuning the procedures to improve control. The corrective actions implemented include:

- Improve housekeeping activities.
- Update the cleaning procedures and chemicals utilized for drains.
- Expand the cleaning program for pallet jacks and forklifts to include hand scrubbing with an alcohol based cleaner.
- Implement a cleaning program for control panels in the RTE area.

The housekeeping activities included the peripheral areas that often get missed and may contribute to the harborage of *L. monocytogenes*. Quaternary ammonium sanitizer was added to the mop buckets utilized for the cafeterias and breakrooms. Additionally, the employee entrance vestibule was sanitized on a daily basis. The drains continued to be a harborage area for *L. monocytogenes;* therefore, a more aggressive program was implemented for drain cleaning.

Due to repetitive findings in Phase I of forklifts and pallet jacks being positive, an expanded cleaning program was implemented at facility X-Ray. This procedure included hand cleaning using an alcohol based cleaner to prevent harborage areas on items being transported throughout the facility. Equipment control panels were a focus because

control panels are covered by production employees at the end of second shift (to protect them from water and chemicals during sanitation) and then first shift production uncovers and starts operations. Therefore, the control panels were not getting cleaned adequately. A thorough procedure was implemented to address the control panels to insure they were getting adequately cleaned on a daily basis. Facility X-Ray had the largest decrease in positives from Phase I to Phase II, with a 74% reduction in *L. monocytogenes* as a result of the implemented corrective actions.

Figure 43 illustrates combined data for the incidence of *L. monocytogenes* and *L.* spp. from all three facilities segmented by zone. Similar to Phase I, all facilities improved between the sampling visits. As expected, the highest incidence levels were from Zone 3, which were the raw processing areas and coolers for raw material storage. In evaluating all plants combined, Zones 1 and 2 were very similar in incidence rates for *L. monocytogenes* and *L.* spp., which indicates that the facilities need to continue to improve the control of contamination entering Zone 1 from the other zones.

Figures 44 through 46 show the incidence of *L. monocytogenes* and *L.* spp. for each plant individually segmented by zone. There were substantial differences between the facilities when evaluated by zone. Facility X-Ray, Visit 2 showed exceptional control of *Listeria* in Zones 1, 2 and 4 compared to the other facilities. Moreover, the improvement between Visit 1 and Visit 2 is evident in Figure 46. In comparing the incidence of *L. monocytogenes* and *L.* spp. between plants and visits, it is evident that there were vast differences between facilities. For example, facility X-Ray (Figure 46) had similar levels at each zone versus facility Victor (Figure 44), which had higher levels of *L.* spp. at all zones. Although facilities maintain Zone 1 as a "protected" area, there

was not a substantial difference in the levels of *Listeria* in Zones 1, 2 and 4. It appeared that as *Listeria* is controlled in the environment, the incidence of *L. monocytogenes* and *L.* spp. equilibrate to the same level.

Figure 47 shows the incidence of *L. monocytogenes* and *L.* spp. for all plants combined stratified by sampling time. As expected from Phase I, there was an increase in incidence of *L. monocytogenes* and *L.* spp. from the pre-operational samples to the operational and end-of-production samples. These data suggest the level of *Listeria* reached a plateau after the start of operations and remained constant throughout the remainder of the production day.

Figures 48 through 50 illustrate the incidence of *L. monocytogenes* and *L.* spp. for each individual plant stratified by sampling time. As discovered in Phase I, it appeared that facilities with more variety of products produced and therefore more complex operations, tended to have higher levels of *Listeria* in the environment and require stricter control systems. Complexity of operation seemed to be related to facilities that at one time, were privately owned and tried to produce a large variety of products to meet several customers' requests. By doing this, the number of lines and product variety continually increased, typically with plant additions and/or modifications that negatively impacted product flow and food safety.

It is important to note that the level observed at pre-op approximately doubled and remained at that level for operational and end-of-production time periods. This further confirmed the importance of effective and consistent sanitation procedures to start the production day with a low level of *Listeria.*

In addition to environmental samples, employee boot samples were obtained to evaluate and compare the incidence of *L.* spp. on RTE employee boots throughout the production shift. Figure 51 illustrates each plant and the incidence levels carried on the employee boots. Consistent with previous discussions, facility X-Ray had the lowest incidence from pre-operational, operational and end-of-production sampling. The pre-operational level was alarming to all of the management staff and further stressed the importance of an effective boot cleaning policy for all employees.

The investigators wanted to compare the incidence of *Listeria* on boot samples from management personnel and RTE personnel in the facility. Figure 52 illustrates the results with management having a substantially higher level over all sampling times than the hourly production employees. The hourly employees had 5%, 5% and 35% for pre-operational, operational and end-of-production sampling times, respectively. The management staff had incidence levels of 30%, 40% and 50% for the same time periods. This information reiterates the importance of procedures and policies for all employees. Typically, management personnel are more mobile and are exposed to a multitude of environments (raw and RTE) during the day versus an employee assigned to one production area. Therefore, training is critical and the policies for boot cleaning and sanitizing must apply to all personnel and visitors that enter the facility to prevent and reduce the contamination potential.

A measuring wheel was utilized to evaluate the distance that an organism could be transferred through a facility. The initial tracking sample was collected in a wet area that was known to contain *L.* spp. Samples were collected at the initial site and then in 0.6 m intervals up to 3 m, then in 1.5 m increments up to 9.0 m. *Listeria* was confirmed

at the 9.0 m sample; therefore, confirming the ability of the target organism to be transferred throughout the facility on wheels, boots, etc. Furthermore, a similar wheel tracking sampling was performed to evaluate the effectiveness of the sanitizer floor foamers. The samples collected after the foamers were negative for *Listeria* for at least 9.0 m from the floor foamer.

Air Samples

Air samples were collected at each facility for each sample time – pre-operational, operational and end-of-production. Sites included areas that had air movement into the facility from outside (i.e. dock areas) and transition areas between RTE and raw areas. Of the 90 samples collected, *Listeria* was not recovered in any of the air samples. This is consistent with a statement made by Dr. Bruce Tompkin, (2002) "a rather common misconception is that air is a notable source of contamination". Similarly, Autio et al. (1998) reported that air mediated contamination could not be proved.

Raw Material

Thimothe et al. (2004), showed a positive relationship between *L. monocytogenes* in raw material to *L. monocytogenes* in the environment (P = .027) and *L. monocytogenes* in the finished product (P = .038). However, it was reported that raw fish seemed not to be the most important factor in the contamination of cold-smoked rainbow trout (Autio et al., 1998) or in cold-smoked salmon (Vogel et al., 2001).

Unpublished industry data show *L. monocytogenes* present in 3.4% to 46% of raw beef and pork raw material samples (16.2% average). Figure 53 highlights the incidence

of *L. monocytogenes* and *L.* spp. in raw meat samples received at all plants combined and for each plant individually. The incidence was higher than anticipated with 25% positive *L. monocytogenes* and 41% *L.* spp. Facilities Victor and X-Ray were relatively consistent in incidence of *L. monocytogenes* and *L.* spp.; however, Facility Yankee had a significantly higher level in the raw meat. In agreement with Lunden, et al. (2003), these data confirm the importance of controlling the environment at all zones to prevent cross-contamination from the raw areas to the RTE areas of the facility since it is a source of the pathogen. The importance of the Zone concept is a way to facilitate a more targeted approach to control the environment and consequently reduce the risk of a *L. monocytogenes* finding a niche in a facility.

Ribotypes

Seventeen different riboprints were identified in Phase II of this project (Figure 54). The distribution of riboprints was similar to that seen in Phase I of this study. The two Phases represent a fall season and a summer season. Although the prevalence of *L. monocytogenes* in the environment is expected to be higher in the summer months the riboprint distribution does not appear to be seasonal. Two riboprints, DUP-1039 and DUP-1052, made up 63% of the total and these appeared to be very common in other industrial environments (Nadon et al., 2004). The same two riboprints (Figure 55) were also the most predominate in raw material samples.

The riboprint distribution within the RTE facility may be the direct result of the riboprints associated with the raw material. Another consideration would be that these riboprints were very common throughout all environments and simply predominate in

both the raw and cooked processing facilities. Nevertheless, for facilities Victor and Yankee, the riboprint "foot print" established in the raw, Zone 3 area seems to carry through the remainder of the facility and reinforce the importance of raw and cooked segregation. One exception is facility X-Ray, which was selected for this study because of its ability to better segregate raw and RTE based on Phase I data. The "footprint" for Zone 3 was not evident in Zone 1. Researchers in Denmark reported that as fish products move through a plant and the product form is changed, the strains of *L. monocytogenes* change (Vogel et al., 2001).

As reported in Phase I, the Phase II riboprints are predominated by isolates not normally associated with human illness. These data support the power of a tool like riboprinting to differentiate clonal variations more specific than biochemical testing or serotyping.

Riboprinting may be of limited value in the initial assessment of a facility's environment. If the sampling is extended from the RTE (Zone 1) to the other contiguous areas and there are two to three major riboprints, then the value of riboprinting is limited. Once the environmental programs are corrected and the incidence of *Listeria* is greatly reduced, the discriminating power of riboprinting to find common sources, track movement and determine niches becomes a powerful tool.

Conclusions

Overall, after implementation of corrective actions, *L. monocytogenes* was present in 9% while *Listeria* spp. were detected in 17% of the environmental samples, compared to 18% and 27% in Phase I, respectively. The overall impact of this study on the three

test plants from Phase I to Phase II was a 50% reduction in incidence of *L. monocytogenes* from 18% to 9%.

It is imperative for the industry to react and take appropriate corrective actions on all *L.* spp. positive findings. The first step in *L. monocytogenes* control is to get a true assessment of the environment by performing mapping studies. This allows the investigators to develop a plan for improvement. Furthermore, it is crucial to focus on the entire facility in implementing a *Listeria* control program. The goal is to prevent a niche through proper design, cleaning, sanitizing, and repair, which should reduce the risk of *L. monocytogenes* finding a harborage site.

The riboprint distribution within the RTE facility may be the direct result of the riboprints associated with the raw material. As reported in Phase I, the Phase II riboprints are predominated by isolates not normally associated with human illness. These data support the power of a tool like riboprinting to differentiate clonal variations more specific than biochemical testing or serotyping.

General corrective actions that should be evaluated by all facilities in the industry include:

- An aggressive drain cleaning and maintenance program for the entire facility, including the raw zones of the facility.
- Implement a captive boot policy, which includes adequate cleaning procedures to be followed at the end of each shift prior to storing boots.
- Installation of properly functioning door foamers, which serve as an effective barrier to reduce the potential spread of contamination through the zones of the facility.

- Housekeeping, which at times is overlooked, is key in all areas and needs to be focused on.
- Control systems and cleaning systems need to be in place for any item that moves from area to area – including people, ladders, forklifts, pallet jacks, carts, dollies, etc.

Table 1: USDA, FSIS Microbial Testing, 2002.

Product Category	% Prevalence for *L. monocytogenes*
Peeled sausage	1.72%
Unpeeled sausage	0.63%
Sliced, diced and shredded	1.96%
Small mass chopped & formed	1.05%
Salads and spreads	0.42%
Small mass whole muscle	0.77%
Large mass whole muscle	0.49%
Large mass chopped & formed	0.93%

Source: FSIS, Microbial Testing Program

Table 2: Yearly Summary of FSIS Random Sampling Program:

Year	% *Lm* Positive
1995	3.02
1996	2.91
1997	2.25
1998	2.54
1999	1.91
2000	1.45
2001	1.32
2002	1.03

Source: FSIS Press Release, Oct. 17, 2003

Table 3: Prevalence (%) of *L. monocytogenes* in RTE Meat and Poultry Products, 1990 through 1999.

Year	Cooked, Roast, Corned Beef	Sliced ham and luncheon meat	Small cooked sausages	Large cooked sausages	Jerky	Cooked poultry products	Salads/ Spreads/ Pates	Fermented sausages
1990	6.38 (22/345)[a]	7.69 (1/13)	4.21 (13/309)	5.32 (5/94)	0.00 (0/25)	2.79 (12/240)	5.48 (19/347)	N/A[b]
1991	4.02 (20/498)	5.48 (4/73)	7.24 (28/387)	4.6 (12/261)	0.00 (0/39)	2.62 (17/649)	3.17 (15/473)	N/A
1992	3.86 (19/492)	7.89 (9/114)	6.03 (21/348)	0.42 (1/239)	0.00 (0/19)	2.01 (7/349)	3.32 (8/241)	N/A
1993	3.04 (13/428)	8.05 (12/149)	5.3 (25/472)	2.13 (7/328)	0.00 (0/39)	1.91 (6/314)	2.19 (6/274)	N/A
1994	2.09 (10/479)	5.46 (13/238)	4.81 (29/603)	1.14 (5/438)	2.22 (1/45)	2.37 (13/549)	2.41 (14/580)	N/A
1995	2.68 (15/560)	5.00 (5/100)	4.09 (25/611)	1.14 (5/438)	0.00 (0/50)	2.25 (20/889)	4.69 (28/597)	N/A
1996	3.35 (17/507)	7.69 (7/91)	3.74 (21/561)	0.95 (4/420)	0.00 (0/43)	3.17 (28/883)	2.17 (12/554)	N/A
1997	2.08 (11/530)	4.20 (12/286)	2.74 (17/621)	1.62 (6/371)	0.00 (0/40)	0.95 (9/946)	2.43 (5/206)	9.26 (10/108)
1998	2.15 (11/511)	4.18 (11/263)	3.49 (26/746)	1.19 (6/506)	1.56 (3/192)	2.22 (19/857)	3.11 (1/225)	2.87 (7/244)
1999	2.71 (25/922)	4.58 (44/960)	1.76 (38/2162)	0.43 (5/1167)	0.00 (0/278)	1.44 (14/970)	1.15 (5/435)	2.09 (10/478)
Cumulative	3.09 (163/5275)	5.16 (118/2287)	3.56 (243/6820)	1.31 (56/4262)	0.52 (4/770)	2.12 (145/6836)	3.03 (119/3932)	3.25 (27/830)

[a]No. of positive samples/No. of samples analyzed.
[b]N/A, not applicable.
Levine et al., 2001

Table 4: Distribution of Serotypes Among *L. monocytogenes Eco*RI Ribotypes

Lineage	*Eco*RI ribotype	1/2a	1/2b	1/2c	3a	3b	3c	4a	4b	4c
I	**DUP- 1038**								41	
I	**DUP- 1042**		26			1	1		19	1
I	**DUP- 1044**	1							31	
I	**DUP- 1052**		6			1			1	
I	**DUP- 1026**		1							
I	**DUP- 1024**		5						2	
I	**DUP- 1043**		1							
I	**DUP- 1027**		2							
II	**DUP- 1062**	8								
II	**DUP- 1030**	30		7	1					
II	**DUP- 1039**	9		1	1					
II	**DUP- 1045**	13								
II	**DUP- 1053**	4								
II	**DUP- 1054**	1								
II	**DUP- 1056**	3								
II	**DUP- 1029**	2								
II	**DUP- 1035**	1								
II	**DUP- 1047**	4								
III	**DUP- 1061**							1		1
III	**DUP- 1059**							4		1
III	**DUP- 10146**	1								
III	**DUP- 10147**									1
III	**DUP- 10145**									1

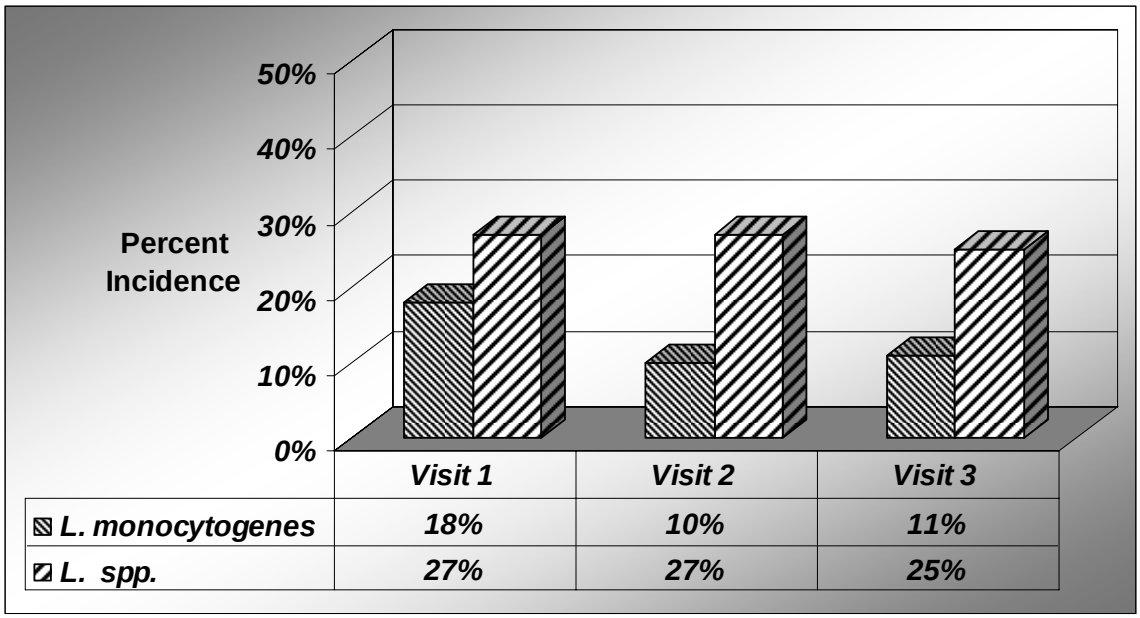
Nadon et al., 2001

Table 5: Recalls Due to *L. monocytogenes*.

Year	No. of Recalls	% of Recalls for *L. monocytogenes*	Approximate kilograms
2000	35	46	8,201,000
2001	25	26	6,693,000
2002	41	33	14,701,000
2003	14	23	25,000
2004	17	31	245,000

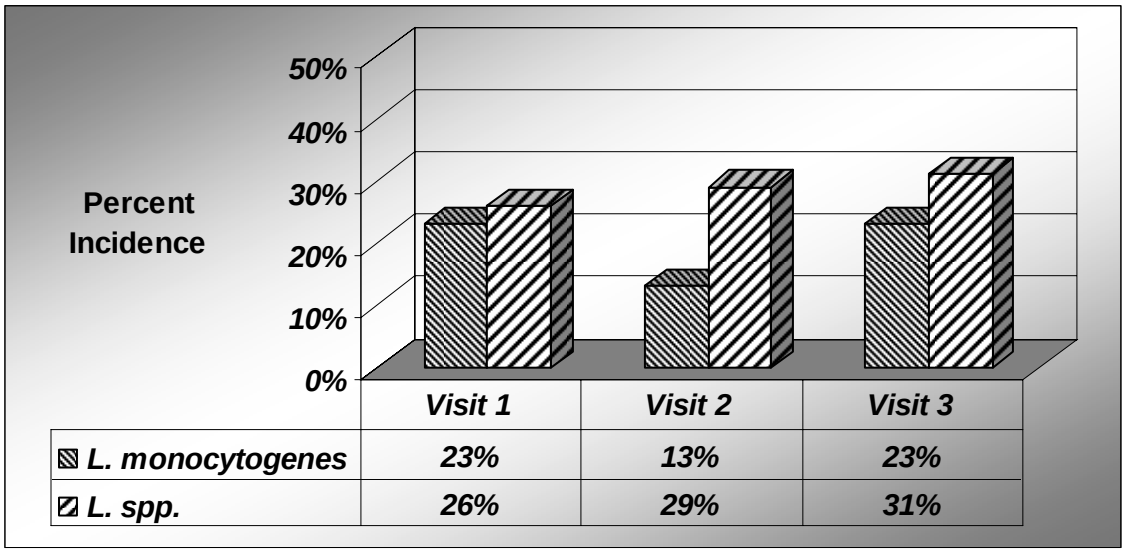
Source: FSIS Recall Information Center

Figure 1. Incidence of *L. monocytogenes* and *L.* spp. Segmented by Visit.



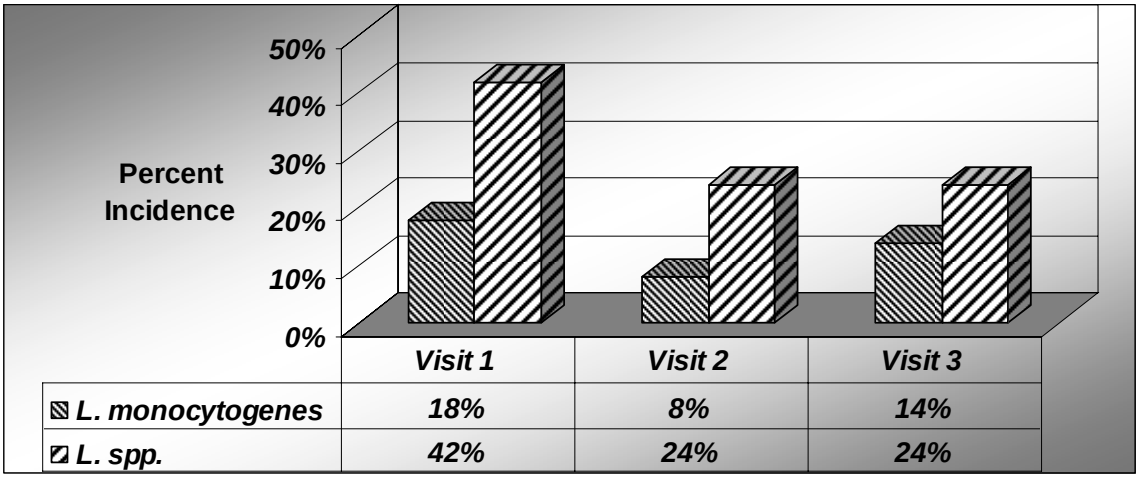
General Notes:
Visit 1, 2 and 3 were made at 30 d intervals to enable plant personnel to develop and implement corrective actions.

Figure 2. Incidence of *L. monocytogenes* and *L.* spp. for Plant Uniform Segmented by Visit.



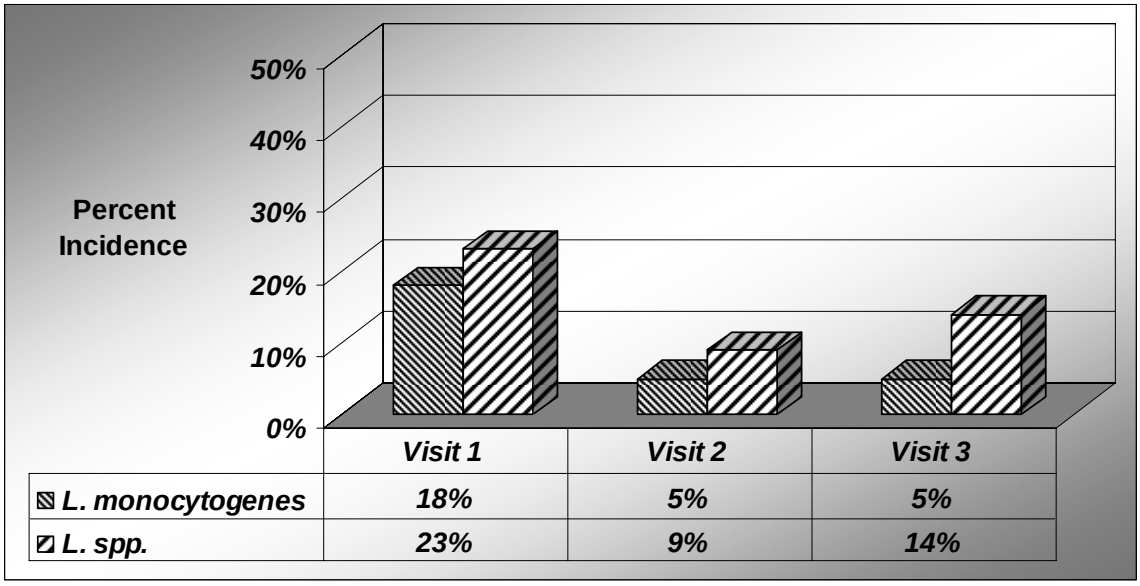
General Notes:
Visit 1, 2 and 3 were made at 30 day intervals to enable plant personnel to develop and implement corrective actions.

Figure 3. Incidence of *L. monocytogenes* and *L.* spp. for Plant Victor Segmented by Visit.



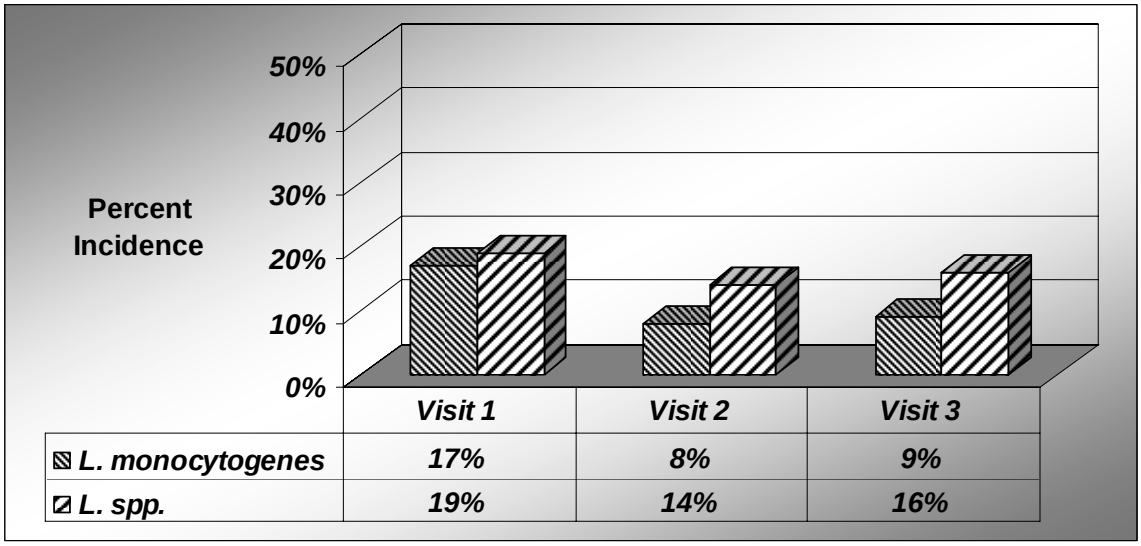
General Notes:
Visit 1, 2 and 3 were made at 30 day intervals to enable plant personnel to develop and implement corrective actions.

Figure 4. Incidence of *L. monocytogenes* and *L.* spp. for Plant X-Ray Segmented by Visit.



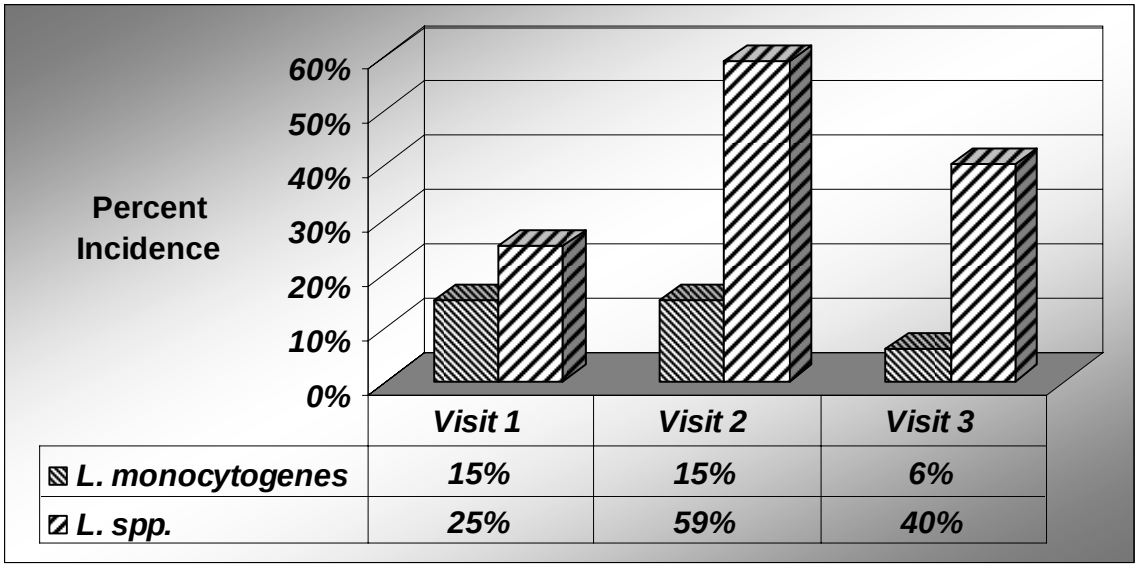
General Note:
Visit 1, 2 and 3 were made at 30 day intervals to enable plant personnel to develop and implement corrective actions.

Figure 5. Incidence of *L. monocytogenes* and *L.* spp. for Plant Yankee Segmented by Visit.



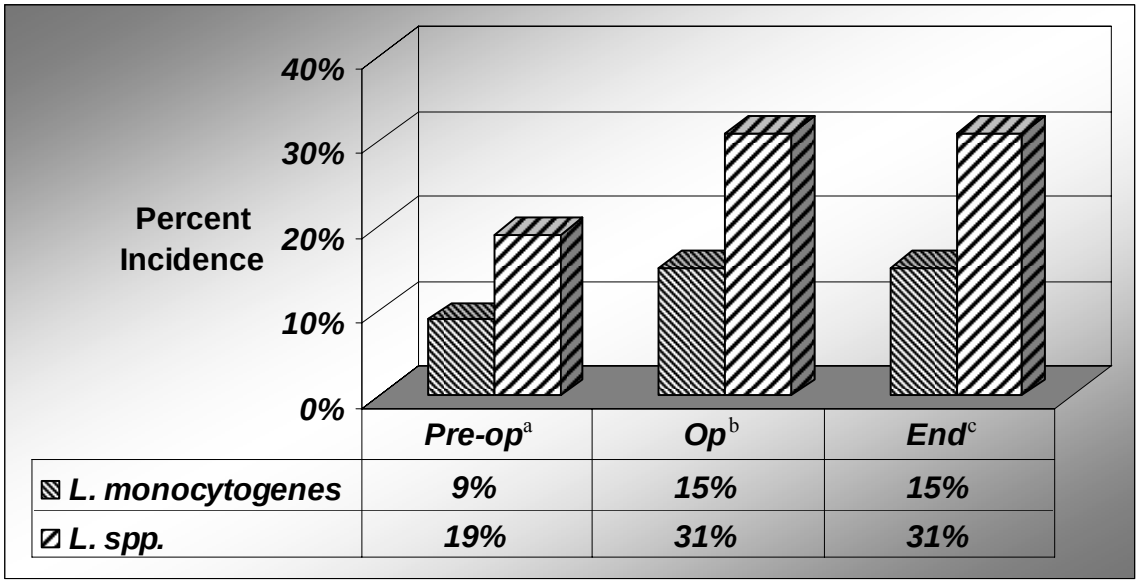
General Note:
Visit 1, 2 and 3 were made at 30 day intervals to enable plant personnel to develop and implement corrective actions.

Figure 6. Incidence of *L. monocytogenes* and *L.* spp. for Plant Zulu Segmented by Visit.



General Note:
Visit 1, 2 and 3 were made at 30 day intervals to enable plant personnel to develop and implement corrective actions.

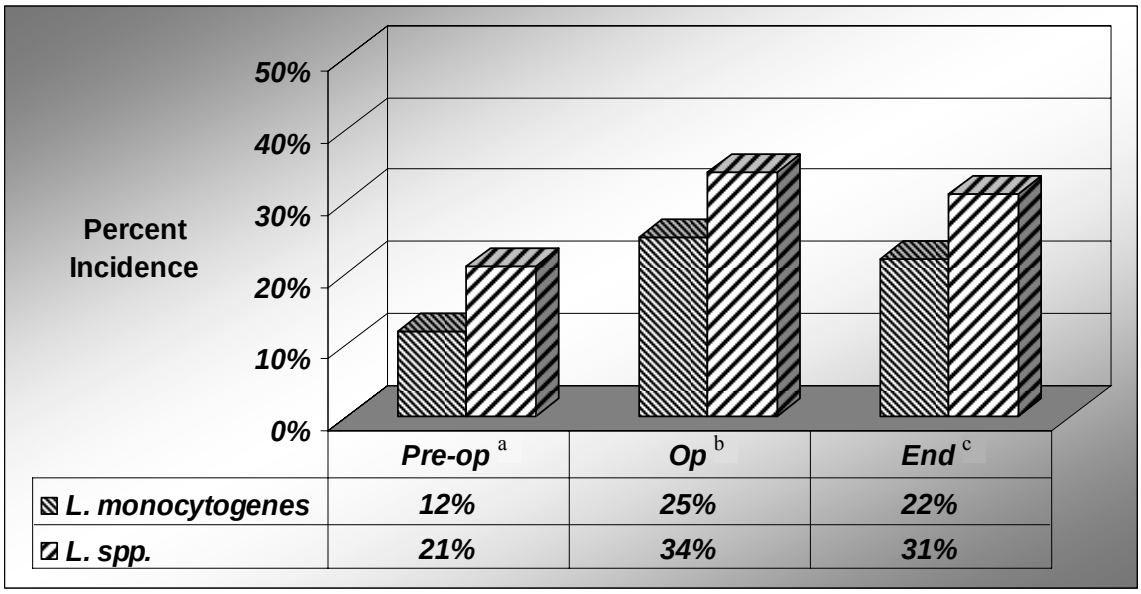
Figure 7. Incidence of *L. monocytogenes* and *L.* spp. in All Plants Segmented by Sampling Time.



	Pre-op[a]	Op[b]	End[c]
L. monocytogenes	9%	15%	15%
L. spp.	19%	31%	31%

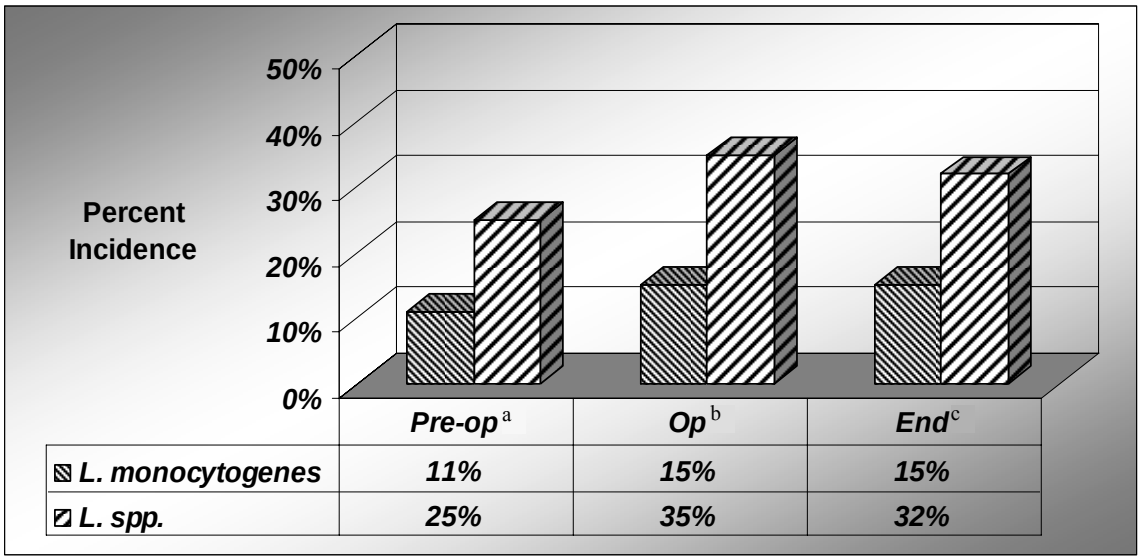
[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

Figure 8. Incidence of *L. monocytogenes* and *L.* spp. in Plant Uniform Segmented by Sampling Time.



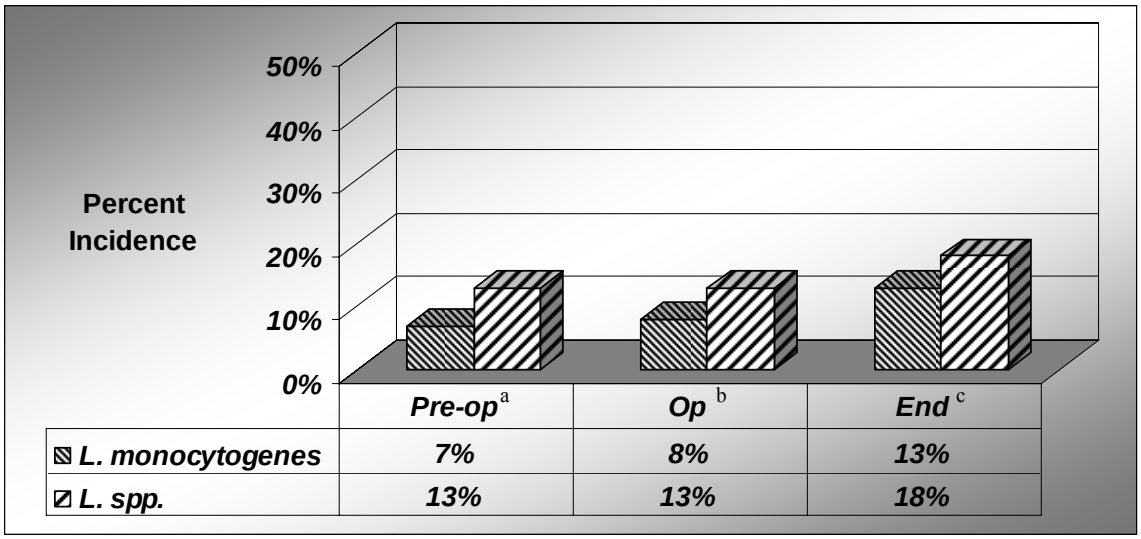
[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

Figure 9. Incidence of *L. monocytogenes* and *L.* spp. in Plant Victor Segmented by Sampling Time.



[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

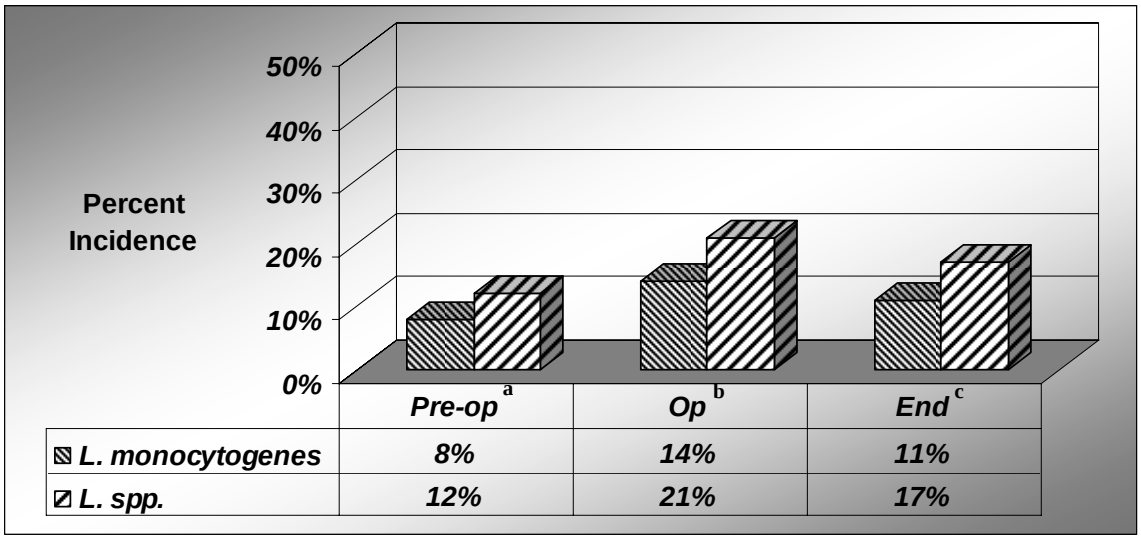
Figure 10. Incidence of *L. monocytogenes* and *L.* spp. in Plant X-Ray Segmented by Sampling Time.



	Pre-op[a]	Op[b]	End[c]
L. monocytogenes	7%	8%	13%
L. spp.	13%	13%	18%

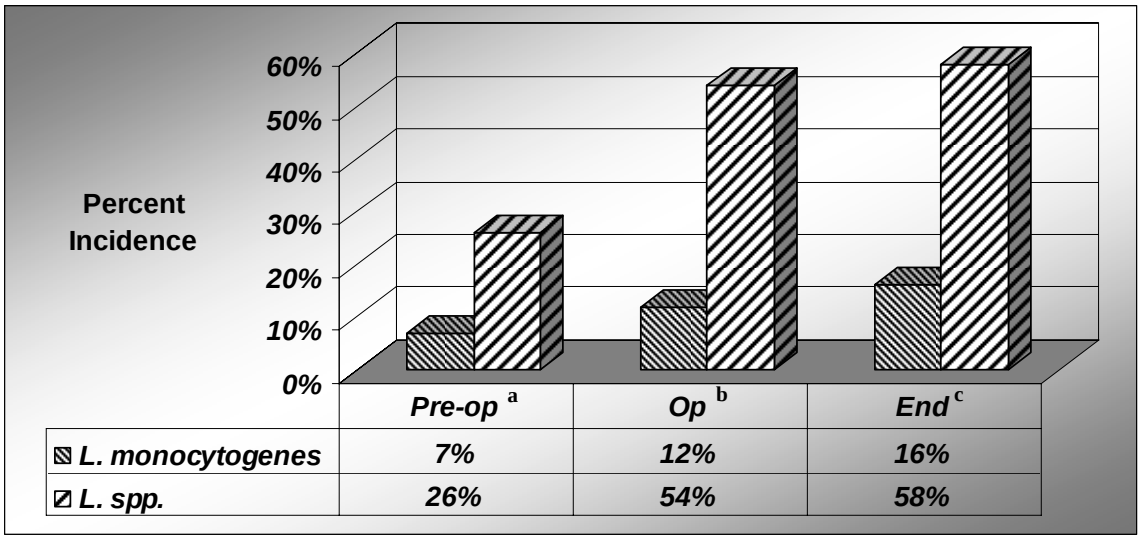
[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

Figure 11. Incidence of *L. monocytogenes* and *L.* spp. in Plant Yankee Segmented by Sampling Time.



[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

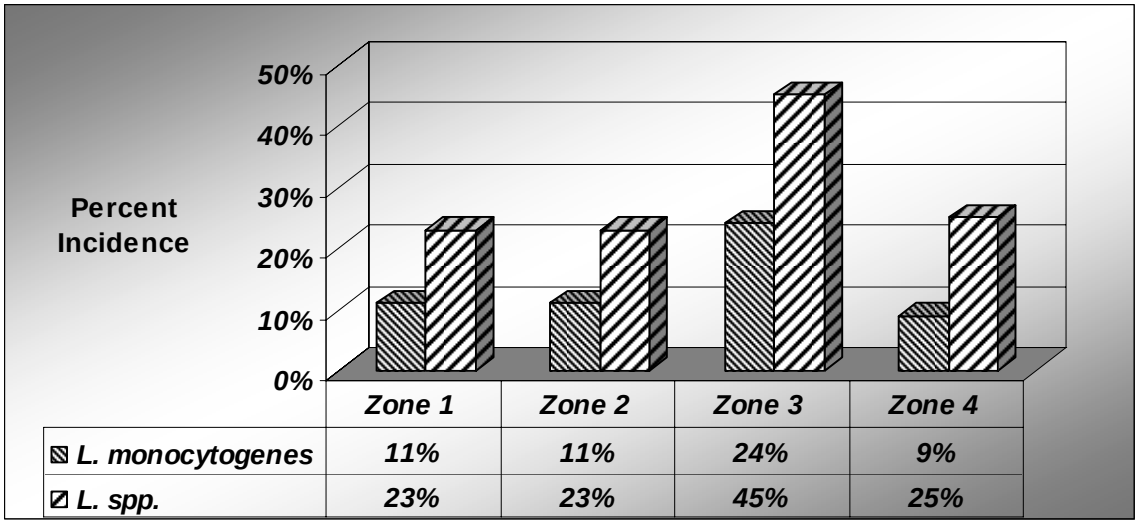
Figure 12. Incidence of *L. monocytogenes* and *L.* spp. in Plant Zulu Segmented by Sampling Time.



	Pre-op [a]	Op [b]	End [c]
L. monocytogenes	7%	12%	16%
L. spp.	26%	54%	58%

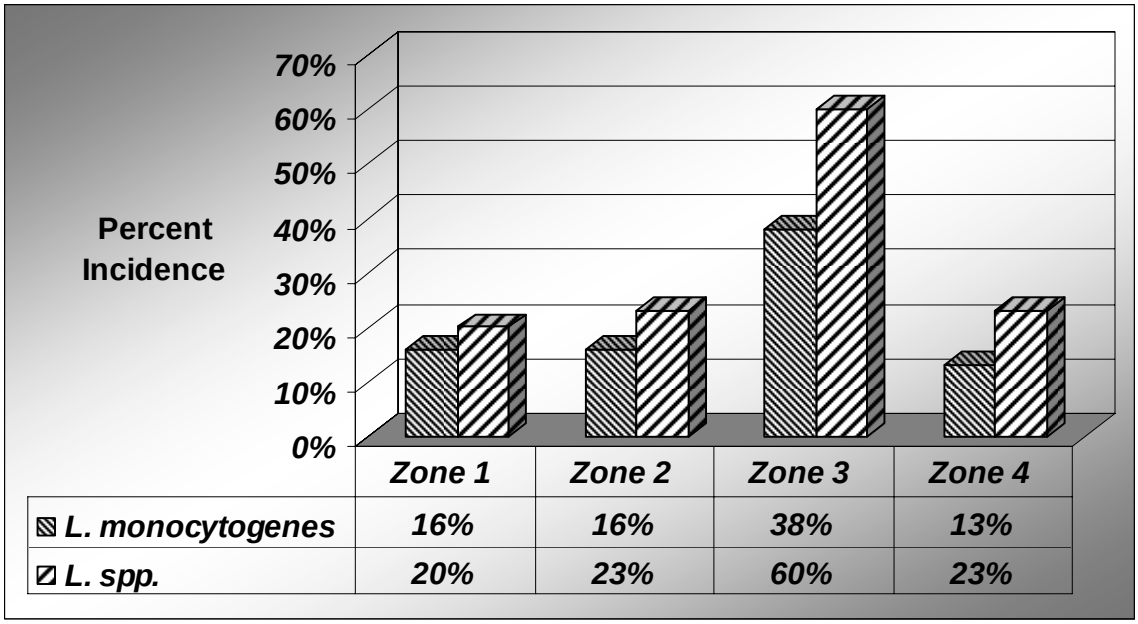
[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

Figure 13. Incidence of *L. monocytogenes* and *L.* spp. in All Plants Segmented by Environmental Zone.



General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

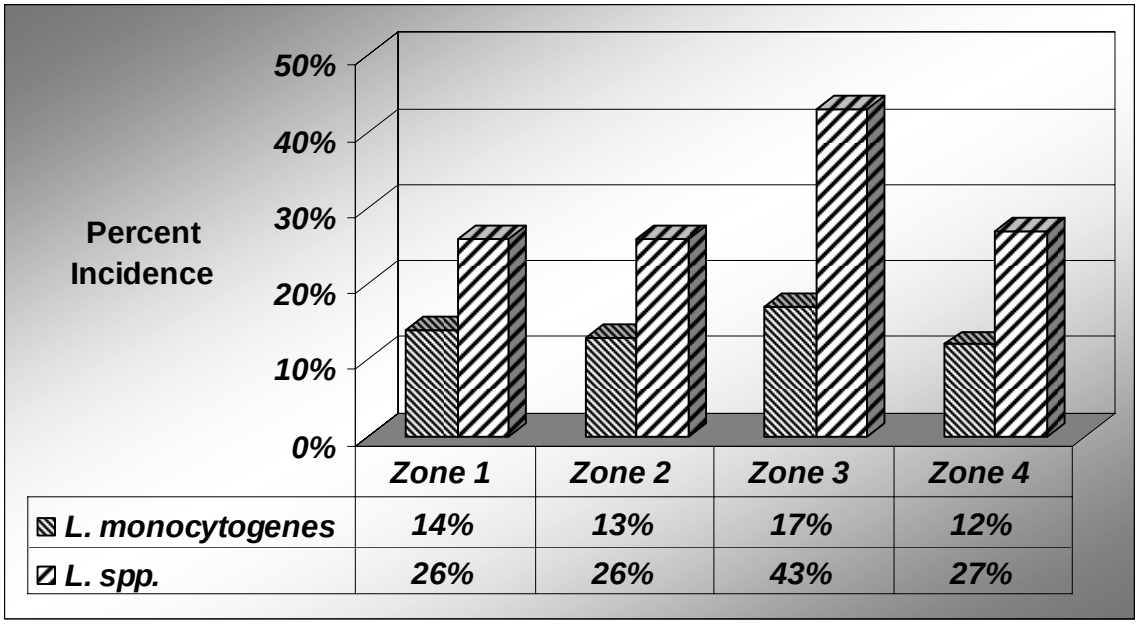
Figure 14. Incidence of *L. monocytogenes* and *L.* spp. in Plant Uniform Segmented by Environmental Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L. monocytogenes	16%	16%	38%	13%
L. spp.	20%	23%	60%	23%

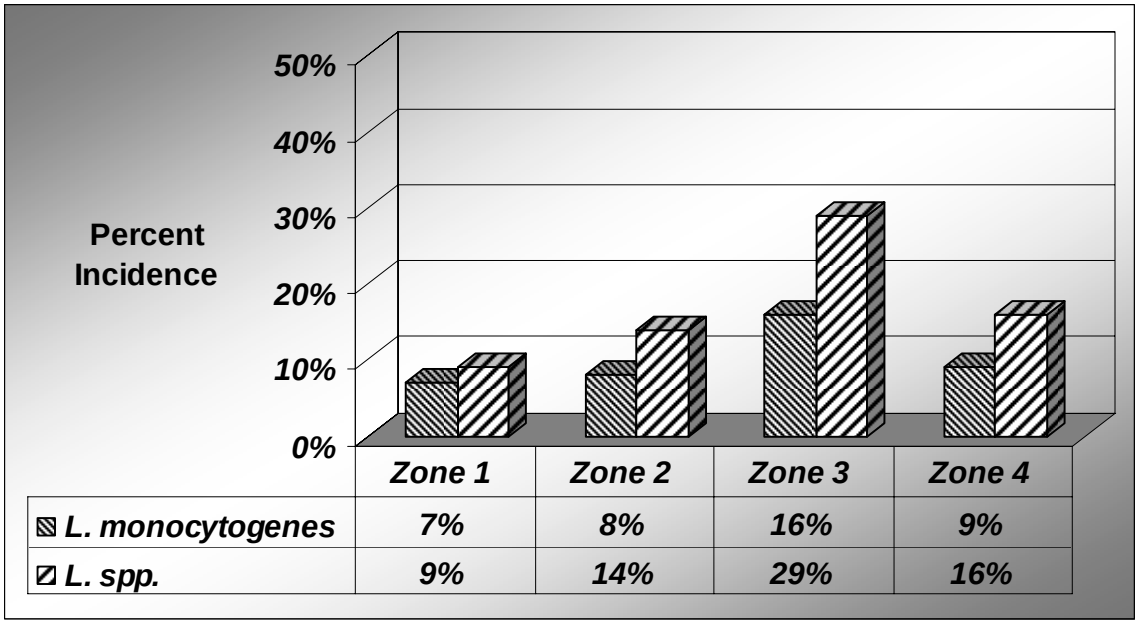
General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 15. Incidence of *L. monocytogenes* and *L.* spp. in Plant Victor Segmented by Environmental Zone.



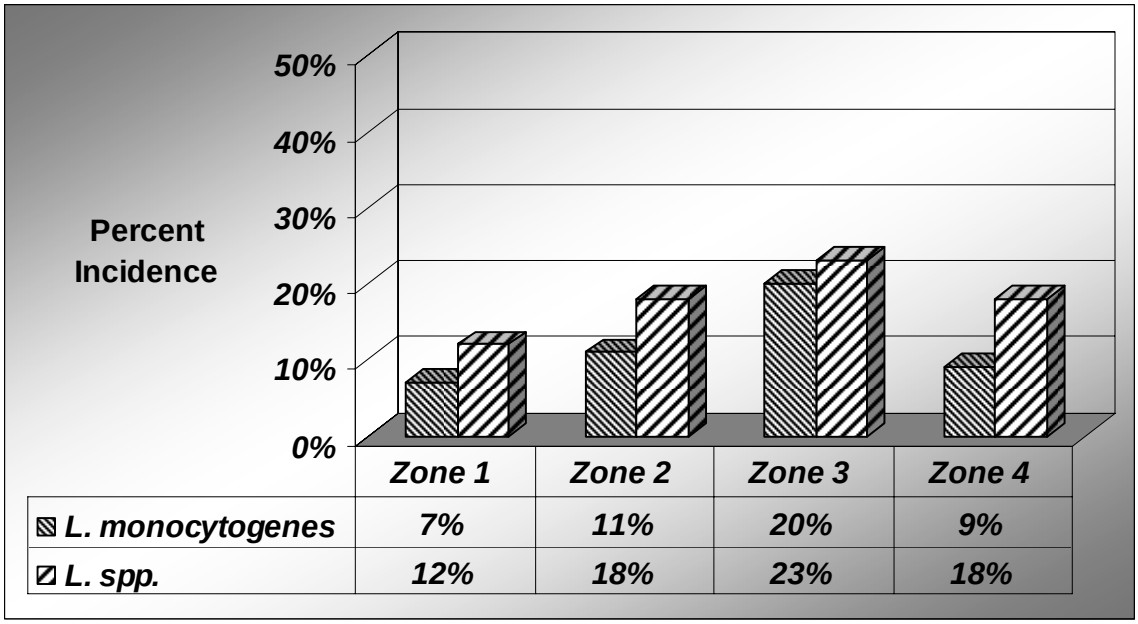
General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 16. Incidence of *L. monocytogenes* and *L.* spp. in Plant X-Ray Segmented by Environmental Zone.



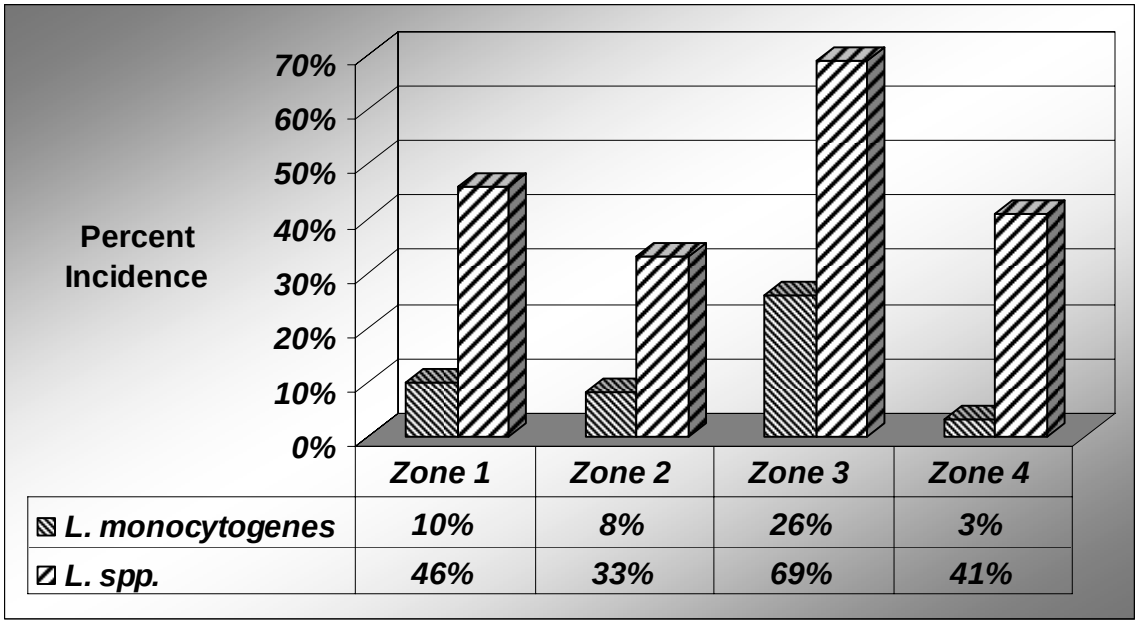
General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 17. Incidence of *L. monocytogenes* and *L.* spp. in Plant Yankee Segmented by Environmental Zone.



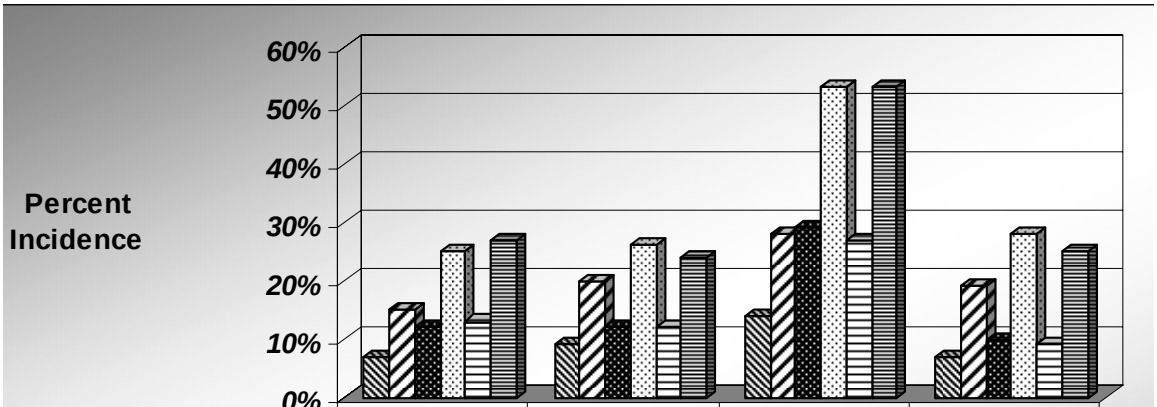
General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 18. Incidence of *L. monocytogenes* and *L.* spp. in Plant Zulu Segmented by Environmental Zone.



General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 19. Incidence of *L. monocytogenes* and *L.* spp. in All Plants Evaluating the Interaction Between Sampling Time by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L.m. Pre-op [1]	7%	9%	14%	7%
L. spp. Pre-op [2]	15%	20%	28%	19%
L.m. Op [3]	12%	12%	29%	10%
L. spp. Op [4]	25%	26%	53%	28%
L.m. End [5]	13%	12%	27%	9%
L. spp. End [6]	27%	24%	53%	25%

[1]***L. m.*** **Pre-op – The percent incidence of *L. monocytogenes* on samples taken before operations started**

[2]***L.*** **spp. Pre-op – The percent incidence of L. spp. on samples taken before operations started**

[3]***L. m.*** **Op – The percent incidence of L. monocytogenes on samples taken at the end of the first shift**

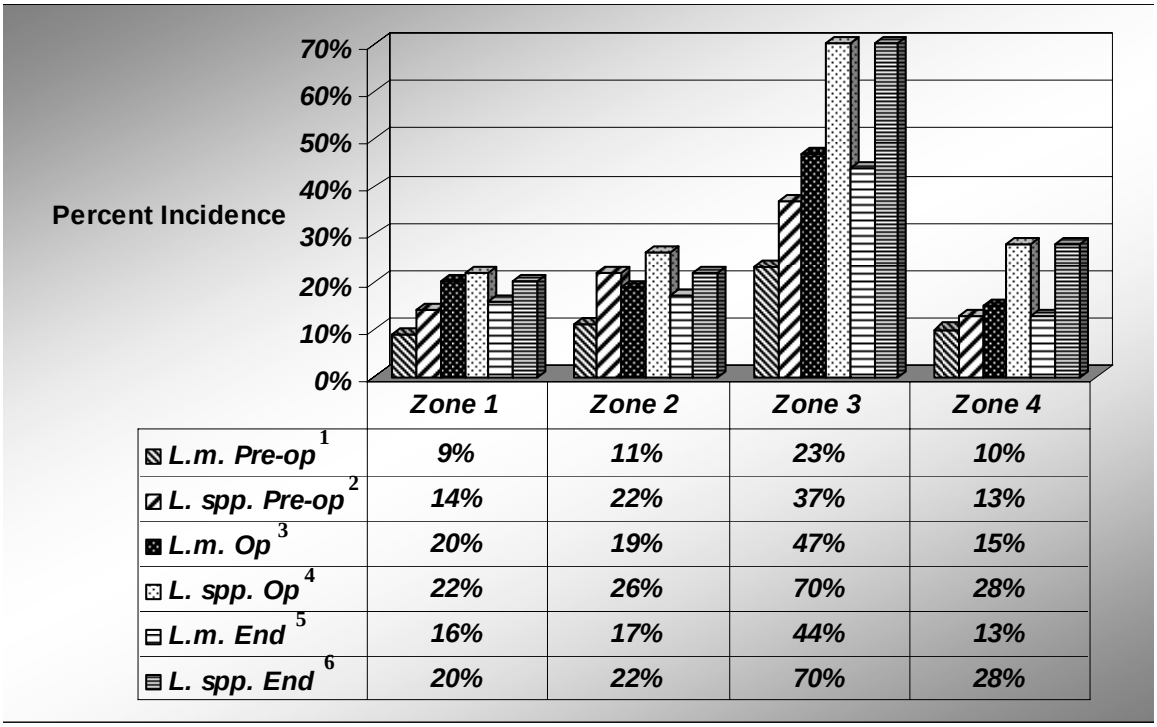
[4]***L.*** **spp. Op – The percent incidence of L. spp. on samples taken at the end of the first shift**

[5]***L. m.*** **End – The percent incidence of L. monocytogenes taken on samples taken during second shift**

[6]***L.*** **spp. End – The percent incidence of *L.* spp. on samples taken during second shift**

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 20. Incidence of *L. monocytogenes* and *L.* spp. in Plant Uniform Evaluating the Interaction Between Sampling Time by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L.m. Pre-op [1]	9%	11%	23%	10%
L. spp. Pre-op [2]	14%	22%	37%	13%
L.m. Op [3]	20%	19%	47%	15%
L. spp. Op [4]	22%	26%	70%	28%
L.m. End [5]	16%	17%	44%	13%
L. spp. End [6]	20%	22%	70%	28%

[1]***L. m.*** **Pre-op – The percent incidence of *L. monocytogenes* on samples taken before operations started**

[2]***L.*** **spp. Pre-op – The percent incidence of *L.* spp. on samples taken before operations started**

[3]***L. m.*** **Op – The percent incidence of *L. monocytogenes* on samples taken at the end of the first shift**

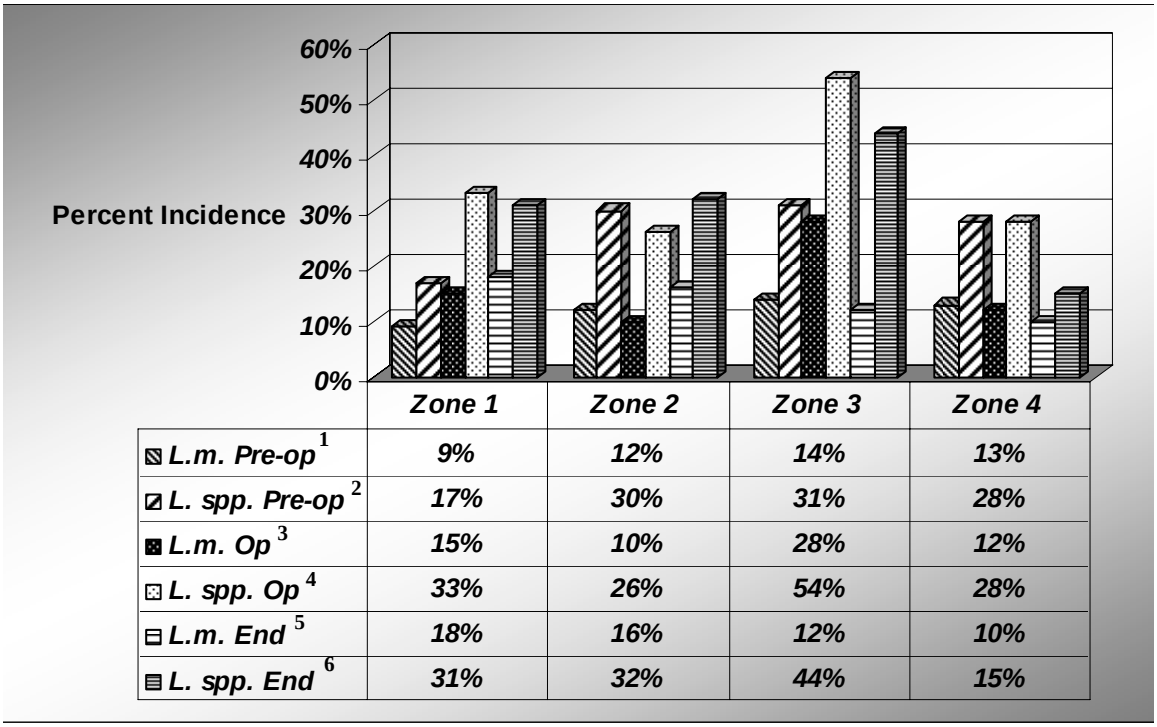
[4]***L.*** **spp. Op – The percent incidence of *L.* spp. on samples taken at the end of the first shift**

[5]***L. m.*** **End – The percent incidence of *L. monocytogenes* taken on samples taken during second shift**

[6]***L.*** **spp. End – The percent incidence of *L.* spp. on samples taken during second shift**

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 21. Incidence of *L. monocytogenes* and *L.* spp. in Plant Victor Evaluating the Interaction Between Sampling Time by Visit.



	Zone 1	Zone 2	Zone 3	Zone 4
L.m. Pre-op [1]	9%	12%	14%	13%
L. spp. Pre-op [2]	17%	30%	31%	28%
L.m. Op [3]	15%	10%	28%	12%
L. spp. Op [4]	33%	26%	54%	28%
L.m. End [5]	18%	16%	12%	10%
L. spp. End [6]	31%	32%	44%	15%

[1]***L. m.*** **Pre-op – The percent incidence of *L. monocytogenes* on samples taken before operations started**

[2]***L.*** **spp. Pre-op – The percent incidence of *L.* spp. on samples taken before operations started**

[3]***L. m.*** **Op – The percent incidence of *L. monocytogenes* on samples taken at the end of the first shift**

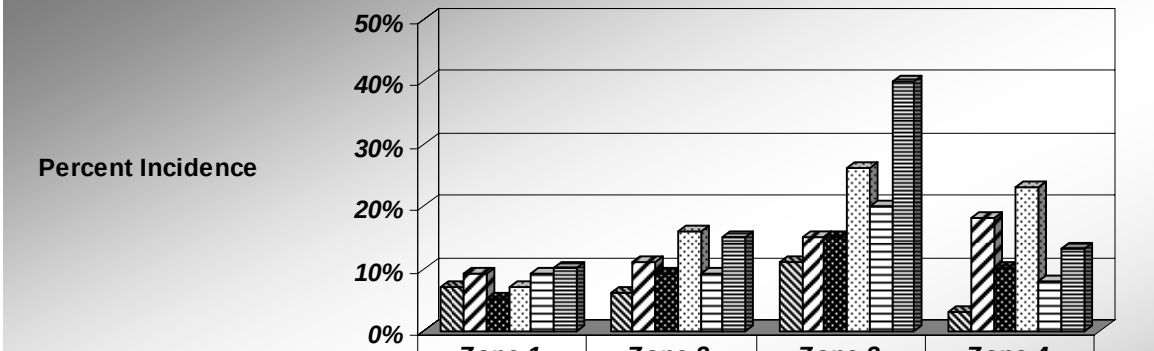
[4]***L.*** **spp. Op – The percent incidence of *L.* spp. on samples taken at the end of the first shift**

[5]***L. m.*** **End – The percent incidence of *L. monocytogenes* taken on samples taken during second shift**

[6]***L.*** **spp. End – The percent incidence of *L.* spp. on samples taken during second shift**

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 22. Incidence of *L. monocytogenes* and *L.* spp. in Plant X-Ray Evaluating the Interaction Between Sampling Time by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L.m. Pre-op [1]	7%	6%	11%	3%
L. spp. Pre-op [2]	9%	11%	15%	18%
L.m. Op [3]	5%	9%	15%	10%
L. spp. Op [4]	7%	16%	26%	23%
L.m. End [5]	9%	9%	20%	8%
L. spp. End [6]	10%	15%	40%	13%

[1]***L. m.*** **Pre-op – The percent incidence of *L. monocytogenes* on samples taken before operations started**

[2]***L.*** **spp. Pre-op – The percent incidence of *L.* spp. on samples taken before operations started**

[3]***L. m.*** **Op – The percent incidence of *L. monocytogenes* on samples taken at the end of the first shift**

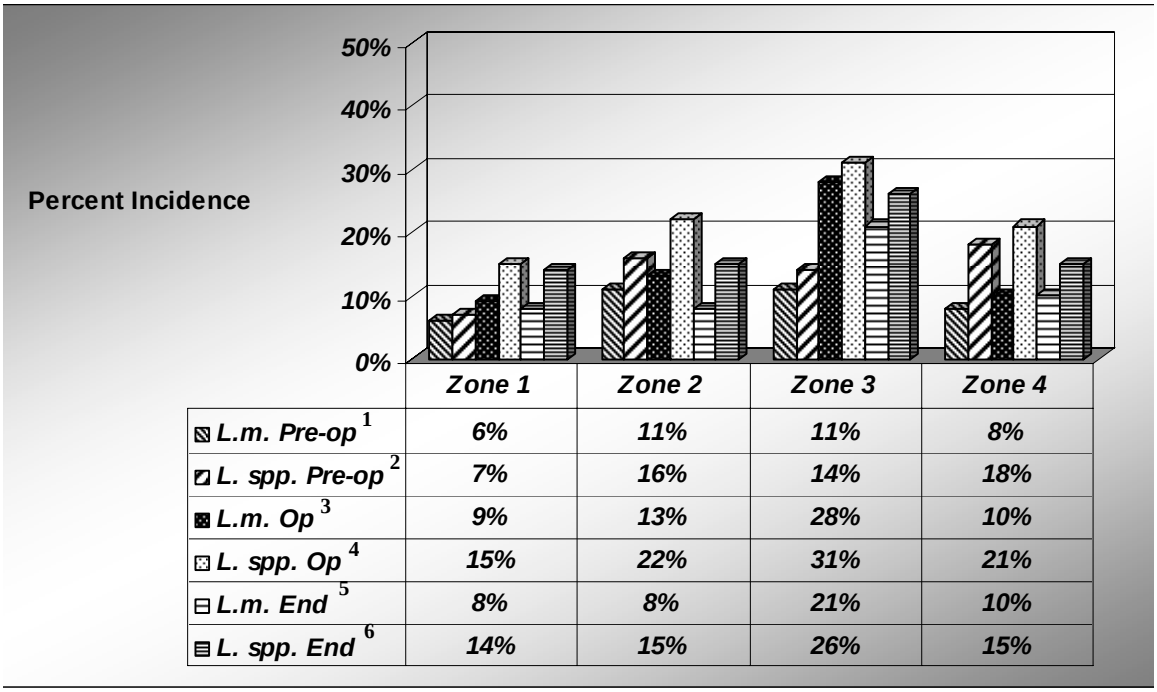
[4]***L.*** **spp. Op – The percent incidence of *L.* spp. on samples taken at the end of the first shift**

[5]***L. m.*** **End – The percent incidence of *L. monocytogenes* taken on samples taken during second shift**

[6]***L.*** **spp. End – The percent incidence of *L.* spp. on samples taken during second shift**

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 23. Incidence of *L. monocytogenes* and *L.* spp. in Plant Yankee Evaluating Sampling Time by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L.m. Pre-op [1]	6%	11%	11%	8%
L. spp. Pre-op [2]	7%	16%	14%	18%
L.m. Op [3]	9%	13%	28%	10%
L. spp. Op [4]	15%	22%	31%	21%
L.m. End [5]	8%	8%	21%	10%
L. spp. End [6]	14%	15%	26%	15%

[1]***L. m.*** **Pre-op – The percent incidence of *L. monocytogenes* on samples taken before operations started**

[2]***L.*** **spp. Pre-op – The percent incidence of *L.* spp. on samples taken before operations started**

[3]***L. m.*** **Op – The percent incidence of *L. monocytogenes* on samples taken at the end of the first shift**

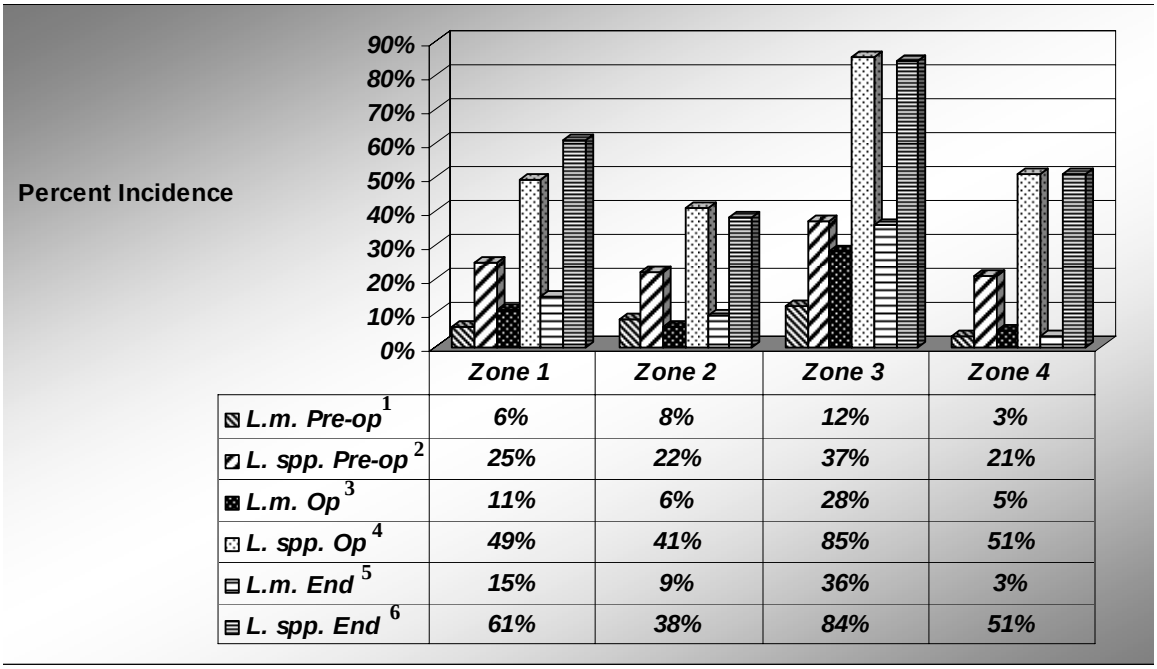
[4]***L.*** **spp. Op – The percent incidence of *L.* spp. on samples taken at the end of the first shift**

[5]***L. m.*** **End – The percent incidence of *L. monocytogenes* taken on samples taken during second shift**

[6]***L.*** **spp. End – The percent incidence of *L.* spp. on samples taken during second shift**

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 24. Incidence of *L. monocytogenes* and *L.* spp. in Plant Zulu Evaluating the Interaction Between Sampling Time by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L.m. Pre-op[1]	6%	8%	12%	3%
L. spp. Pre-op[2]	25%	22%	37%	21%
L.m. Op[3]	11%	6%	28%	5%
L. spp. Op[4]	49%	41%	85%	51%
L.m. End[5]	15%	9%	36%	3%
L. spp. End[6]	61%	38%	84%	51%

[1]***L. m.*** **Pre-op – The percent incidence of *L. monocytogenes* on samples taken before operations started**

[2]***L.*** **spp. Pre-op – The percent incidence of *L.* spp. on samples taken before operations started**

[3]***L. m.*** **Op – The percent incidence of *L. monocytogenes* on samples taken at the end of the first shift**

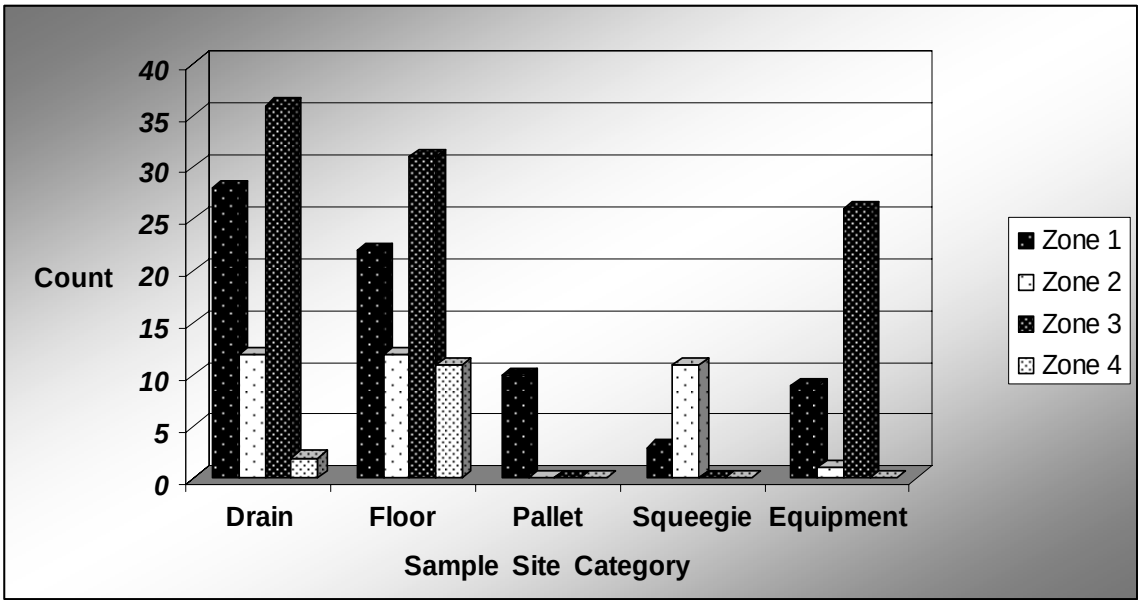
[4]***L.*** **spp. Op – The percent incidence of *L.* spp. on samples taken at the end of the first shift**

[5]***L. m.*** **End – The percent incidence of *L. monocytogenes* taken on samples taken during second shift**

[6]***L.*** **spp. End – The percent incidence of *L.* spp. on samples taken during second shift**

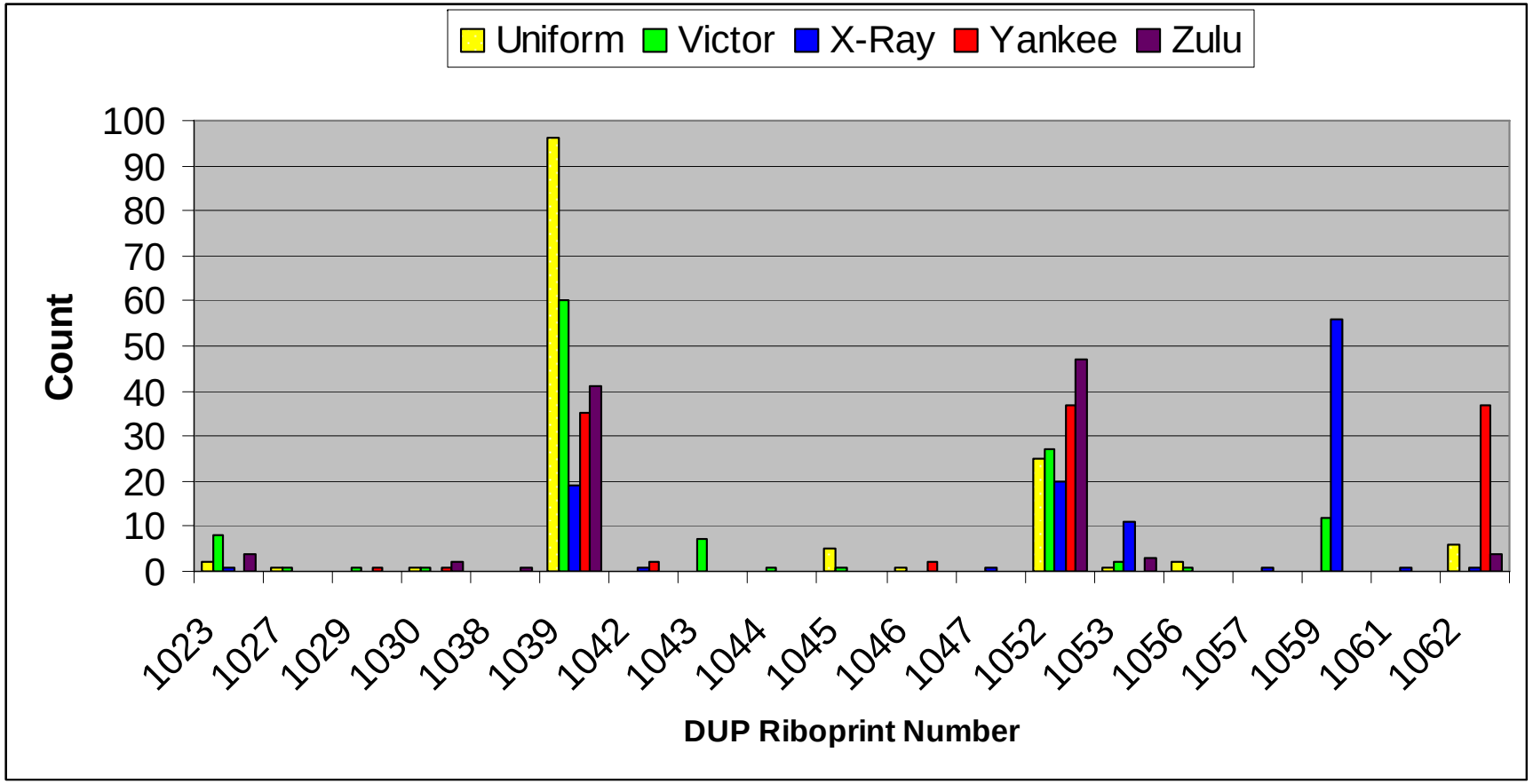
General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 25. The Number of *L. monocytogenes* Positives by Sample Site Category in Each Zone.



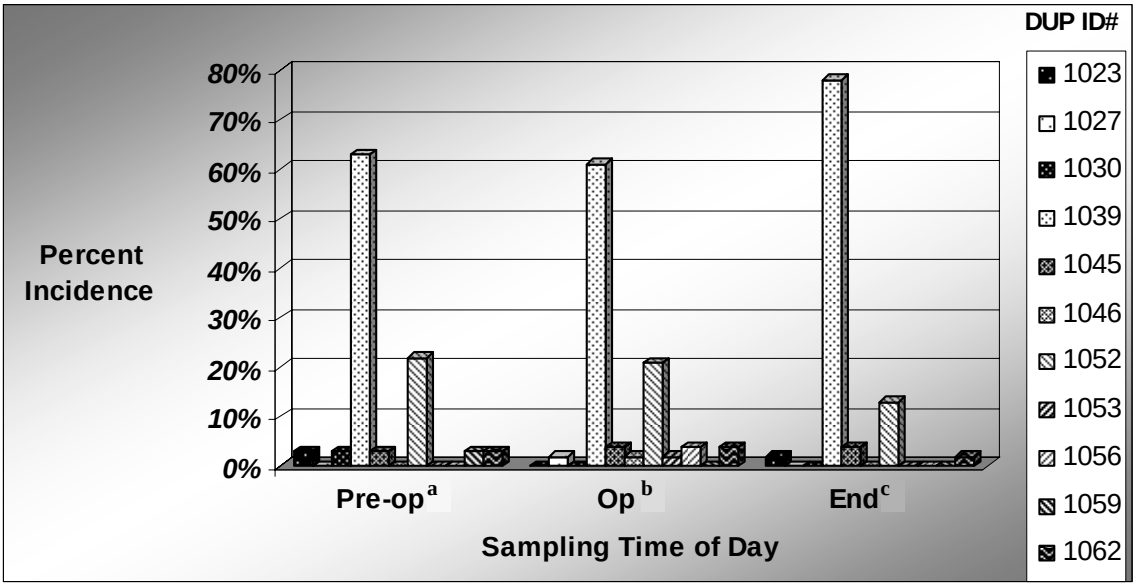
General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 26. DUP Ribotype Numbers by Individual Plant.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

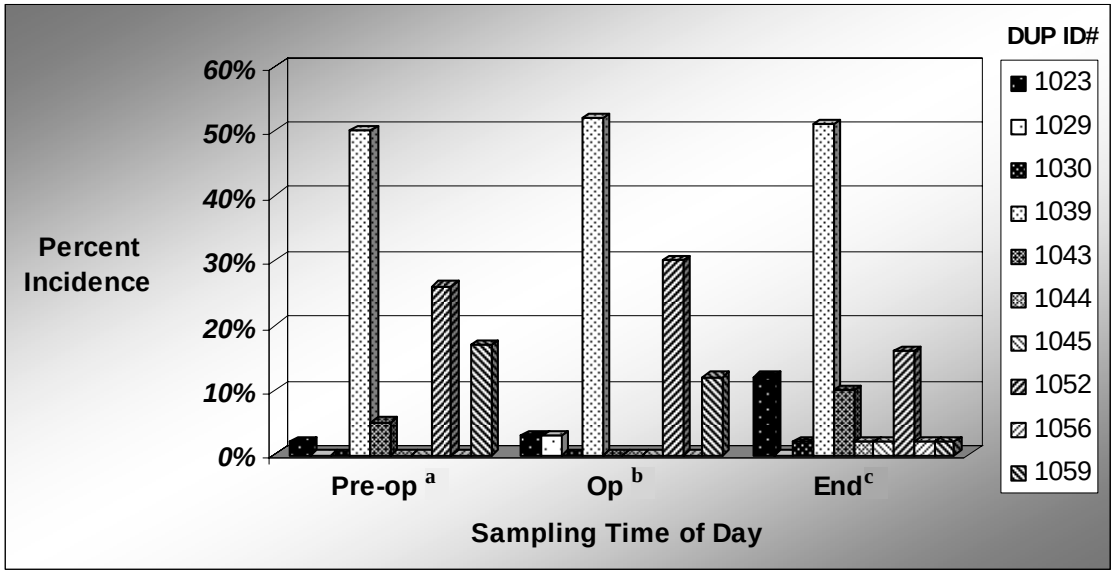
Figure 27. DUP Numbers Segmented by Sampling Time for Plant Uniform.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

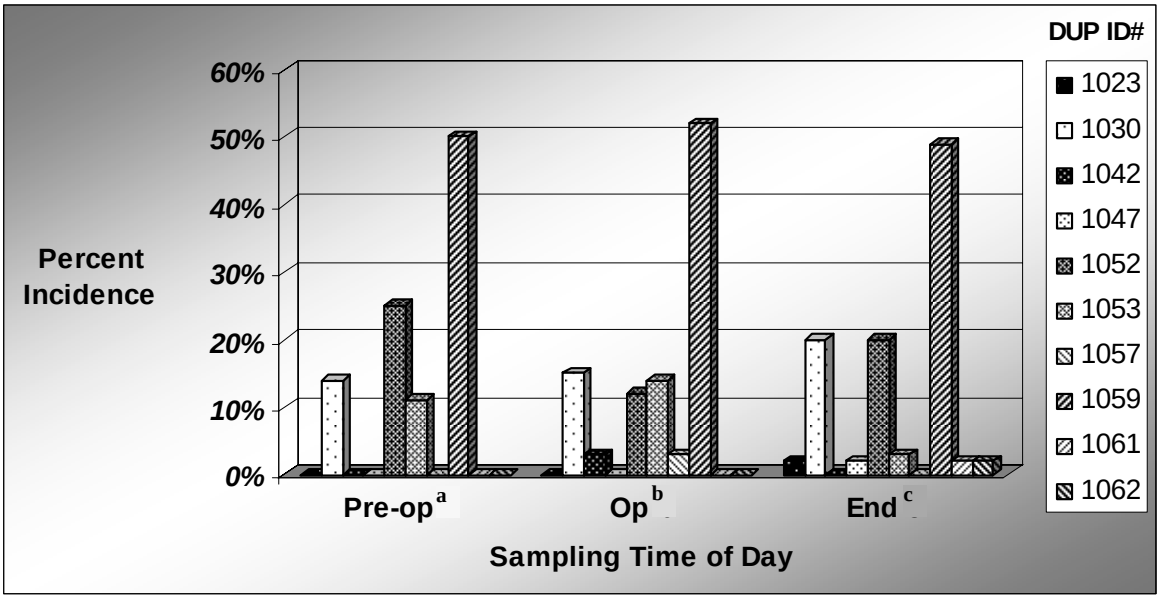
Figure 28. DUP Numbers Segmented by Sampling Time for Plant Victor.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

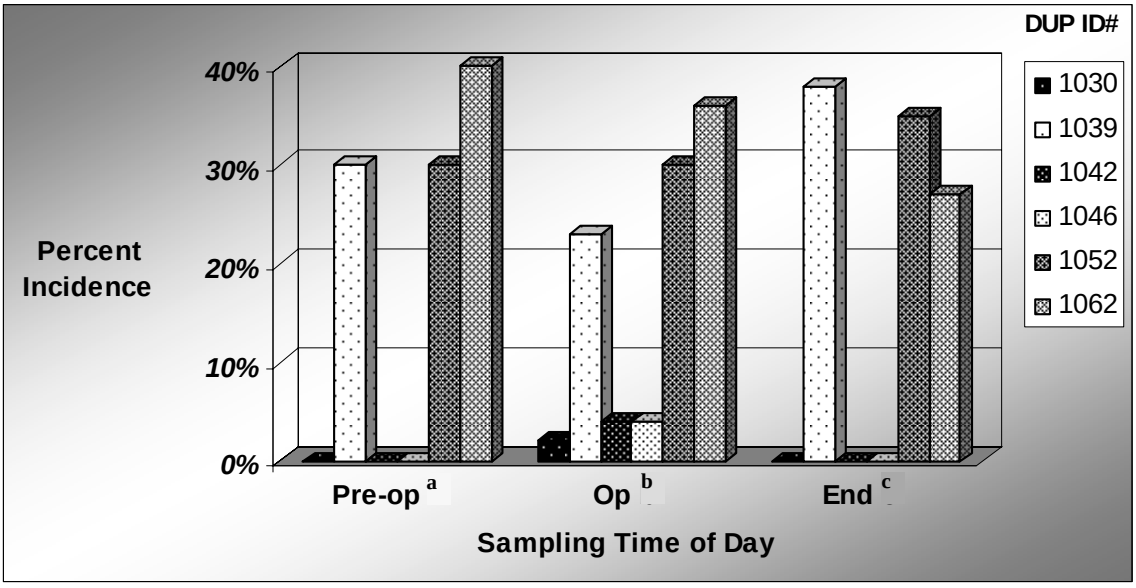
Figure 29. DUP Numbers Segmented by Sampling Time for Plant X-Ray.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

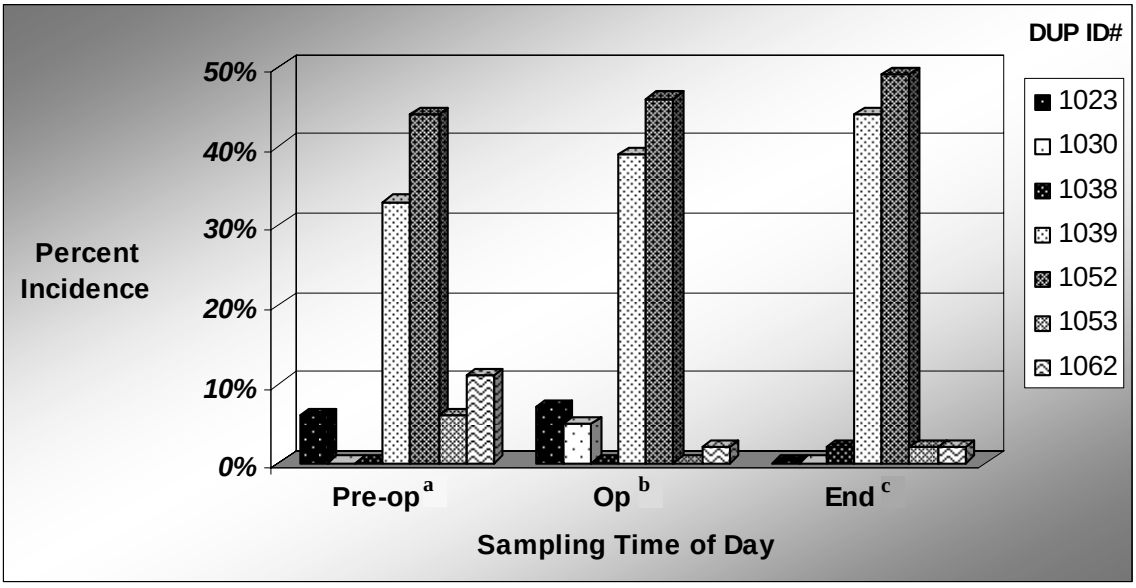
Figure 30. DUP Numbers by Sampling Time for Plant Yankee.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

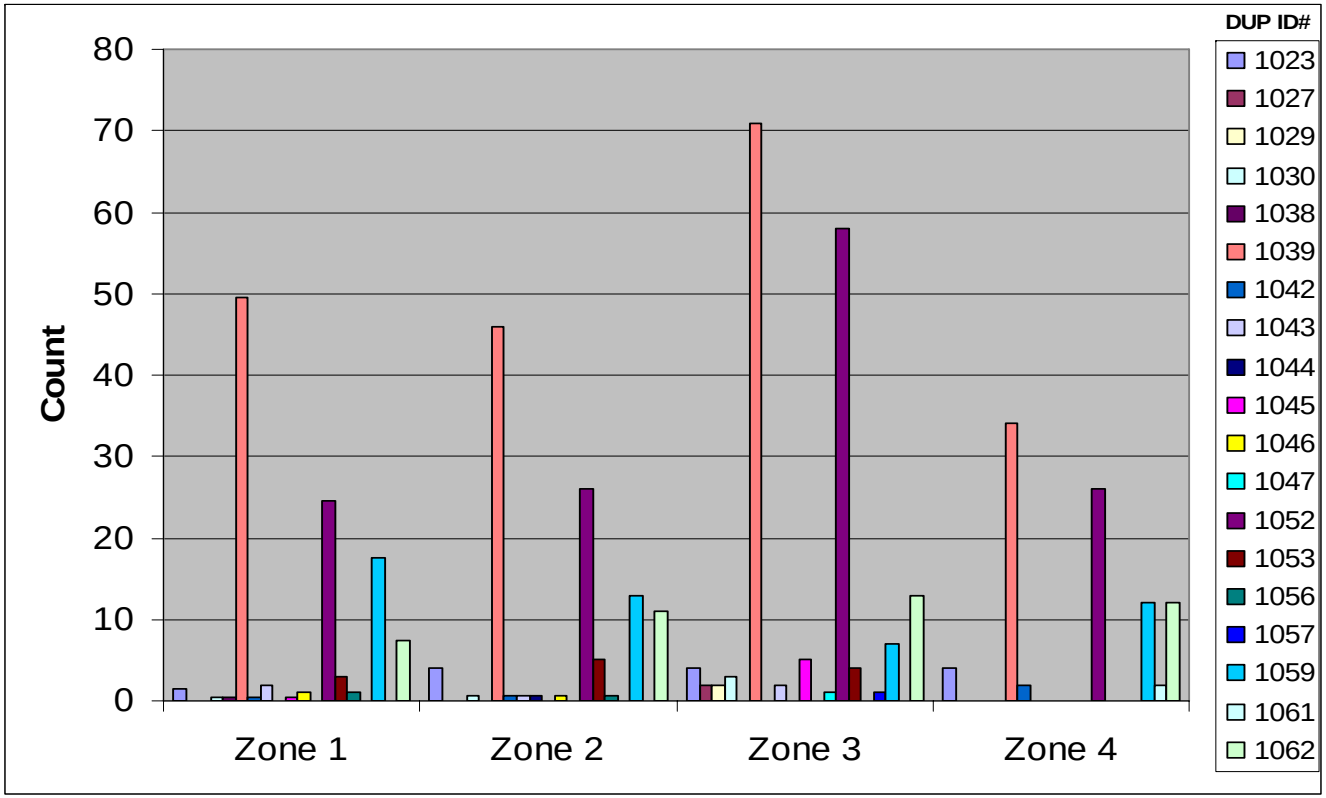
Figure 31. DUP Numbers Segmented by Sampling Time for Plant Zulu.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

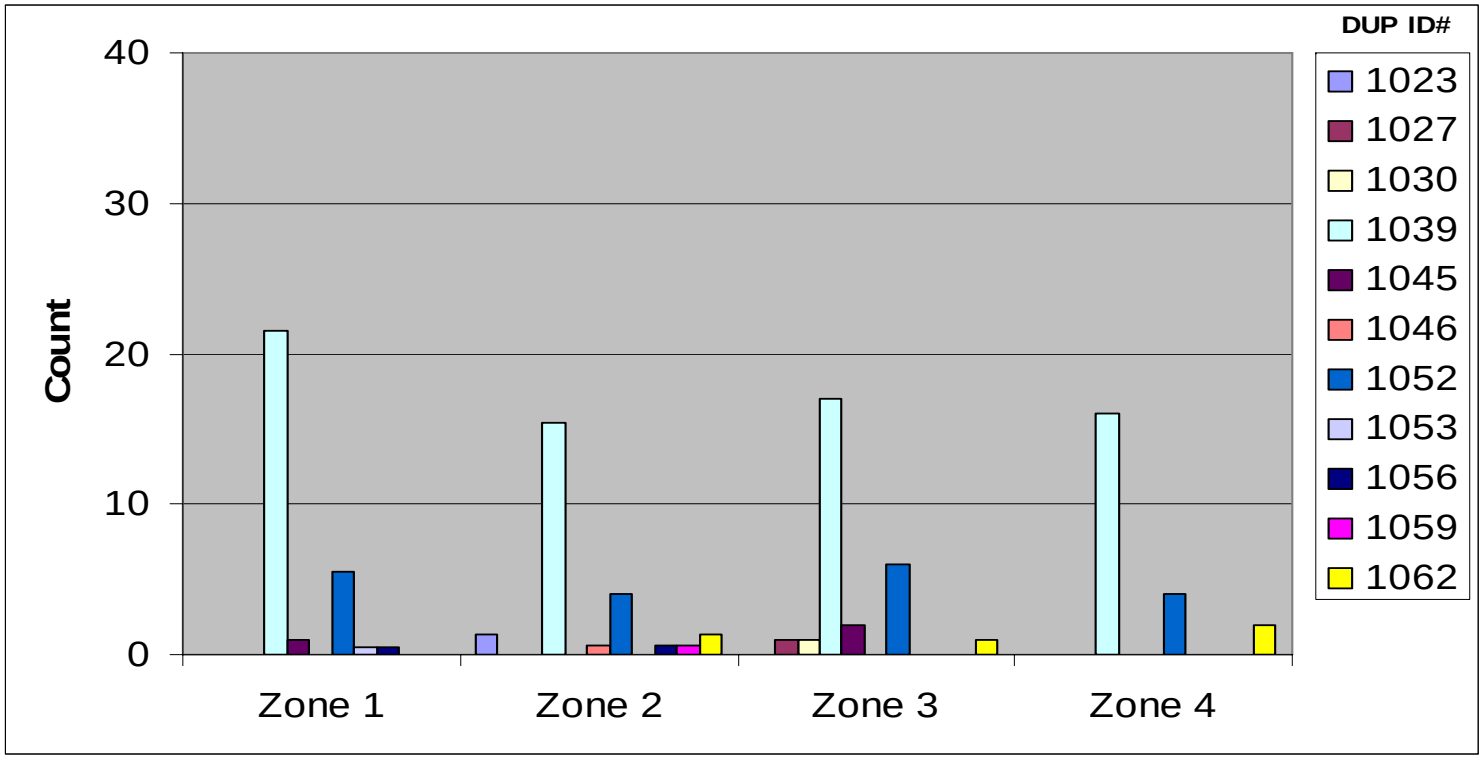
Figure 32. For All Plants – The Number of Each Ribotype in Each Zone.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

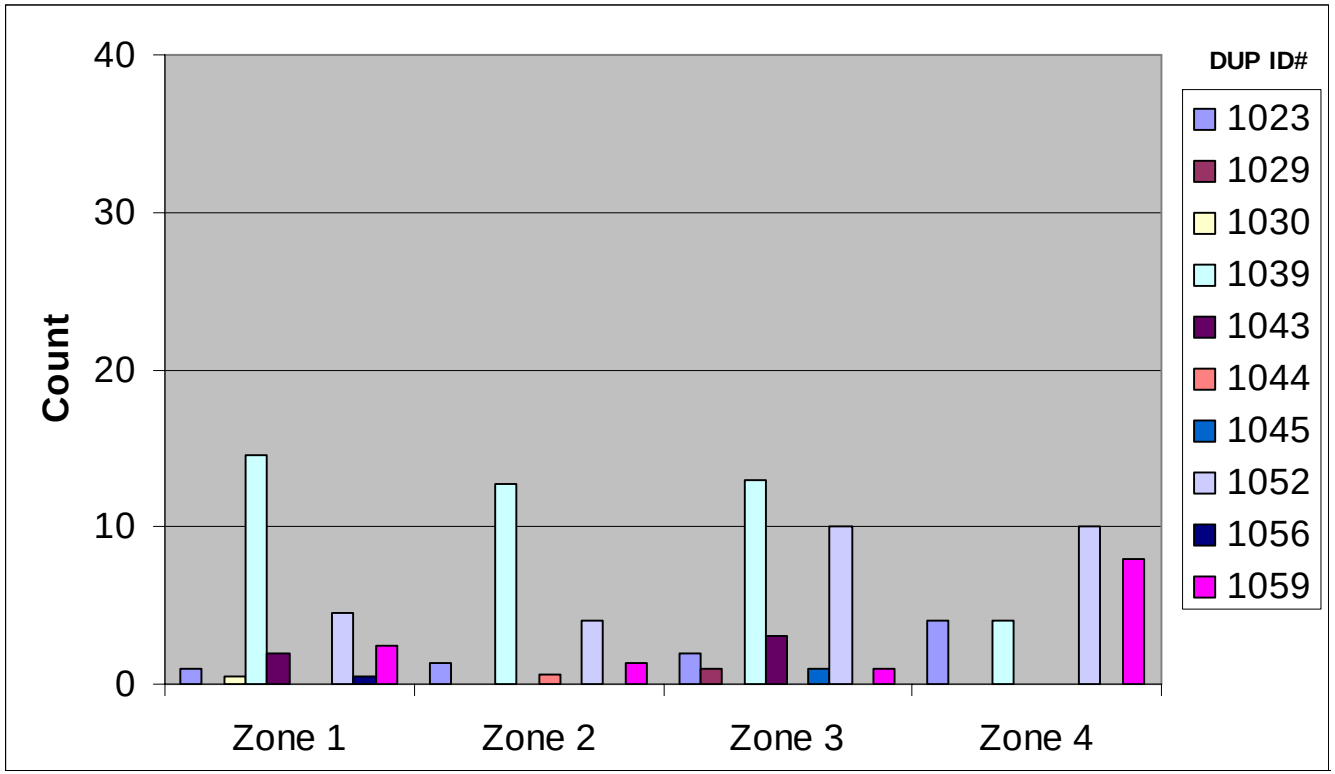
Figure 33. For Plant Uniform – The Number of Each Ribotype in Each Zone.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

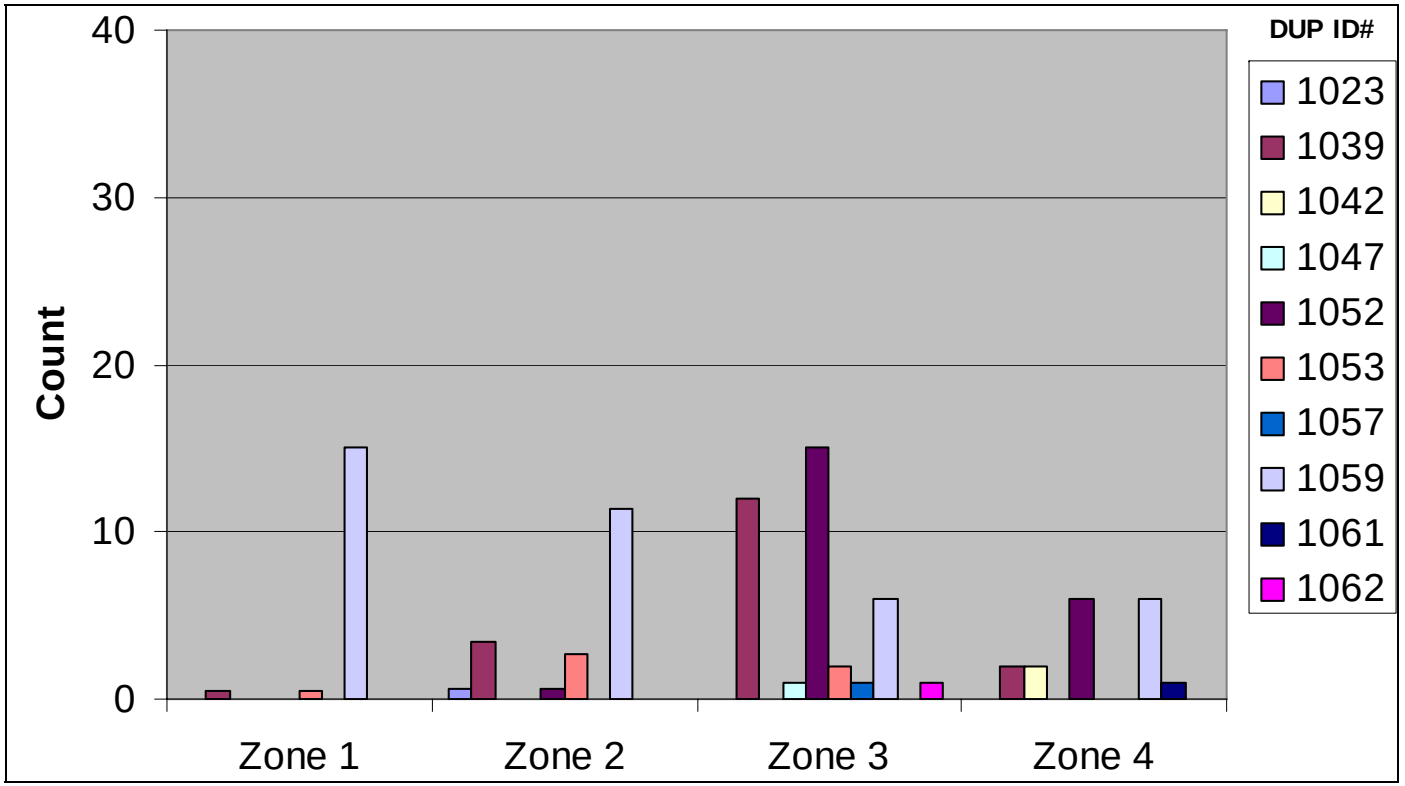
Figure 34. For Plant Victor – The Number of Each Ribotype in Each Zone.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

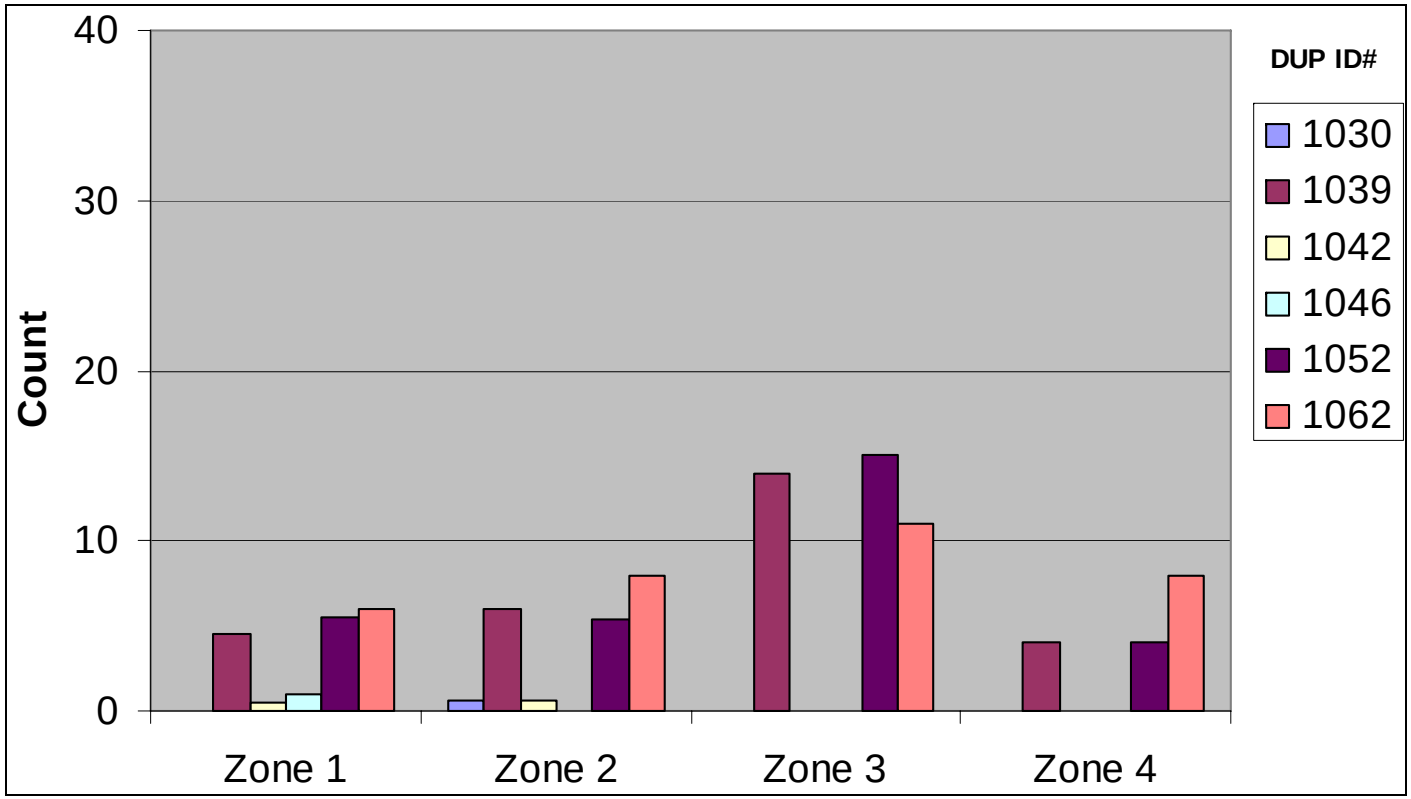
Figure 35. For Plant X-Ray – The Number of Each Ribotype in Each Zone.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

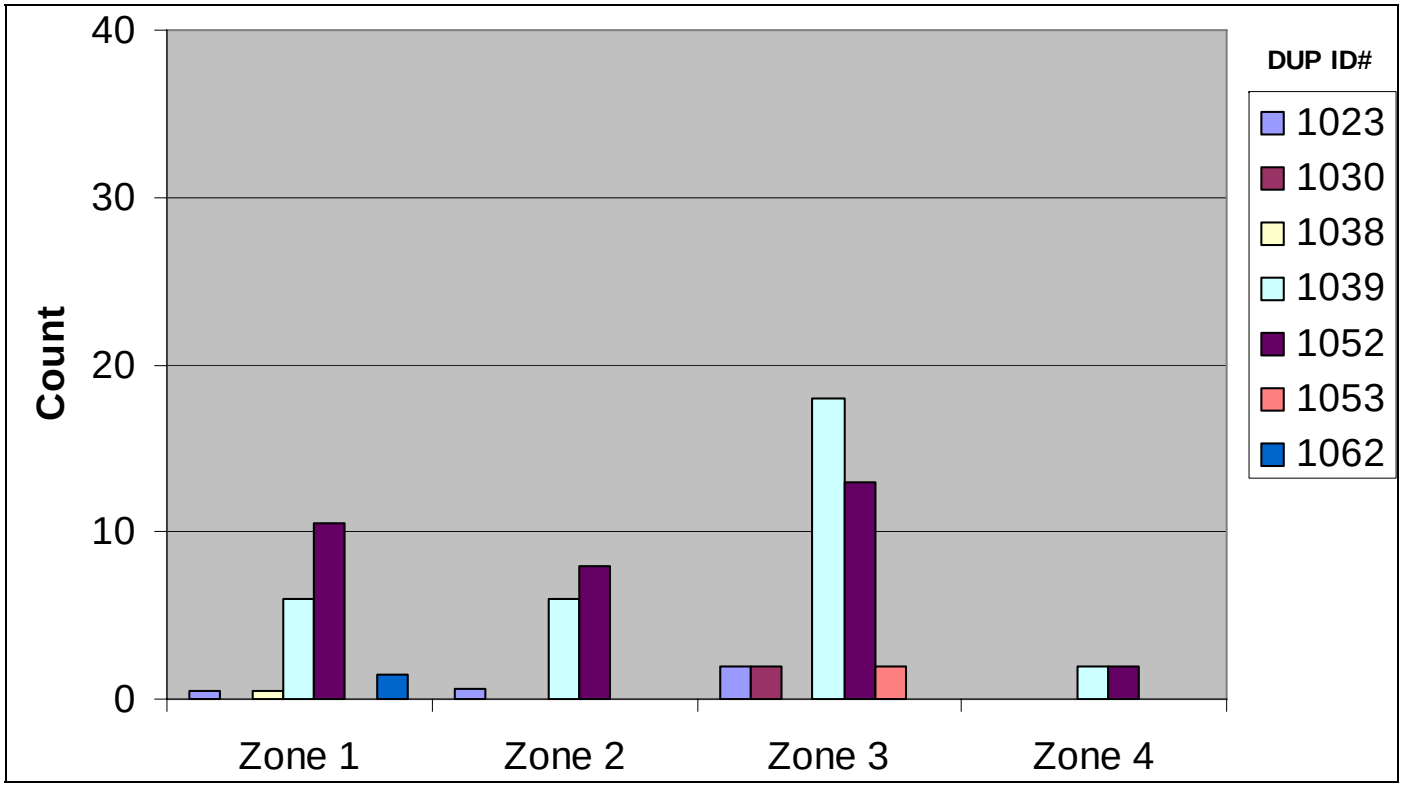
Figure 36. For Plant Yankee – The Number of Each Ribotype in Each Zone.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

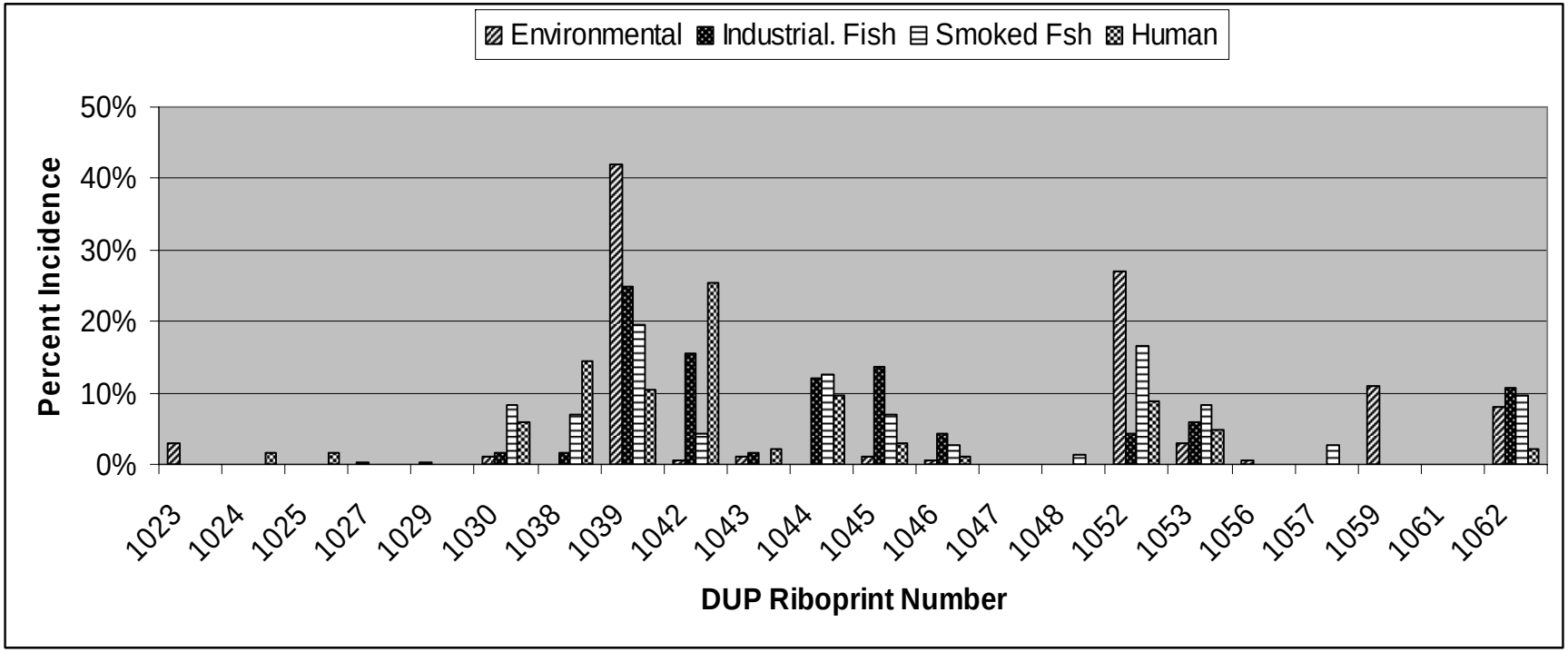
Figure 37. For Plant Zulu – The Number of Each Ribotype in Each Zone.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

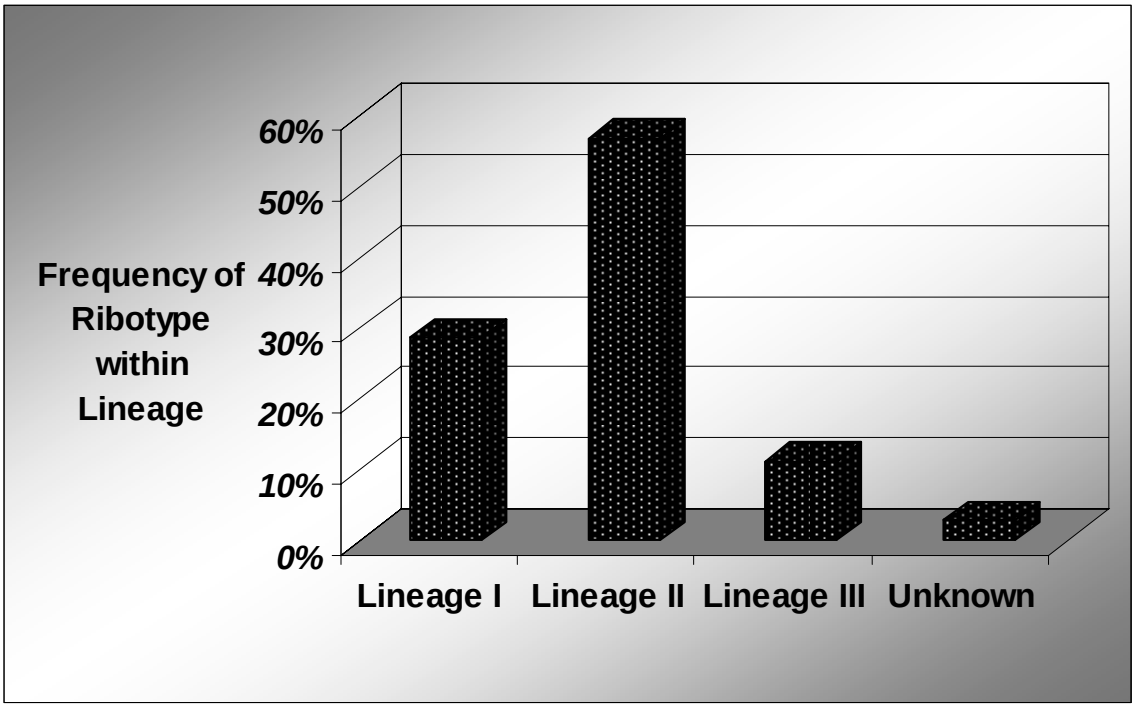
Figure 38. Comparison of Percentage of Ribotypes in this Study Compared to Others.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Environmental – Represents the percentage of ribotypes from the 600 *L. monocytogenes* isolates typed in Phase I of this study.
Industrial Fish – Represents 117 industrial fish isolates typed (Norton et al., 2001).
Smoked Fish – Represents 72 smoked salmon isolates typed (Gendel and Ulaszek, 2000).
Human – Represents 275 human isolates typed (Norton et al., 2001).

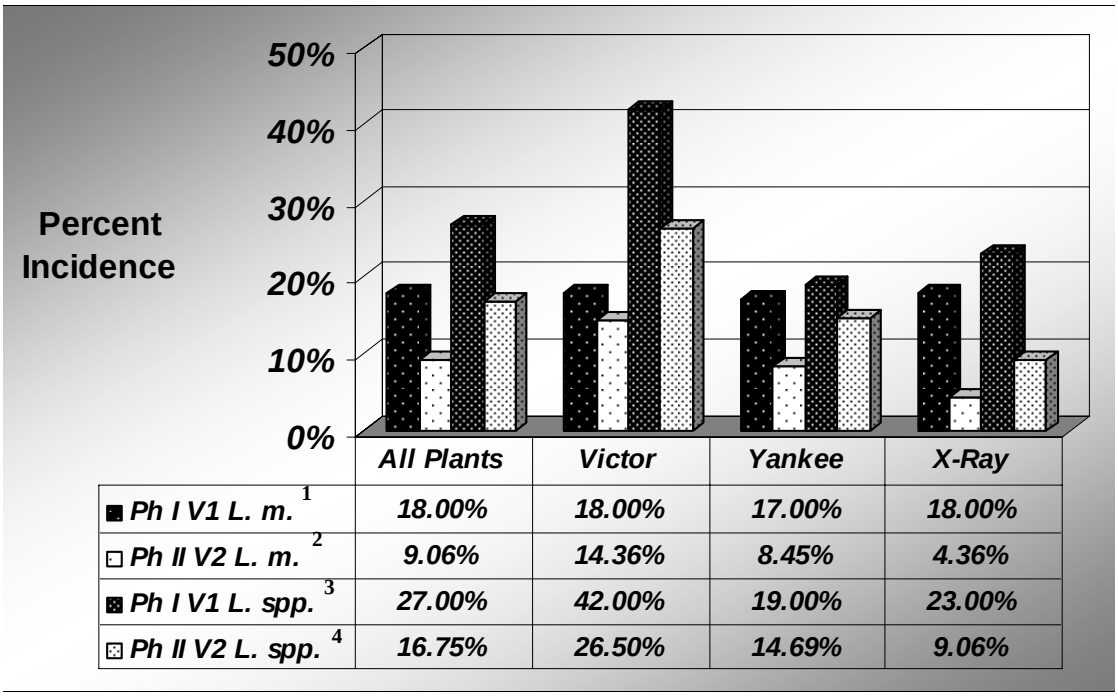
Figure 39. Ribotype by Lineage.



General Note:
Lineage I, II and III refers to a genetic grouping by serotype. Correlating serotype and ribotypes gives a strong indication of which lineage group each ribotype falls into.

Correlating serotypes and ribotypes consistent with Nadon et al., (2001), and segregating ribotypes by genetic lineage, the *L. monocytogenes* ribotyped in this study fell into Lineage I, II, and III, 28.7%, 56.9% and 11.3% respectively. There were 3% of the ribotypes with no determined lineage.

Figure 40. Percent Reduction in *L. monocytogenes* and *L.* spp. in All Plants and Each Plant from Phase I to Phase II.



	All Plants	Victor	Yankee	X-Ray
Ph I V1 L. m. [1]	18.00%	18.00%	17.00%	18.00%
Ph II V2 L. m. [2]	9.06%	14.36%	8.45%	4.36%
Ph I V1 L. spp. [3]	27.00%	42.00%	19.00%	23.00%
Ph II V2 L. spp. [4]	16.75%	26.50%	14.69%	9.06%

[1]**Ph I V1 *L. m.*: The percent incidence of *L. monocytogenes* from the first visit of Phase I.**

[2]**Ph II V2 *L. m.*: The percent incidence of *L. monocytogenes* from the second visit of Phase II.**

[3]**Ph I V1 *L.* spp.: The percent incidence of *L.* spp. from the first visit of Phase I.**

[4]**Ph II V2 *L.* spp.: The percent incidence of *L.* spp. from the second visit of Phase II.**

Figure 41. Percent Reduction in Each Plant of *L. monocytogenes*.

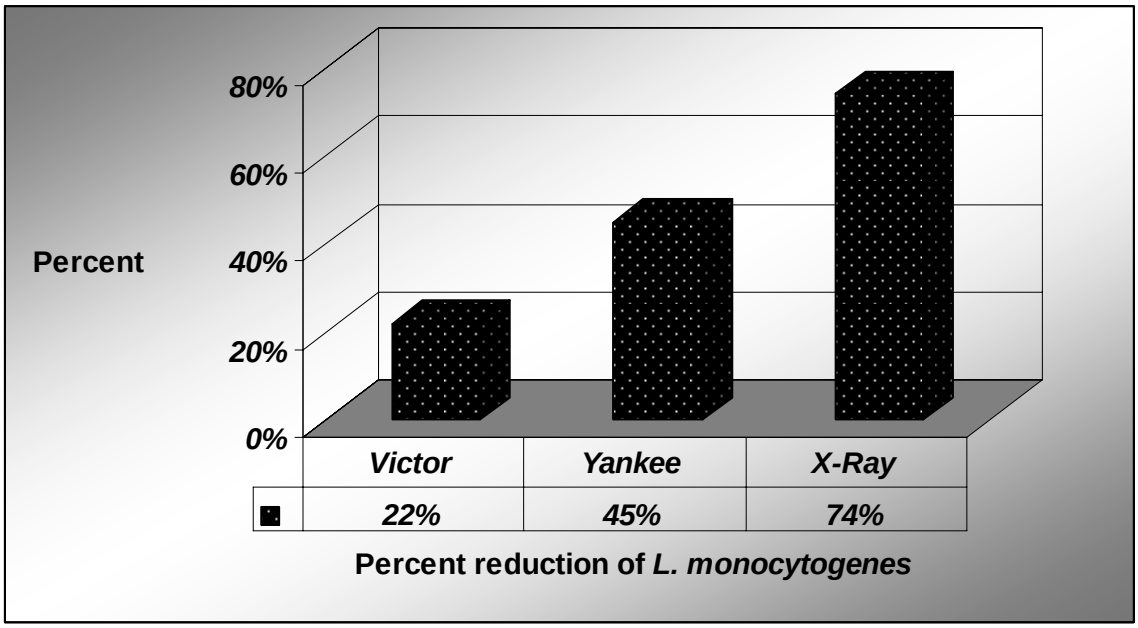
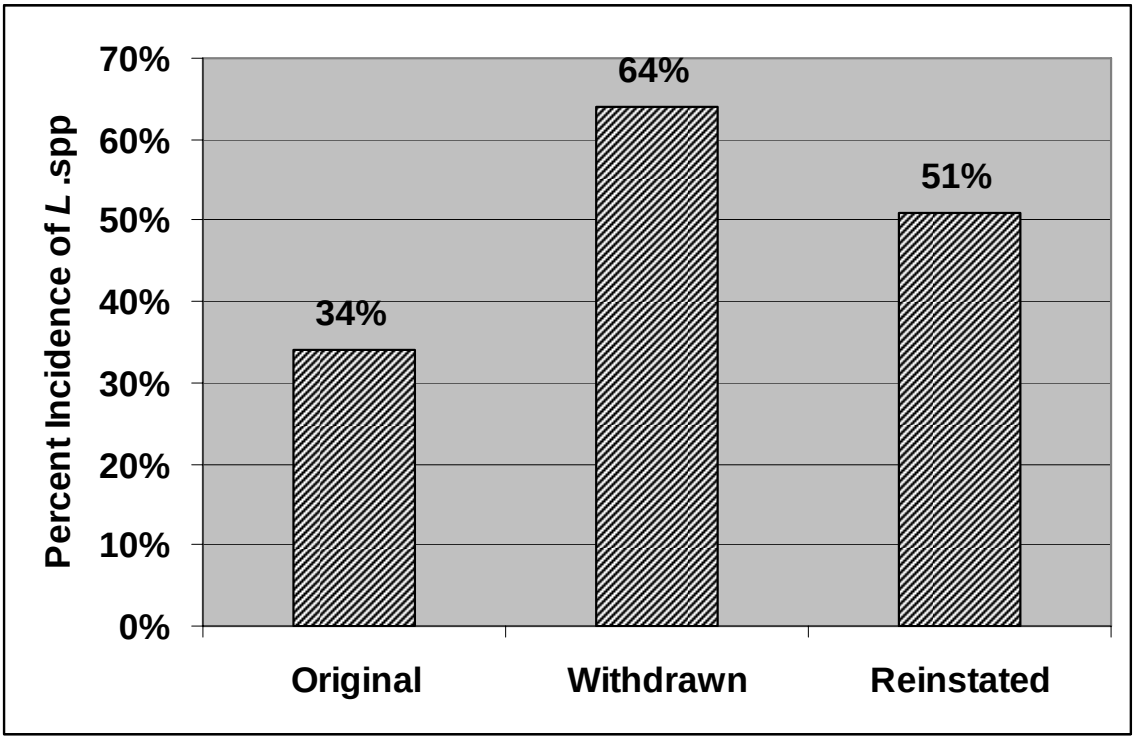


Figure 42. Incidence of *L*. spp. as a Result of Change in Boot Policy.



General Note:

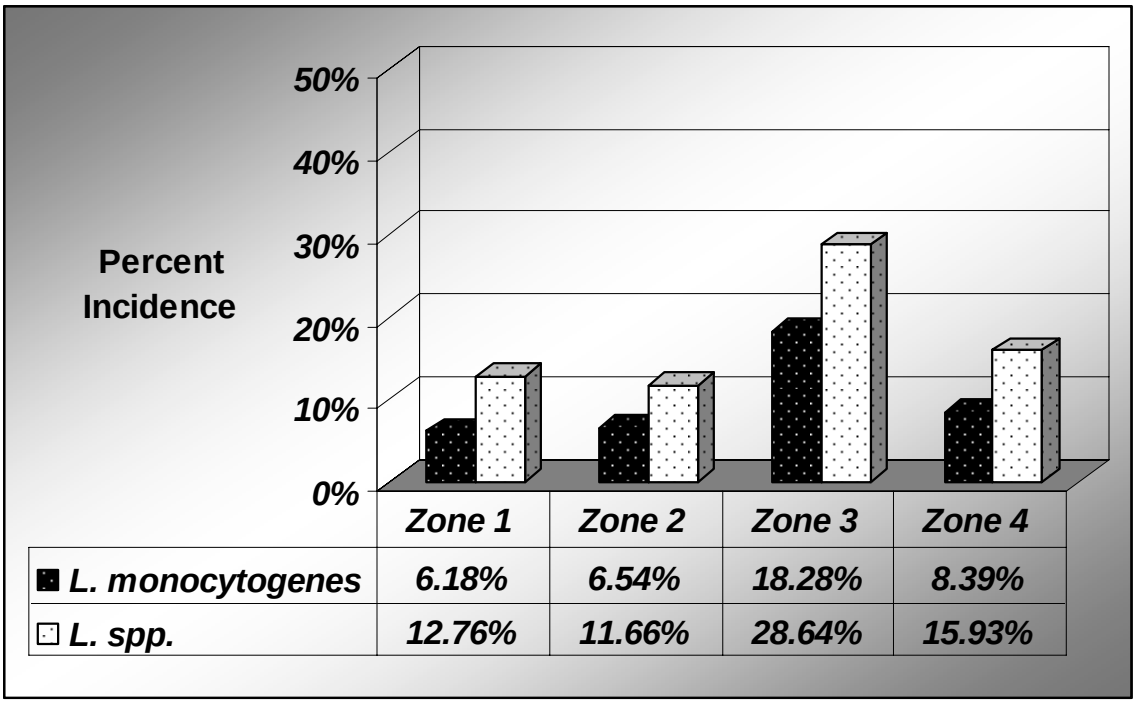
Using in-plant data, this depicts the percent incidence of *L*. spp. on samples taken from foot ware.

The "Original" percentage was the percent incidence when the initial policy of the plant providing boots for employees which had to be worn only in the plant.

The "Withdrawn" percentage was the percent incidence when, as a cost saving measure, plant management withdrew the policy and employees could wear their own foot ware (in and out of the plant).

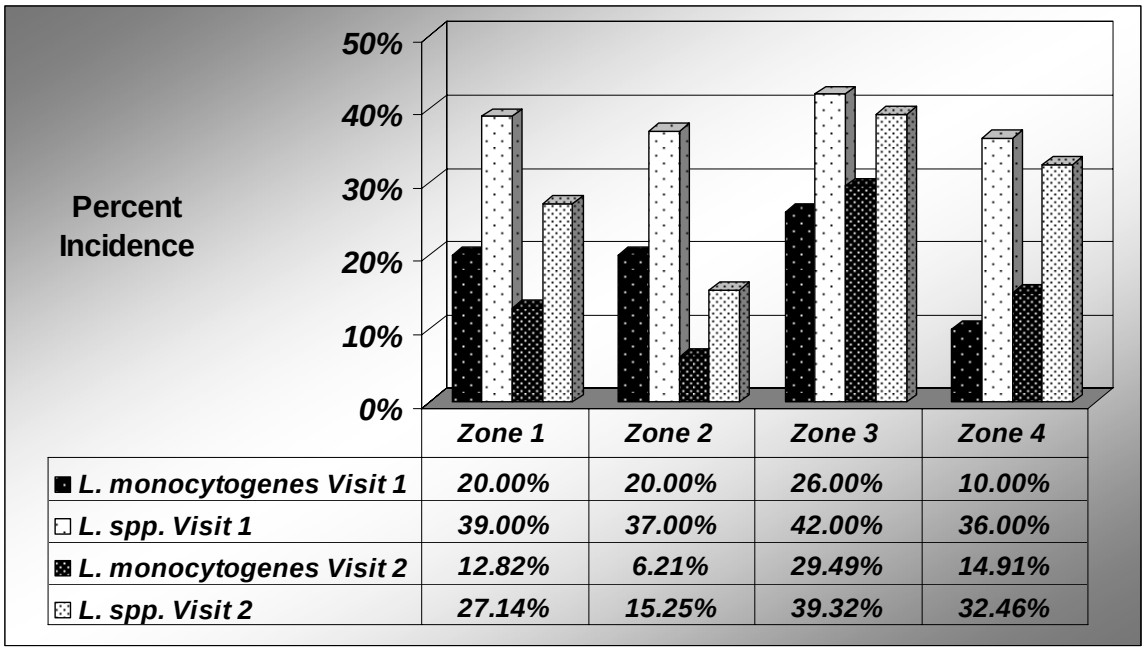
The "Reinstated" percentage shows the percent incidence of reinstating the original boot policy upon noting the dramatic rise in *L*. spp. after withdrawal of the policy.

Figure 43. Phase II: Incidence of *L. monocytogenes* and *L.* spp. for All Plants Segmented by Zone.



General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

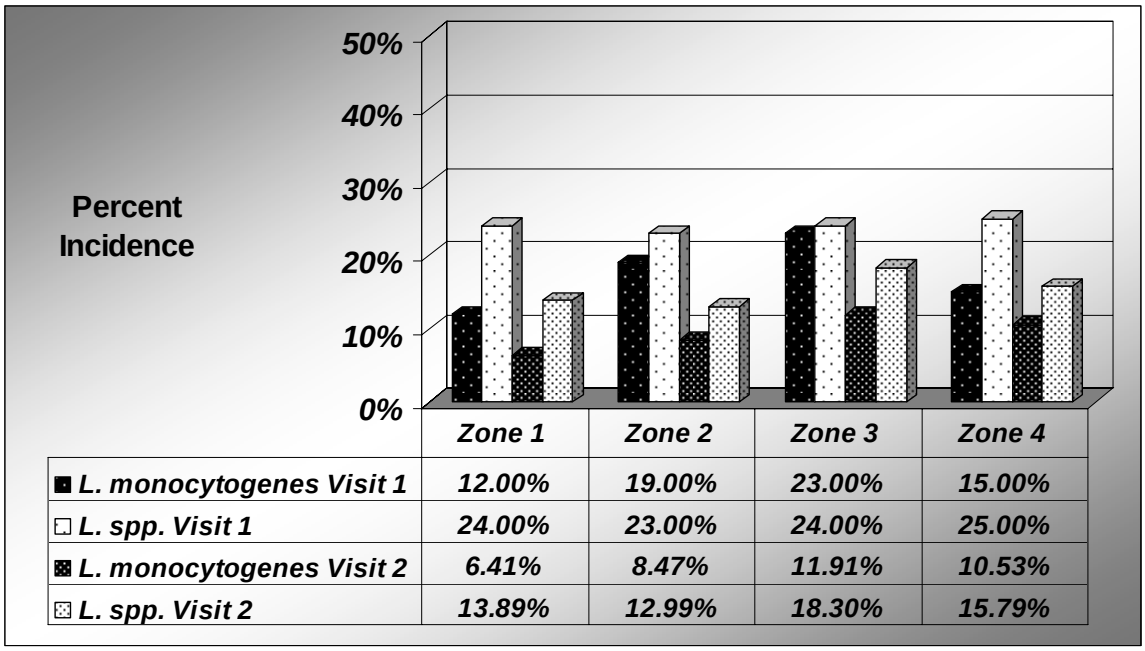
Figure 44. Phase II: Incidence of *L. monocytogenes* and *L.* spp. for Plant Victor Segmented by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L. monocytogenes Visit 1	20.00%	20.00%	26.00%	10.00%
L. spp. Visit 1	39.00%	37.00%	42.00%	36.00%
L. monocytogenes Visit 2	12.82%	6.21%	29.49%	14.91%
L. spp. Visit 2	27.14%	15.25%	39.32%	32.46%

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

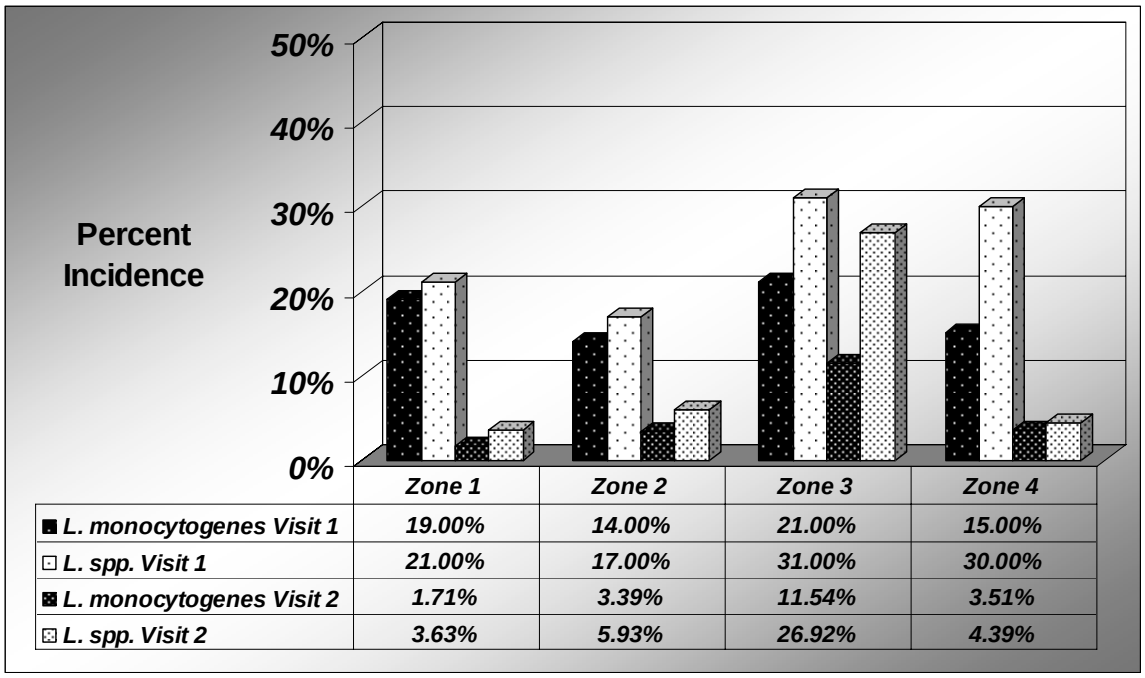
Figure 45. Phase II: Incidence of *L. monocytogenes* and *L.* spp. for Plant Yankee Segmented by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L. monocytogenes Visit 1	12.00%	19.00%	23.00%	15.00%
L. spp. Visit 1	24.00%	23.00%	24.00%	25.00%
L. monocytogenes Visit 2	6.41%	8.47%	11.91%	10.53%
L. spp. Visit 2	13.89%	12.99%	18.30%	15.79%

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

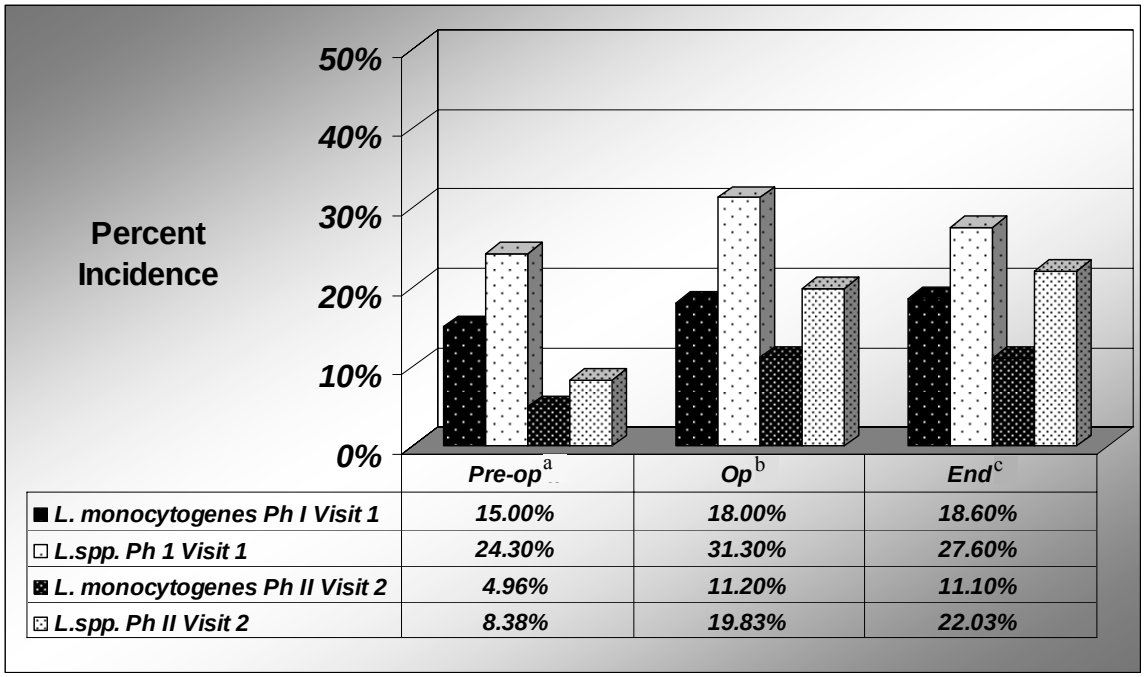
Figure 46. Phase II: Incidence of *L. monocytogenes* and *L.* spp. for Plant X-Ray Segmented by Zone.



	Zone 1	Zone 2	Zone 3	Zone 4
L. monocytogenes Visit 1	19.00%	14.00%	21.00%	15.00%
L. spp. Visit 1	21.00%	17.00%	31.00%	30.00%
L. monocytogenes Visit 2	1.71%	3.39%	11.54%	3.51%
L. spp. Visit 2	3.63%	5.93%	26.92%	4.39%

General Note:
Zone 1 – Final slice/packaging areas and coolers storing ready-to-eat (RTE) products.
Zone 2 – Common areas (RTE and raw material may cross), hallways and cook areas.
Zone 3 – Raw material storage and processing areas and coolers.
Zone 4 – Cafeteria, welfare facilities, dry storage and maintenance.

Figure 47. Phase II: Incidence of *L. monocytogenes* and *L.* spp. in All plants by Sampling time at the first visit of Phase I to the second visit of Phase II.



	Pre-op[a]	Op[b]	End[c]
L. monocytogenes Ph I Visit 1	15.00%	18.00%	18.60%
L.spp. Ph 1 Visit 1	24.30%	31.30%	27.60%
L. monocytogenes Ph II Visit 2	4.96%	11.20%	11.10%
L.spp. Ph II Visit 2	8.38%	19.83%	22.03%

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

General Note:

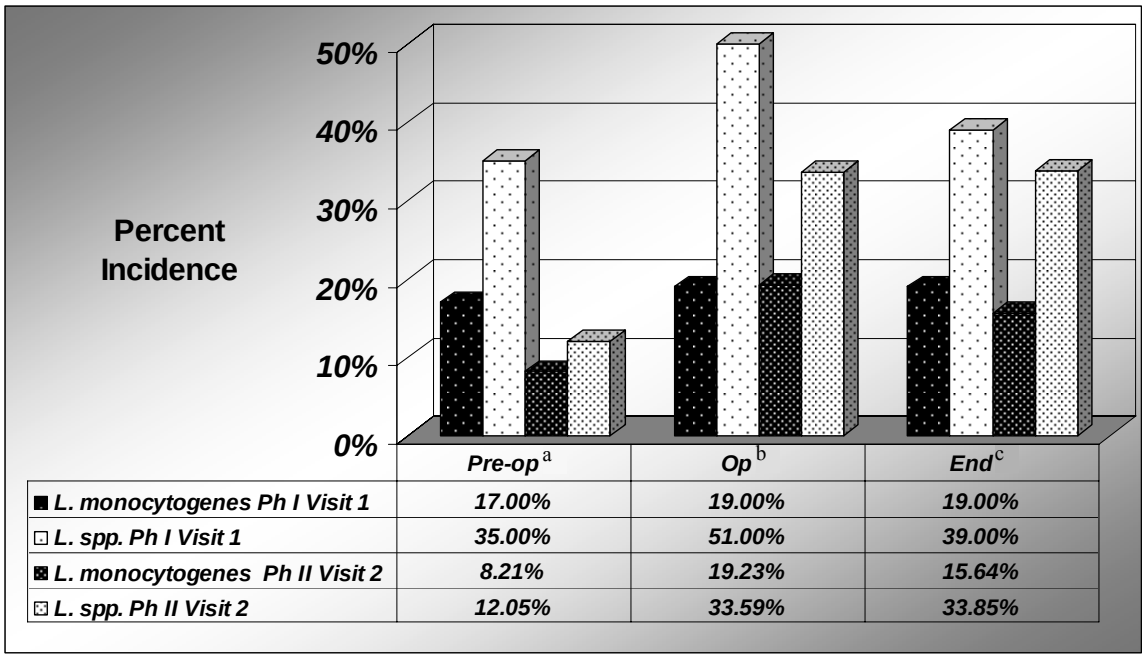
Ph I V1 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the first visit of Phase I.

Ph II V2 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the second visit of Phase II.

Ph I V1 *L.* spp. – The percent incidence of *L.* spp. from the first visit of Phase I.

Ph II V2 *L.* spp. – The percent incidence of *L.* spp. from the second visit of Phase II.

Figure 48. Phase II: Incidence of *L. monocytogenes* and *L.* spp. in Plant Victor by sampling time at the first visit of Phase I to the second visit of Phase II.



	Pre-op [a]	Op [b]	End [c]
■ *L. monocytogenes Ph I Visit 1*	17.00%	19.00%	19.00%
□ *L. spp. Ph I Visit 1*	35.00%	51.00%	39.00%
▩ *L. monocytogenes Ph II Visit 2*	8.21%	19.23%	15.64%
▨ *L. spp. Ph II Visit 2*	12.05%	33.59%	33.85%

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

General Note:

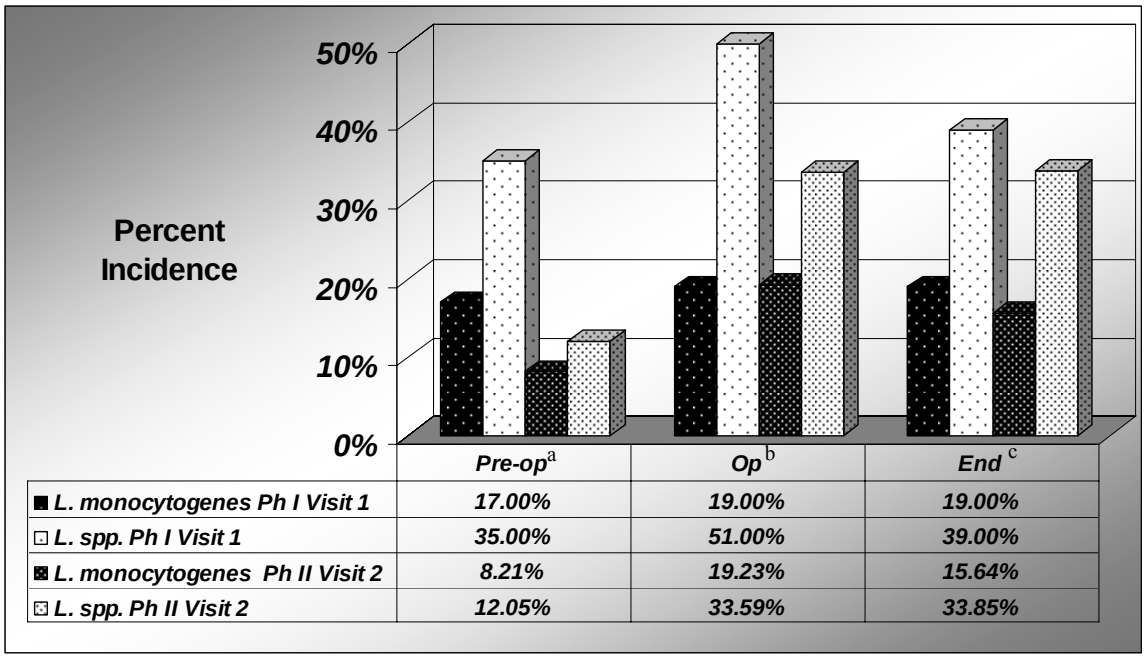
Ph I V1 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the first visit of Phase I.

Ph II V2 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the second visit of Phase II.

Ph I V1 *L.* spp. – The percent incidence of *L.* spp. from the first visit of Phase I.

Ph II V2 *L.* spp. – The percent incidence of *L.* spp. from the second visit of Phase II.

Figure 49. Incidence of *L. monocytogenes* and *L.* spp. in Plant Yankee by Sampling time at the first visit of Phase I to the second visit of Phase II.



	Pre-op[a]	Op[b]	End[c]
L. monocytogenes Ph I Visit 1	17.00%	19.00%	19.00%
L. spp. Ph I Visit 1	35.00%	51.00%	39.00%
L. monocytogenes Ph II Visit 2	8.21%	19.23%	15.64%
L. spp. Ph II Visit 2	12.05%	33.59%	33.85%

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

General Note:

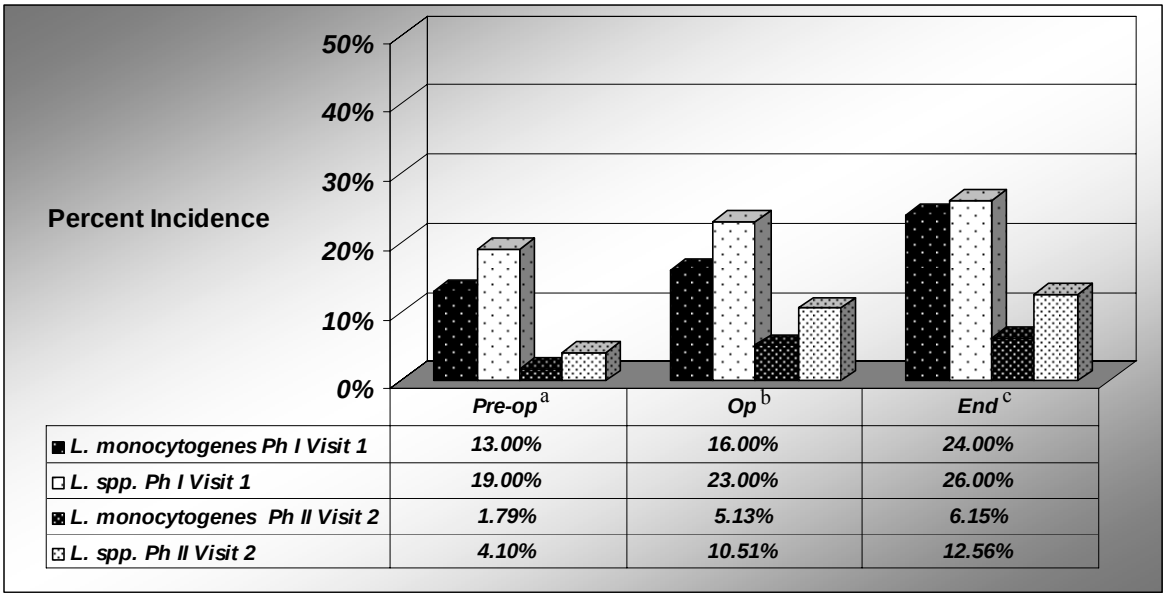
Ph I V1 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the first visit of Phase I.

Ph II V2 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the second visit of Phase II.

Ph I V1 *L.* spp. – The percent incidence of *L.* spp. from the first visit of Phase I.

Ph II V2 *L.* spp. – The percent incidence of *L.* spp. from the second visit of Phase II.

Figure 50. Incidence of *L. monocytogenes* and *L.* spp. in Plants X-Ray by Sampling Time at the First Visit of Phase I to the Second Visit of Phase II.



	Pre-op [a]	Op [b]	End [c]
L. monocytogenes Ph I Visit 1	13.00%	16.00%	24.00%
L. spp. Ph I Visit 1	19.00%	23.00%	26.00%
L. monocytogenes Ph II Visit 2	1.79%	5.13%	6.15%
L. spp. Ph II Visit 2	4.10%	10.51%	12.56%

[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

General Note:

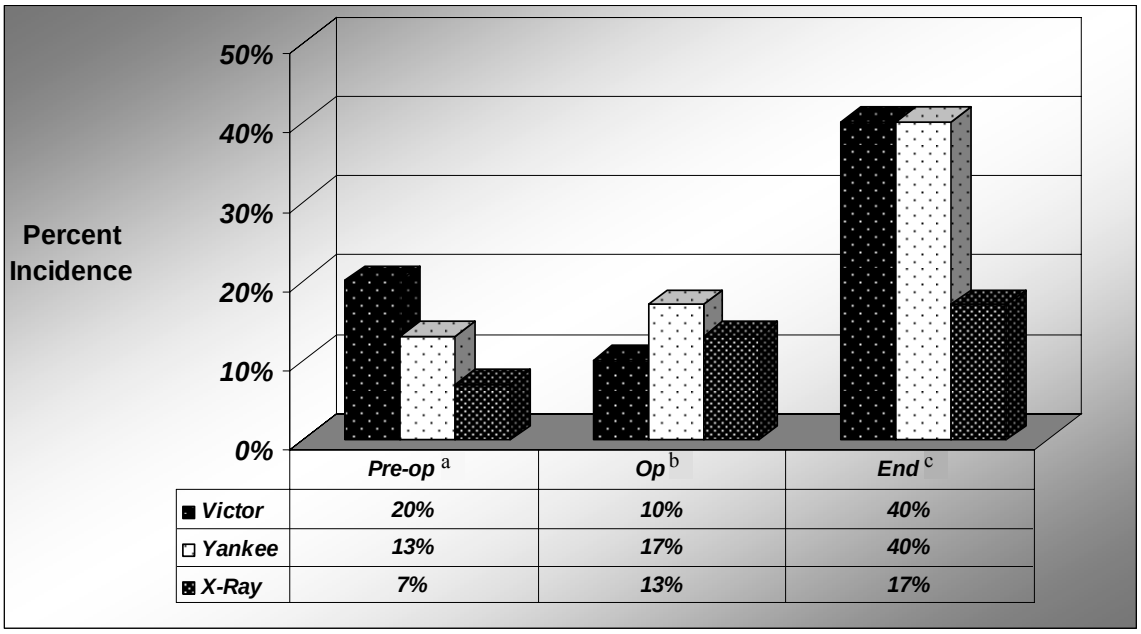
Ph I V1 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the first visit of Phase I.

Ph II V2 *L. monocytogenes* – The percent incidence of *L. monocytogenes* from the second visit of Phase II.

Ph I V1 *L.* spp. – The percent incidence of *L.* spp. from the first visit of Phase I.

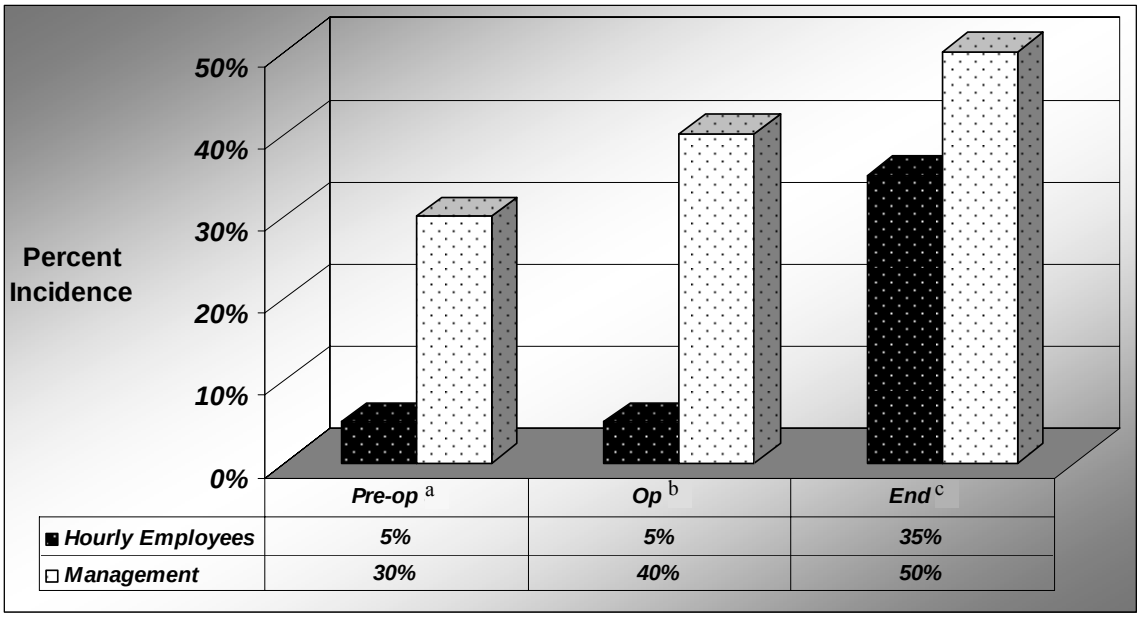
Ph II V2 *L.* spp. – The percent incidence of *L.* spp. from the second visit of Phase II.

Figure 51. Phase II: Incidence of *L.* spp. from Employee Boot Samples Segmented by Time at All Plants.



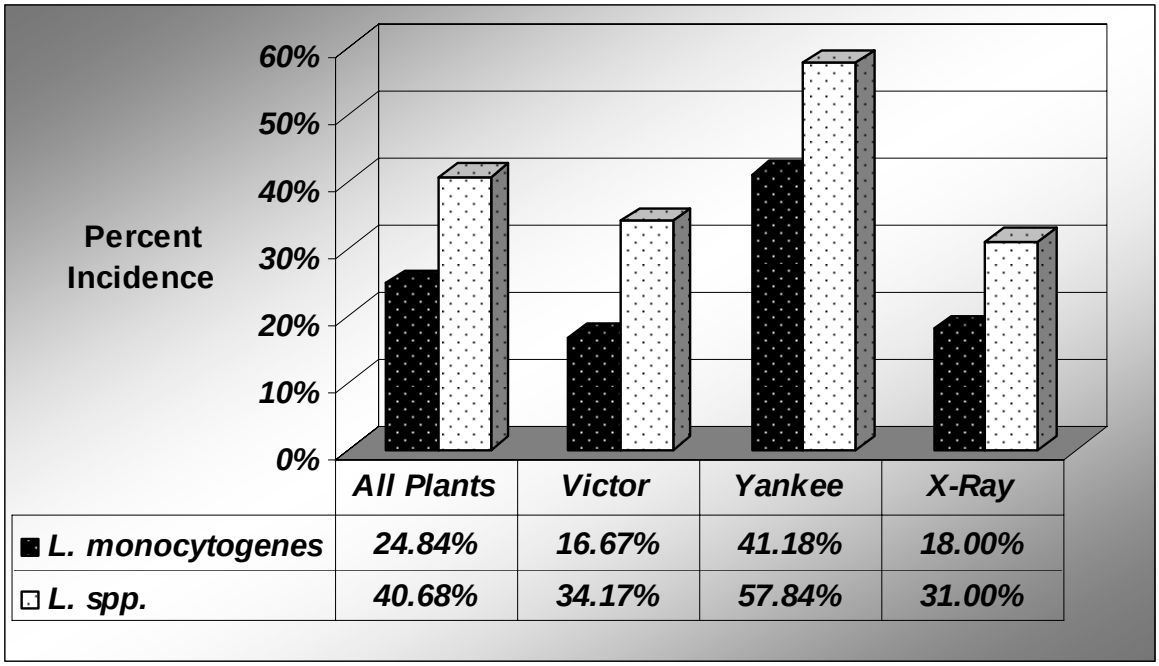
[a]Pre-op: Samples were taken prior to start of operations
[b]Op: Samples taken at the end of first shift
[c]End: Samples taken toward the end of second shift

Figure 52. Incidence of *L.* spp. on Footware of Management vs. Hourly Production Personnel Segmented by Time at Plant Yankee.



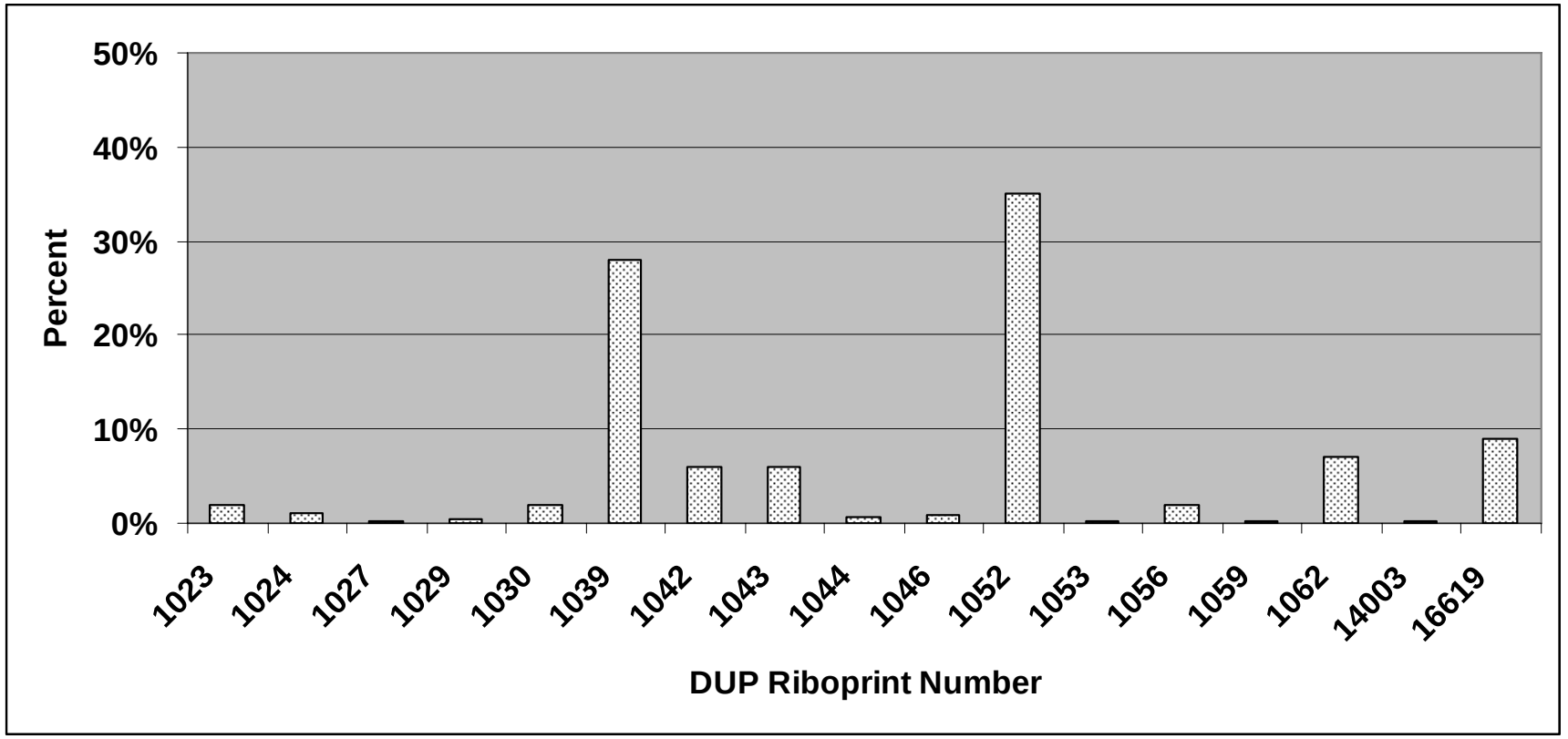
[a]**Pre-op: Samples were taken prior to start of operations**
[b]**Op: Samples taken at the end of first shift**
[c]**End: Samples taken toward the end of second shift**

Figure 53. Incidence of *L. monocytogenes* and *L.* spp. in Beef Raw Material (Trimmings) Samples for all Plants and Individual Plants.



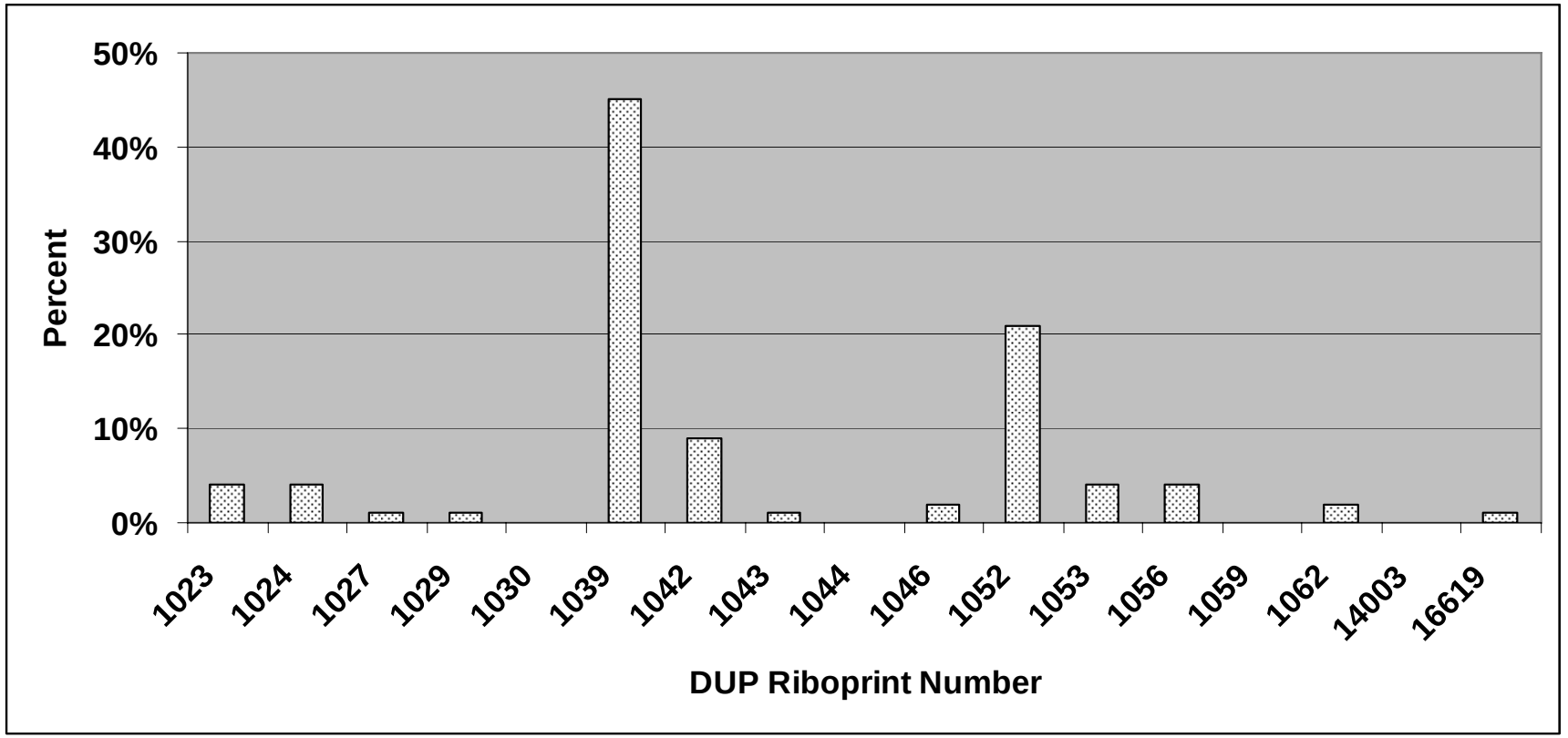
	All Plants	Victor	Yankee	X-Ray
L. monocytogenes	24.84%	16.67%	41.18%	18.00%
L. spp.	40.68%	34.17%	57.84%	31.00%

Figure 54. Phase II: Distribution of Riboprints Cumulative for All Plants.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

Figure 55. Riboprints of Lm Positive Samples from Beef Trimmings.



General Note:
DUP Number – Each riboprint pattern was compared to a library of ribotype patterns supplied by Qualicon, a DuPont company, and given its corresponding DUP identification number.

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VITA

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Candidate for the Degree of

Doctor of Philosophy

Dissertation: INCIDENCE AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* IN THE PROCESSING ENVIRONMENT OF FULLY COOKED, READY-TO-EAT BEEF PRODUCTS

Major Field: Food Science

Biographical:

Personal Data: Born in Friona, Texas on July 21, 1953, the son of Robert and Elsie Lloyd

Education: Graduated from Adrian High School, Adrian, Texas in May 1971; received a Bachelor of Science degree in Animal Science from Texas A&M University, College Station Texas, August of 1975; received a Master of Science degree in Animal Science from South Dakota State University, Brookings, South Dakota, December of 1978; Completed the requirements for Doctor of Philosophy degree in Food Science from Oklahoma State University, Stillwater, Oklahoma in December 2005.

Experience: Raised on a farm in rural Deaf Smith County, Texas. Employed by Monfort, Inc., Greeley Colorado (1978 – 1994); Employed by National Cattlemen's Beef Association, Denver, Colorado (1994 – 1996); Employed by the U.S. Meat Export Federation, Denver, Colorado (1996 – 2000); Employed by Foodbrands America and Tyson Foods, Inc., Oklahoma City, Oklahoma (2000 – present).

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Title of Study: INCIDENCE AND CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* IN THE PROCESSING ENVIRONMENT OF FULLY COOKED, READY-TO-EAT BEEF PRODUCTS

Pages in Study: 118 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and method of Study: Commercial ready-to-eat (RTE) facilities (five in Phase I, three in Phase II) were divided into four zones based on the contamination risk to the RTE product being produced. These zones include: Zone 1 (final slicing/packaging of RTE products); Zone 2 (common areas including hallways and cooling areas); Zone 3 (raw material receiving and storage); and Zone 4 (cafeteria and dry storage). Test facilities produced a variety product types (i.e. hot dogs, roast beef, sliced luncheon meats, pepperoni, tacos, taco meat, pastrami and pizza toppings). In this investigation, environmental samples (Phase I, n = 6,030; Phase II, n = 3,000) were collected from pre-selected, non-product contact sites in RTE facilities located in diverse geographical regions of the U.S. Raw material meat samples and air samples were collected and evaluated for *L. monocytogenes* in Phase II. An outside laboratory performed *Listeria* analysis and ribotyping.

Findings and Conclusions: Using information gathered during this project to develop corrective actions, there was a 50% overall reduction in *L. monocytogenes* positive samples from Phase I to Phase II. There were 19 and 17 different ribotypes identified in the isolates from Phase I and Phase II, respectively. The predominant ribotypes in this study were DUP-1039, DUP-1052, DUP-1059 and DUP-1062. Thirteen different ribotypes were identified from the 80 raw meat isolates evaluated, with DUP-1039 and DUP-1052 comprising 45% and 21% of the isolates, respectively. *Listeria monocytogenes* was not recovered from any of the air samples collected. In general, based on these data, the industry needs to continue to focus on floors, drains and traffic patterns in order to maintain control of *Listeria* and reduce the potential for cross contamination. *L.* spp. control must be implemented, monitored and maintained in adjoining areas to the RTE area in order to maintain adequate control in the processing facility.

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