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A Surveillance of Luncheon Meats for the Occurrence of *Listeria monocytogenes* in Grocery Stores in Tennessee

Jacob Henry Stevens
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Jacob Henry Stevens entitled "A Surveillance of Luncheon Meats for the Occurrence of *Listeria monocytogenes* in Grocery Stores in Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

F. Ann Draughon, Major Professor

We have read this thesis and recommend its acceptance:

David Golden, Svetlana Zivanovic

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Svetlana Zivanovic

Acceptance for the Council:

Anne Mayhew
Vice Chancellor and
Dean of Graduate Studies

(Original signatures are on file with official student records)

A SURVEILLANCE OF LUNCHEON MEATS FOR THE OCCURRENCE OF*LISTERIA MONOCYTOGENES* IN GROCERY STORES IN TENNESSEE

A Thesis
Presented for the
Masters of Science
Degree
University of Tennessee, Knoxville

Jacob Henry Stevens
May 2006

DEDICATION

I dedicate this to God, Abby, and Ms. “Lena Beth” who all taught me one thing or another both great and small. I am reminded of this verse, although I am not a very religious person, it reminded me to dig deep and go far:

Do you see a man skilled in his work? He will serve before kings; he will not serve before obscure men. –Proverbs 22:29

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I'm waiting for the time when I can finally say, this has all been wonderful but now I'm on my way. "Down with Disease" ~Phish

ABSTRACT

The current United States Department of Agriculture (USDA) and Department of Health and Human Services Food and Drug Administration (FDA) policy of 'zero tolerance' for *Listeria monocytogenes* in ready to eat (RTE) foods has prompted the food industry to increase food safety and sanitation practices in order to reduce and inhibit the growth of *L. monocytogenes*. In order to better understand the point of contamination of luncheon meats, a survey of its occurrence was performed in grocery stores throughout Tennessee. Approximately 1000 manufactured-packaged and 1000 deli-sliced luncheon meat samples were analyzed using the USDA-FSIS analytical method with minor modifications over a 10-month period. The overall occurrence of the organism was 0.8%. There were a significantly higher percentage ($P<0.05$) of positive deli-sliced samples than manufactured-packaged luncheon meat samples. Most of the positive samples had a population of less than 0.3 MPN/g, but *L. monocytogenes* was never recovered; in a few cases the population exceeded 110 MPN/g. Chromogenic agar provided more rapid confirmation of *L. monocytogenes* than modified oxford agar (MOX) agar. Results from this study showed that increased food safety practices are needed in grocery store deli environments in order to reduce occurrence of lunchmeat due to excessive, yet necessary handling.

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INTRODUCTION

The United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) regulate the manufacture, packaging, and storage of foods introduced into interstate commerce in the United States. The USDA is responsible for the regulation of meat, poultry, and egg products, while the FDA is responsible for all other food items. Ready-to-eat (RTE) foods receive substantial regulatory attention due to their propensity for post-processing contamination by foodborne pathogens and because they may be stored for long periods under refrigeration. This poses a particular problem with *Listeria monocytogenes* due to its ability to grow at refrigeration temperatures. Currently, the U.S. regulatory policy on *L. monocytogenes* in RTE foods is "zero tolerance", which requires that the organism be not detected in a 25-g sample of foods (Norrung 2000, Shank et al. 1996). If *L. monocytogenes* is detected, the food item is considered adulterated and unfit for human consumption by the USDA (United States Code, 2005a) and/or the FDA (United States Code, 2005b).

Some countries, such as Italy and Australia (Norrung 2000, Gianfranceschi et al. 2003) take the same stance as the U.S. regarding zero tolerance for *L. monocytogenes*. However, other countries including Germany, Netherlands, England, and France accept a tolerance level for *L. monocytogenes* of below 100 CFU/ml (g) at the point of consumption. Canada and Denmark establish tolerances based upon a category structure for RTE foods. (Norrung 2000, Farber and Harwig 1996). Category 1 foods (highest risk), luncheon meats and other delicatessen items that have been causally linked to outbreaks of

listeriosis, are regulated most strictly. Foods in this category that are adulterated with *L. monocytogenes* trigger a Class I recall with consideration of public alert. Category 2 contains all other RTE foods, which are capable of supporting growth of *L. monocytogenes.* Foods in this category are subject to class II recalls and possible consideration of public alert. Category 3 contains two types of RTE food products: those supporting growth with a <10-day shelf life and those not supporting growth. These products receive the lowest priority in terms of inspection, and products contaminated with *L. monocytogenes* of <100 CFU/g are within compliance (Farber and Harwig 1996). While some argue that zero tolerance is an unrealistic and unattainable goal, safety and quality programs such as Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices (GMP), in combination with additional post-processing lethality steps have been successful in reducing the occurrence of post-processing contamination with *L. monocytogenes*. In 2002, the Centers for Disease Control and Prevention (CDC) active foodborne disease surveillance network (FoodNet) reported that *L. monocytogenes* illnesses had the highest hospitalization rate (86.7% of reported cases) and the highest mortality rate (18.0%) of all foodborne pathogens (CDC 2004). While *L. monocytogenes* was responsible for less than 1% of total infections, the organism was responsible for 19 of 51 (37%) human deaths. New cases of human listeriosis have declined over the past 14 years, and in 2002 there were 0.27 cases per 100,000 population. Tennessee, a state participating in FoodNet surveillance, reported only three infections in 2002. The incidence of new cases of listeriosis

decreased 23% during this time period. These declines indicate important progress toward achieving the U.S. Department of Health and Human Services Healthy People 2010 objectives of reducing the incidence of listeriosis to 0.25 cases per 100,000 population by the end of the decade (CDC 2004). However, further work in preventing contamination in post-processing environments is necessary in order to reach the goal.

CHARACTERISTICS

L. monocytogenes is a Gram-positive facultative anaerobic and opportunistic microorganism that is capable of growing between -0.4 and 50°C in a wide array of environments including high salt and acidic conditions (Farber and Peterkin 1991, CFSAN-FDA 2005). It is the causative agent of listeriosis, which includes symptoms of diarrhea, nausea, body aches, and vomiting. Meningitis and septicemia often occurs in infected neonates. Although listeriosis can occur in healthy individuals, it is most problematic in high-risk populations that are immune compromised, such as cancer and AIDS patients, the elderly, neonates, and pregnant women (CFSAN 2005, Farber and Peterkin 1991). The organism can cross the placental barrier of pregnant women resulting in stillbirth, miscarriage and subsequent infection of neonates.

Murray *et al*. (1926) first described this organism as a pathogenic organism in diseased rabbits. Since then, *L. monocytogenes* has been isolated from a wide-array of environmental, water, and food samples. In addition, *L. monocytogenes* has been isolated from healthy and sick humans and other mammals (Farber and Peterkin 1991). Initially it was thought that *L.*

monocytogenes was only present in the environment as a soil-borne bacterium. However, in the 1980s a number of outbreaks were traced back to RTE foods that were contaminated with *L. monocytogenes* (Farber and Peterkin 1991, Kozak *et al*. 1996). Since that time, *L. monocytogenes* has been isolated from all categories of food. Due to its resilient nature, *L. monocytogenes* is capable of surviving at low levels under adverse environmental conditions. Despite proper processing steps, it is likely that RTE foods are contaminated during post-processing steps posing hazards to high-risk populations.

RISK ASSESSMENT

In a system as complex as the production of food, many factors affect both the likelihood and severity of foodborne disease (Lammerding and Fazil 2000). To effectively manage food safety in any environment, a systematic means of examining these factors is necessary. Risk assessment is a scientific and mathematically based investigation that provides an estimate of the probability and impact of adverse health effects attributable to potentially contaminated foods (Lammerding and Fazil 2000, Rocourt *et al*. 2003, Hitchings and Whiting 2001). Generally, the process asks three questions: (1) what agents are present in the food and capable of causing adverse health affects? (2) What is the likely frequency and level of consumption? (3) What is the nature of adverse effects? The answers to these questions are integrated to define a risk characterization (Lammerding and Fazil 2000). Once a risk assessment has been made, the information provides investigators with a better understanding of a particular organism and where gaps in general information exist.

In 2003, the USDA Food Safety and Inspection Service FSIS (2003) released information from a risk assessment for *L. monocytogenes* in RTE foods. The scope of this assessment involved: (1) providing insight into the relationship between *Listeria* species on food contact surfaces and *L. monocytogenes* in RTE meat and poultry products, and (2) evaluating the effectiveness of food contact surface testing and sanitation regimes. The assessment estimated potential levels of consumer exposure and characterized the likely impact of this exposure on three age-based subpopulations: prenatal, elderly, and intermediate age. As a result of this risk assessment, the investigators were able to categorize ready to eat foods into 23 categories based on risk of listeriosis among the three categories of populations. Deli meats in this assessment ranked the highest in this category.

OUTBREAKS INVOLVING RTE LUNCHEON MEATS

Under the current "zero tolerance" policy, ready to eat food meat items that have tested positive during routine FSIS microbiological sampling is considered adulterated. The food items are condemned unfit for human consumption and subject to government seizure and recall. Food companies have the option of voluntary recalls if the product has left the premises of the company (Jay 2000). Due to the speed and efficiency of shipping in refrigerated freightliners, the potential exists that contaminated food products could be available for retail purchase and consumption before a recall is announced.

A number of outbreaks that resulted in numerous illnesses and deaths have been a result of RTE meat products being contaminated with *L.*

monocytogenes. In August 1998, 40 cases of listeriosis that resulted in 4 deaths (one fetus and three elderly persons) were associated with consumption of hotdogs. Strains similar to those cultured from infected patients were isolated in an opened package of hotdogs. As a result, the manufacturer voluntarily recalled specific lots of hot dogs and other RTE meat products that may have been contaminated (CDC 1998).

In 2000, a multi-state outbreak of listeriosis occurred resulting in 29 cases that included four deaths and five miscarriages. The CDC implicated turkey deli meat as the source of infection. The manufacturer stopped shipping their product and issued a voluntary recall on potentially contaminated processed turkey and chicken deli meat (CDC 2000).

In 2002, the CDC reported an outbreak of listeriosis from precooked sliceable turkey deli meat distributed in the northeastern part of the United States. A total of 46 cases, ten fatalities, and 3 miscarried or stillborn children were linked to eating the contaminated deli meat. As a result, the manufactured recalled over twenty seven million pounds of fresh and frozen RTE turkey and chicken products (CDC 2002).

OCCURRENCE IN RTE LUNCHEON MEATS

Considering the ability of *L. monocytogenes* to grow over a wide range of temperature, pH, and salt concentration (Farber and Peterkin 1991), it is not surprising that the organism can be found in meat and meat products stored under proper refrigeration temperatures. The occurrence of *L. monocytogenes* in RTE foods is well documented, but there are very few published studies that

focus primarily on the surveillance of this organism in retail RTE sliced luncheon meats. Many studies that surveyed RTE foods for the presence of *L. monocytogenes* evaluated minimal luncheon meat samples among a myriad of other food items (Gombas *et al.* 2003, Levine *et al*.2001, Gillespie *et al*. 2000, Mena *et al.* 2004, Borges *et al.* 1999, Sakate *et al*. 2004).

Gombas *et al*. (2003) performed a thorough survey of the occurrence and population of *L. monocytogenes* in RTE foods collected in California and Maryland over a two-year period. Samples were collected from both the delicatessen and refrigerated cases of major supermarkets (73%) and smaller grocery stores (23%). Samples were screened and examined for the presence or *L. monocytogenes* in RTE cheeses, bagged salads, various seafood and pasta salads, and luncheon meats. *L. monocytogenes* was found in luncheon and deli meats with an occurrence of 2.7% and 0.4% from the delicatessen and refrigerated cases, respectively. The contamination level of *L. monocytogenes* ranged from less than 0.1 to 10^4 CFU/g.

Levine *et al*. (2001) collected and tested RTE meat and poultry products from approximately 1,800 federally inspected processing plants over a 10-year period for the presence of *L. monocytogenes* and other foodborne pathogens. Overall, *L. monocytogenes* was present in 2.8% of the samples (31,000 analyzed). The percentage of positive samples ranged from 0.52% in jerky to 5.16% in sliced ham and luncheon meats. For each individual year between 1990 and 1999, a decline was noted in the prevalence percentage indicating improvements in plant sanitation and reduction of post-process contamination.

Gillespie *et al.* (2000) examined cold, sliced RTE meats collected from catering establishments in the United Kingdom. A total of 3494 samples were collected and analyzed for the presence *of L. monocytogenes*. The organism was present in 13 samples (0.4%); however, only five samples that contained levels deemed unacceptable in the United Kingdom. The population of *L. monocytogenes* in positive samples was less than 10^3 CFU/g.

Wang and Muriana (1994) surveyed 20 brands of frankfurters for the presence of *L. monocytogenes* purchased from supermarkets. They compared the isolation from the internal meats and the liquid exudates surrounding the frankfurter. The organism was isolated in 7 of 93 exudate samples (7.5%) and was not isolated from internal meat samples, indicating that contamination most likely occurred post-processing. *L. monocytogenes* was the predominant *Listeria* spp. found among the commercially available frankfurters tested. The contamination level of the exudate using the MPN three tube methods ranged from 0.34 to 2.3 MPN/ml.

Ojeniyi *et al*. (2000) investigated the presence of *L. monocytogenes* at critical control points in a Danish turkey processing plant and its poultry providers. *L. monocytogenes* was isolated from 4 of 55 (7.3%) RTE turkey products and 12 of 101 (11.9%) raw turkey products. Subsequently, none of the samples taken directly from the turkey house was positive for the bacterium. Ojeniyi postulated that the post-processing contamination occurred due to poor sanitation at the plant.

Coillie *et al*. (2004) sampled a total of 252 RTE food products. These were mostly meat and fish products that were usually prepackaged and purchased from major and low-budget Belgian retail markets for the presence of *L. monocytogenes*. The organism was recovered from 23.9% (33 of 138) of the fish and 14.3% (11 of 77) of meat products. Contamination levels were generally low (<10 CFU/g), but levels greater than 100 CFU/g were detected in some of the seafood products.

Inoue *et al.* (2000) surveyed retail foods, including RTE salmon in five cities in Japan over a two-month period in 1999 for the presence and contamination level of *L. monocytogenes.* The organism was isolated in 5 of 92 (5.4%) smoked salmon examined, with contamination levels not exceeding 4.3 MPN/g. *L. monocytogenes* was isolated in 3.3% (7 of 213) of the total samples examined, with overall contamination levels ranging from less than 0.3 to over 400 MPN/g.

In a similar study, Nakamura *et al.* (2004) investigated the occurrence of *L. monocytogenes* in RTE fish products in Osaka City, Japan. His team purchased a total of 95 items from local retail stores over one year. *L. monocytogenes* was isolated in 13% (12 of 95) of the samples, all from cold-smoked salmon and trout. The organism was more prevalent in the summer than in the winter; 75% of the samples (9 of 12) were isolated between June and September. The population of *L. monocytogenes* was all less than 100 CFU/g.

Gianfranceschi *et al.* (2003) collected 4185 raw and RTE food products from food production plants, supermarkets, and small food stores throughout Italy

from 1990-1999. Six percent (269 of 4185) of the RTE food products were positive for *L. monocytogenes.* The current policy in place in Italy for controlling *L. monocytogenes* is zero tolerance for RTE foods with the application of HACCP and GMPs in processing facilities.

Borges *et al*. (1999) surveyed the occurrence of *L. monocytogenes* in four types of salami belonging to five different brands purchased in retail markets in Rio de Janeiro, Brazil. The pathogen was detected in 13.3% (11 of 81) of samples of Italian-style salami. In a similar study in Brazil, Sakate *et al.* (2003) evaluated the occurrence and population of *L. monocytogenes* in 45 samples of pre-sliced, vacuumed-packaged salami purchased in federally inspected retail stores in Sao Paulo. The organism was isolated from 3 of 45 samples (6.7%) with an average population of 9.2 MPN/g.

Wilson (1995) examined over 8000 RTE foods for the presence of *Listeria* spp., specifically *L. monocytogenes* from retail displays in Ireland. A total of 5% of the samples were positive for *Listeria* spp. with *L. monocytogenes* being isolated most. *L. monocytogenes* was isolated in less than 1% of the samples (49 of 8360). Fourteen of the samples had a population of greater than 100 CFU/gram of food sample.

Rorvik *et al.* (1991) investigated foods purchased from retail stores, processing plants, and butcher shops in Norway for *L. monocytogenes.* A total of 105 RTE meats including fermented sausage and processed meats were analyzed. The organism was recovered from 3.8% of the samples analyzed.

Mena *et al*. (2004) examined the occurrence of *L. monocytogenes* in different food products including RTE delicatessen meat items purchased from Portugese producers and retailers. A total of 3% of the 132 samples were positive for *L. monocytogenes*.

LISTERIA MONOCYTOGENES AND *LISTERIA* SPP. ON FOOD CONTACT SURFACES

On February 27, 2001 FSIS issued a proposed rule to require that all establishments that produce RTE meat and poultry products conduct environmental testing of food contact surfaces for *Listeria* spp. after lethality treatment and before final product packaging (FSIS, 2001). Post-processing contamination has been recognized as the mode of transmission of *L. monocytogenes* from food contact surfaces to the food item. Safe food handling of RTE foods is a key factor in the reduction of foodborne illness and outbreaks. In their review, the FDA's Center for Food Safety and Applied Nutrition (CFSAN) noted that out of a total of 72 articles that described 81 outbreaks believed to have occurred due to poor hygiene by food workers, *L. monocytogenes* was not a causative agent among 16 pathogens mentioned (CFSAN-FDA 1999). Given the ubiquitous nature of the *L. monocytogenes*, the potential exists for an outbreak to occur, particularly when environmental or personnel sanitation is poor. In the description of their findings (CFSAN-FDA 1999), the majority of the outbreaks involved the transmission of foodborne pathogens by food workers' hands (specifically mentioned in 34 outbreaks). Poor personal hygiene, lack of hand washing, improper glove use, unsanitary contact surfaces, cross

contamination, and improper holding temperatures were also associated with outbreaks. The majority (93%) of the cases involved workers who were ill either prior to or at the time of the outbreak.

In 2000, the FDA released a report that established a baseline on the occurrence off foodborne disease risk factors within the retail segment of the food industry. The data were collected during routine field inspections of retail facilities, including deli departments. In a summation of their findings, 74% of the delis were in compliance with the 1998 Food Code. Risk factors that were cited as out of compliance were incidents of poor personal hygiene and improper food holding times (FDA 2000)

Zivkovic *et al.* (1998) analyzed a total of 94 swabs taken from various workstations on both the "ready cooked" and raw chicken production lines. A total of 37 of 94 (39.4%) samples were positive for *Listeria* spp., suggesting that a major source of contamination comes directly from the work and production environment of a processing plant. The study did not publish distinction in species of the *Listeria* isolates; however the occurrence of *Listeria* spp. in general indicates the potential for *L. monocytogenes* to be present on contact surfaces.

Sergelidis *et al*. (1997) investigated the presence of *L. monocytogenes* in domestic, retail, and industrial refrigerators in Greece. They also determined the effectiveness of refrigerators in maintaining proper temperature in order to determine if a positive correlation between temperature abuse and organism presence existed. Refrigerator handles and locations that were commonly in

contact with food products were swabbed to determine the presence of *L. moncytogenes.* A total of 395 refrigerators and 107 handles were examined. The organism was isolated from 1.8% of refrigerators (7 of 395) and 1.4% of handles (2 of 138). The dairy and meat plant maintained temperatures less than 4°C in refrigerators throughout the course of the study; however the retail and domestic refrigerators had instances of temperatures in the range of 8 to 13°C. Despite the temperature abuse observed in home refrigerators, Sergelidis *et al*. (1997) could not conclude that a correlation existed between temperature abuse and organism presence. Since *L. monocytogenes* was found on and in refrigerators, these surfaces could be potential sources of contamination for RTE foods.

A number of studies have investigated the transfer of *L. monocytogenes* from RTE meats and cheese to food contact surfaces (Vorst *et al.* 2005, Vorst *et al*. 2003, Lin *et al*. 2004). Vorst *et al*. (2005) inoculated one-year-old stainless steel blades of different grade (304 and 316, respectively) with a six strain *L. monocytogenes* cocktail at levels of 10^8, 10^5, and 10^3 CFU/blade. Chubs of bologna, salami, and roast turkey were sliced (30 slices), diluted in phosphate buffer solution, and plated onto modified Oxford agar (MOX). The transfer of 10^8 CFU/blade resulted in logarithmic decreases while transfer of 10^5 and 10^3 were sporadic. Greater tailing of *L. monocytogenes* was observed on grade 304 blades, which physically appear rougher after one year's use, than 316 blades ($P< 0.05$). In a separate study, Vorst *et al*. (2003) investigated the transfer of *L. moncytogenes* (10^6 CFU/cm^2) to different areas of a deli meat slicer. The

investigators were able to recover and quantify *L. monocytogenes* on the metal blade guard, blade, collection area, and table. Lin et al. (2004) inoculated a deli meat slicer blade with a population 10^3 CFU of a 5-strain *L. monocytogenes* cocktail. The authors investigated differences in recovery of the species from bologna, roast beef, and oven roasted turkey that had had been sliced and vacuum packaged for 30 days. The number of *L. monocytogenes* positive samples increased during storage of turkey meat but decreased for salami and bologna due to the additional growth inhibitors.

CHROMOGENIC MEDIA FOR THE RECOVERY OF *LISTERIA MONOCYTOGENES*

Currently, USDA and FDA methods are the most commonly used protocols in the U.S. to detect and isolate *Listeria* spp. in food items. Warburton *et al.* (1991) performed a comparative study of both methods and found the USDA method to be slightly more efficient in isolating the organism in foods. Both methods rely on the use of MOX and PALCAM agars to select for *Listeria* spp. Typical listeriae colonies growing on these media are detected by the activity of the enzyme β-D-glucosidase. Grey-green colonies occur when the enzyme hydrolyses esculin (Gracieux *et al*. 2003, Reissbrodt 2004). Blackish-brown halos become evident due to the reaction of the breakdown product, esculin, with ferric iron. A disadvantage of conventional media is that *L. monocytogenes* is not differentiated from other *Listeria* spp., potentially allowing the underestimation of the population of *L. monocytogenes* within a bacterial population. Additional biochemical and hemolytic testing must take place in

order to differentiate *L. monocytogenes* from other *Listeria* spp. In most cases, definitive results can take up to 5 to 7 days (Gracieux *et al*. 2003).

Chromogenic media have an advantage over conventional media by not only selecting for, but also differentiating *L. monocytogenes* from other *Listeria* spp. through the utilization of characteristic enzymes and virulence genes specific to *L. monocytogenes.* This advantage allows for detection, upon enrichment, of *L. monocytogenes* in a more timely, convenient, and less expensive manner. The FDA Bacteriological Analytical Manual (BAM) protocol currently recommends the use of chromogenic plating upon isolation of suspected *Listeria* colonies on conventional media.

The first chromogenic agar to be marketed for *Listeria* was Agar *Listeria* (ALOA™), according to Ottaviania and Agosti (Reissebrodt 2004). In the ALOA™ medium, the chromogenic compound, X-glucoside, is added as substrate for the detection of β-glucosidase, which is common for all *Listeria* spp. A series of antimicrobial agents also found in PALCAM provides ALOA™ with its selectivity characteristics (Vlaemynck *et al.* 2000, Leclercq, 2004). The differentiation of *L. monocytogenes* from other *Listeria* spp. is based on the production of a phosphatidylinositol-specific phospholipase C (PI-PLC) by *L. monocytogenes* strains that cleaves the specific purified substrate added to the medium. *L. monocytogenes* produces a bluish turquoise colony with an opaque clear-cut halo (Vlaemynck *et al*. 2000, Sacchetti *et al*. 2003, Reissbrodt, 2004). On ALOA™, *L. ivanovii*, *Staphylococcus aureus*, certain *Enterococcus* spp. and

Bacillus cereus can be mistaken for *L. monocytogenes* due to their ability to produce phospholipase C, atypical morphology, or the lack of selectivity (Vlaemynck *et al*.2000, Karpiskova *et al.* 2000, Leclercq 2004).

Rapid L'Mono medium is based on the chromogenic detection of PI-PLC, which is demonstrated by hydrolysis of X-inositol phosphate contained in the agar that produces a blue staining of *L. monocytogenes* colonies (Leclercq 2004). *L. monocytogenes* is unable to ferment xylose that is present in the medium. *L. ivanovii* utilizes the carbohydrate allowing for the differentiation of the two species by the appearance of bluish-green colonies surrounded by a yellow halo surrounding *L. ivanovii* colonies (Reissbrodt 2004).

Comparison studies of chromogenic versus conventional media including Oxford, MOX, and PALCAM have focused primarily on selectivity and recovery of *L. monocytogenes* in both spiked and naturally contaminated food samples. Additional analyses have compared the sensitivity and specificity of both types of culture media with the aim of determining the efficiency of chromogenic media by comparing convenience, cost, and analysis time. Karpiskova *et al*. (2000) investigated 990 food samples obtained from retail markets for the presence of *L. monocytogenes* on plated Oxford, PALCAM, and Rapid L'Mono agar media with the aim of shortening the time for a more precise identification and confirmation. On chromogenic media, typical *L. monocytogenes* isolates were selected and identified up to 24 hours faster than the conventional medium. Additional confirmatory testing had to be performed on the presumptive positives of the conventional medium. The investigators found the chromogenic medium to be

“very convenient and useful” and noted that the chromogenic medium provided a “non-negligible saving of time”.

Sacchetti *et al.* (2003) performed a similar comparative study in which the sensitivity and specificity of the plating media were calculated. The chromogenic media produced a higher percentage of positive samples for *L. monocytogenes* (39.4% with ALOA, 34.8% with Rapid L’Mono) than with conventional media (22% with PALCAM and Oxford). ALOA gave no false negatives. Rapid L’Mono gave 6 (4.5%) false negatives, while the total for the conventional media was 23 (17.4%) (Sacchetti *et al.,* 2003) The chromogenic media had no false positives, while the conventional media produced 33 false positives (25%).

Vlaemynck *et al.* (2000) investigated the sensitivity, specificity, and selectivity of ALOA in comparison to conventional plating media using naturally and artificially contaminated samples of dairy, cheese, and meat products. ALOA detected 4.3% more positives from naturally contaminated dairy and meat samples. ALOA had 13.9% false negatives compared to 38.9% using the conventional media to detect *L. monocytogenes* in food samples. In samples that were artificially contaminated with mixed listeriae populations, ALOA recovered more colonies than Oxford and PALCAM. No false positives or false negatives were detected using the chromogenic agar. *L. monocytogenes* was not detected on Oxford agar from primary and secondary enrichments in 12 of 56 (21.4%) positive samples that were all detected on ALOA.

RESEARCH OBJECTIVES

The primary objective of this study was to determine the occurrence of *L. monocytogenes* in RTE luncheon meats using molecular and traditional microbiological technology to screen the samples and to assess the levels of the organism in the positive samples using the USDA three-tube MPN test. The study consisted of the collection of 2000 samples over a 10-month period that extended across the state of Tennessee. The first objective for this project was to compare the recovery of *L. monocytogenes* on chromogenic and conventional culture media using increased sample sizes and modified dilutions. The second objective for this project was to compare the occurrence and level of contamination of *L. monocytogenes* in sliced-to-order luncheon meat from deli departments and manufacturer-packaged luncheon meat. Results from this study will provide greater insight into the occurrence of the organism and the level of compliance by food processors.

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PART II
A COMPARISON OF PLATING MEDIA AND DILUTION RATIOS FOR RECOVERING *LISTERIA MONOCYTOGENES* FROM LUNCHEON MEAT PURCHASED FROM RETAIL GROCERY STORES THROUGHOUT TENNESSEE

ABSTRACT

While rapid detection methods have become quicker and more convenient, classic microbiological culture methodology remains more efficient, yet slow, at detecting and isolating *Listeria monocytogenes* in ready-to-eat foods. Recently, chromogenic plating agar have shown promised for reducing confirmation time by selecting for and differentiating microorganisms by targeting exclusive genes and enzymes. Over 1500 luncheon meat samples were surveyed using the USDA microbiological method in order to compare the recovery of *L. monocytogenes* and other *Listeria* species on MOX agar and two chromogenic plating media (Rapid'L. Mono and CHROMagar™ *Listeria).* No significant differences ($P > 0.05$) were found among the three agars in recovery of *L. monocytogenes* and *Listeria* species. Rapid'L. Mono had the highest sensitivity (100%) and specificity (100%) for detecting *L. monocytogenes*, while MOX agar was the most efficient in detecting *Listeria* species. Results from this study showed that chromogenic agars were able to efficiently detect and identify *L .monocytogenes* thereby reducing the need for time-consuming confirmation of non-*L. monocytogenes* colonies.

INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis. While symptoms are limited and quite rare in healthy adults, the illness is more problematic in immune compromised populations. Listeriosis is characterized by flu-like symptoms that include diarrhea, nausea, body aches, and vomiting. Miscarriages and stillbirths have been linked to pregnant women consuming

contaminated food items. (Farber and Peterkin 1991, CFSAN-FDA 2004). FoodNet reported (2004) that *L. monocytogenes* had the highest hospitalization and the highest mortality rate for all foodborne pathogens, yet it was only responsible for less than 1% of total infections reported.

L. monocytogenes is a Gram-positive, facultative anaerobe that is characterized by its ubiquitous and resilient nature. In their extensive review, Farber and Peterkin (1991) noted that *L. monocytogenes* is capable of surviving and growing in a wide range of pH, temperature, water activity (Aw), and salt concentrations. However, the foodborne pathogen is not particularly resistant to heat thus easily destroyed during processing of food items. *L. monocytogenes* can survive under adverse conditions such as the inhospitable environment of food contact surfaces thereby potentially contaminating post-processed foods. As a result, the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) are currently enforcing a policy of zero tolerance in order to prevent exposure of the pathogen to high risk populations from consumption of RTE foods contaminated in the post-processing steps (Shank *et al.* 1996). Food safety and quality programs such as Hazard Analysis Critical Control Points (HACCP) and Good Manufacturing Practices (GMPs) have been implemented in order to control the potential of spread and cross contamination of foodborne pathogens in food processing and storing environments. In addition, post-lethality treatments and/or additional antimicrobials in food formulations are put in place to inhibit *L. monocytogenes.* Food-processing plants are routinely inspected for the presence of *L.*

monocytogenes on their production line and RTE products in order to ensure effectiveness of processing and sanitation and to determine compliance with federal laws. Violations could result in fines, seizures, recalls, and adverse reports in the media.

The USDA and FDA have developed analytical methods to test for the presence of *L. monocytogenes* in food items within their jurisdictions (Hitchins 2003). The FDA method consists of single enrichment step using Buffered Listeria enrichment broth (BLEB). The USDA method incorporated a two-step enrichment consisting of Listeria enrichment broth- University of Vermont Formulation (UVM-1) as a primary enrichment and Fraser broth for the secondary enrichment. The FDA method uses PALCAM and modified oxford agar (MOX) as their selective agars, while the USDA method only employs the use of MOX.

Recently, the scientific community has seen the advent of chromogenic culture media. Chromogenic substrates are compounds that react by changing color due to the reaction with of targeted enzymes present in bacteria exclusive to particular Gram reactions, genera, or species of bacteria (Manafi 2000). This reaction provides differentiation of species, whereas conventional agars, with few exceptions, are unable to differentiate species. Selective agents are added to inhibit bacterium *(Bacillus, Lactobacillus, Enterococcus*, etc.) containing targeted enzymes. This results in additional sub-culturing and biochemical testing to identify the species of a bacterium (Gracieux *et al.* 2003). For most *Listeria* chromogenic agars, differentiation is based on the presence of phospholipase C

(PIPLC). This enzyme is present in pathogenic *L. monocytogenes* and *L. ivanovii* (Reissbrodt 2004, *Sacchetti et al.* 2003). Colonies of these species will appear on CHROMagar™ *Listeria* plates as turquoise colonies with a clear halo. However, additional confirmation testing is necessary to differentiate between the two species. Another agar, Rapid L Mono, in addition to PIPLC detection, differentiates all species of *Listeria* based on xylose fermentation. Currently, the FDA method recommends the use of chromogenic agar in conjunction with PALCAM and MOX plates.

Traditionally, conventional enrichment methods for detection and recovery of *L. monocytogenes* generally involve 25g samples in 225 ml of enrichment broth (1:10 ratio) and varying in the number of enrichment steps. Due to the resilient nature of *L. monocytogenes*, it is possible that the organism can survive at low numbers in adverse environments, and then contaminate RTE foods during post processing. Increasing the sample size and/or decreasing the enrichment broth volume could improve recovery of the organism in RTE luncheon meats by five fold. However, under current conventional protocols that utilize a 1:10 ratio for enrichment, the expense of preparation and storage of large volumes of culture media could be a problem when analyzing large number of samples. Reducing the enrichment broth volume by using modified dilution ratios may overcome such problems. Little research on recovery of pathogens using different enriched ratios has been conducted.

The first objective of this study was to compare the efficiency of Rapid L'Mono, CHROMagar™ *Listeria*, and MOX agar plates in recovering *L.*

monocytogenes from luncheon meat purchased at retail stores throughout Tennessee. The second objective was to compare the qualitative and quantitative recovery of *L. monocytogenes* from artificially inoculated luncheon meat using modified and conventional dilution schemes.

MATERIALS AND METHODS

Sample Screening

The efficiency of various agar media selective for *Listeria* species was compared using the USDA microbiological method for isolation and identification of *L. monocytogenes* from meat, poultry, and egg products. (McClain and Lee 1998). A test portion of 125g of luncheon meat was blended with 200ml of *Listeria* enrichment broth- University of Vermont formulation (UVM-1) (Acumedia Manufacturers, Inc., Baltimore, MD) and stomached for 2 minutes at normal speed. An additional 125ml of UVM-1 was added and hand massaged for a final 1:4 dilution. The homogenate was incubated for 24 h at 32°C. 0.1ml was then transferred to 10ml of Fraser Broth (FB, Acumedia Manufacturers, Inc. Baltimore, MD) and incubated for 48 h at 35°C. Samples from positive FB tubes, indicated by a blackened appearance, were streaked onto Modified Oxford Agar (MOX, Acumedia Manufacturers, Inc. Baltimore, MD), Rapid'L. Mono agar (RLM, BioRad, Hercules, CA), and CHROMagar™ *Listeria* agar (CHROM, Beta Dickson, Franklin Lakes, NJ). MOX, RLM, and CHROM for 48 h were incubated at 37, 35, and 35°C, respectively. Additionally, known *L. monocytogenes* and *L. innocua* were streaked onto the various plating media to serve as standards for

comparison of typical colony morphology and quality control of biochemical testing media.

Biochemical Confirmation

Suspected *L. monocytogenes* colonies were picked and placed in trypticase soy broth containing 0.6% yeast extract (TSB-YE) (Difco™, Sparks, MD) and incubated for 24 h at 30°C. TSB-YE cultures were observed using phase contrast microscopy to determine the presence of tumbling motility of rod shape organisms. Positive cultures (Table 1, appendix) were tested for carbohydrate fermentation of 0.5% rhamnose, mannitol, and xylose. In conjunction with carbohydrate tests, trypiticase soy agar containing 10% Horse Blood (TSA-B) (Remel, Inc., Lenexa, KS.) were stabbed with the suspected *L. monocytogenes* culture and incubated for 36 h at 35°C in order to observe for hemolytic activity. Cultures that were positive for tumbling motility, rhamnose fermentation, and were hemolytic activity genetically confirmed using GeneQuence™ *Listeria monocytogenes*-specific DNA probe (Neogen Corporation, East Lansing MI.).

Sensitivity and Specificity

Sensitivity and selectivity of test media for recovery and isolation of *L. monocytogenes* in luncheon meat was determined as described by Restanino *et al*. (1999). Since MOX agar is only a selective agar, *Listeria* species (*L. innocua, L. welshmeri, L. grayii, and L. ivanovii*) as well as ”Listeria-like” bacteria (*Bacillus, Lactobacillus,* etc.) are considered false positives. CHROMager™ *Listeria* can only differentiate between pathogenic and non-pathogenic Listeria species;

therefore *L. ivanovii* would be considered a false positive in addition to any other bacteria that appears as blue colonies surrounded with a opaque halo. Rapid L'Mono differentiates all *Listeria* species, so colonies that were similar in appearance to *L. monocytogenes* that were later confirmed as negative are considered false positives.

% Sensitivity = 100 X [true positives/(true positives + false negatives)]

% Specificity = 100 X [true negatives/(true negatives + false positives)]

Enumeration Using Most Probable Number (MPN)

Luncheon meat samples (125g) were blended with 200ml of UVM-1 enrichment broth and homogenized by stomaching at normal speed for 2 min. Then 175ml or 925ml of UVM were added to obtain 1:4 and 1:10 dilution ratios, respectively. The dilutions were inoculated with approximately 1, 10, and 100 CFU/g of *L. monocytogenes*. A nine-tube MPN assay was performed. Three aliquots (10, 1, and 0.1 ml) of the sample homogenate representing 0.1, 0.01, and 0.001 g of the original sample were dispensed into the three sets of tubes. The tubes were incubated at 32°C for 24 h and 0.1 ml from each tube was transferred to 10 ml Fraser Broth and incubated up for to 48 hours at 35°C. After incubation, tubes were examined for the presence of hydrolyzed esculin as indicated by a blackened appearance. The MPN was calculated using the tables provided by the USDA. Blackened tubes at the highest dilution were streaked onto RLM and MOX agar to determine the presence of *Listeria monocytogenes*. Typical *L. monocytogenes* colonies on MOX were confirmed utilizing biochemical testing consisting of carbohydrate fermentation, motility, and blood hemolysis.

Growth Comparison

Duplicate luncheon meat samples (containing no curing agents) of 125g were aseptically placed in Whirl-PAK™ bags and inoculated with 10 CFU/125g of *L. monocytogenes.* A volume of 200ml of UVM-1 was added and the samples were homogenized by stomaching at medium speed for 2 min; 175 and 925ml of UVM-1 were added to obtain 1:4 and 1:10 dilution ratios, respectively. The UVM-1 homogenates were incubated at 32°C. Samples (1 ml) were taken every three hours, diluted in .1% Peptone water (Beta, Dickinson and Company, Sparks MD.), and surface plated (0.1ml) on to CHROM agar. The plates were incubated at 35°C for 48 h. Positive *L. monocytogenes,* as indicated by a clear-cut halo surrounded a bluish-turquoise colonies, were confirmed using phase contrast microscopy, sugar fermentation (Rhamnose, Xylose, and Mannitol), and blood hemolysis.

Statistical Analysis

Data was analyzed using SPSS 13.0 (2004) statistical software package for Windows in order to compare the means. Significance of differences were seat at $P<0.05$.

RESULTS

Selective Plating Media Comparison

Over a 10-month period, a total of 1555 luncheon meat samples purchased from grocery stores throughout Tennessee were analyzed for the presence of *L. monocytogenes.* Due to the type of samples being analyzed, the USDA-FSIS microbiological method, with slight modifications, and additional

culture media were employed (McClain and Lee 1998). Since the number of positives is unknown in naturally contaminated samples the number of overall total positive samples that were genetically confirmed using Neogen Gene-Trak provided the true value. A total of six samples (0.3%) were positive for *L. monocytogenes*. All test agars detected *L. monocytogenes* from the six positive samples indicating that there were no significant differences among the media ($P > 0.05$). Based on the parameters of the study, either CHROM or RLM would be suitable replacements for MOX agar, and both provide more rapid species confirmation.

The sensitivity and specificity of the selective plating media are shown in Table 2. All figures and tables are located in the appendix. Under the conditions of the study MOX, CHROM, and RLM had 25, 4, and 0 false positives, respectively. All three media had a sensitivity of 100% and RLM had the highest specificity at 100%.

Comparison of Modified and Conventional Dilutions

There were no significant differences ($P>0.05$) in the MPN/ml between 1:4 and 1:10 dilutions when inoculated at 10 and 100 CFU/g (Table 3). However, when comparing the growth of *L. monocytogenes* in modified (1:4) and traditional (1:10) dilutions in UVM-1 enrichment broth for 24 h, the 1:4 dilution provided equal or greater population ($P< 0.05$) of *L. monocytogenes* than the 1:10 dilution. Growth of the organism was not detected on CHROM until the sixth sampling time (15 h) (Figure 1). At 24 hours, the population of *L. monocytogenes* was approximately 1 log higher in the modified dilution on CHROM. Under the

conditions of the study, modified dilutions could be an alternative to traditional dilutions when analyzing large sample sizes.

DISCUSSION

Selective Plate Medium Comparison

There was no significant difference (P>0.05) between RLM, MOX, and CHROM for detecting *L. monocytogenes* in luncheon meat. MOX agar had 25 false positive while RLM and CHROM had 0 and 4 false positives, respectively. None of the media provided false negatives. RLM allows for differentiation between *Listeria* species, whereas additional differentiation tests are necessary to distinguish between strains of *Listeria* appearing on CHROM and MOX. Under the conditions and results of this study, RLM is superior to CHROM in confirming the presence of *L. monocytogenes*. A number of studies found that chromogenic plating media have higher recoveries of *L. monocytogenes* than conventional media (Karpiskova *et al.* 2000, Bauwens *et al.* 2003, Vlaemynck *et al*. 2000, Sacchetti *et al*. 2003). There are conflicting reports regarding RLM. Karpiskova *et al*. (2000) found the chromogenic plating medium to give “results comparable to PCR” (Polymerase Chain Reaction). Gracieux et al. (2003), however, noted that RLM was only capable of accurately detecting virulent strains of *L. monocytogenes*, while detection was significantly lower in less virulent strains compared to other chromogenic and conventional agars.

Comparison of Modified and Conventional Dilutions

There were no significant differences (P>0.05) between the MPN of conventional (1:10) and modified (1:4) dilutions of meat samples in UVM-1 broth.

However, 1:4 dilutions provided equal or better results ($P<0.05$) than 1:10 dilutions when the growth of *L. monocytogenes* was monitored for 24 hours in UVM-1 broth. The use of 1:4 dilutions when analyzing larger sample sizes saved on the expense and handling of large amounts of culture media while maintaining a 50 sample per week schedule. Traditional analytical methods for detection, isolation, and identification of *L. monocytogenes* prescribe 25-gram samples in 225-ml enrichment broth (Hitchens 2002; McClain and Lee 1998). In this study, the sample size was increased to 125 grams in order to increase the chances of detection 5 fold. While the aim of our study was not to compare the detection between 125 and 25-gram samples, the occurrence of *L. monocytogenes* (0.8%) in lunchmeats was lower than that reported in other studies that used traditional dilution schemes (Gombas *et al.* 2003, Gillespie *et al*. 2000, Wilson 1995, Uyttendaele *et al.* 1999, Levine *et al*. 2001). There are two possible explanations as to why this is the case. The first possibility is that the occurrence would be higher in countries that do not have the “zero tolerance” policy in place regarding *L. monocytogenes* in RTE foods. The second possibility is that as time progresses the occurrence of *L. monocytogenes* in RTE foods in the US will continue to decrease signifying better sanitation and food safety practices by industrial and retail facilities. Levine *et al*. (2001) found a gradual decrease in occurrence of *L. monocytogenes* in RTE foods in the US over a 10-year period. However, further studies in comparing increased and traditional samples sizes in modified and traditional dilutions and various analytical methods demands further attention. Few studies have compared the recovery of *L. monocytogenes* in RTE

foods using increased and traditional sample sizes. Lin *et al*. (2004) found significantly higher ($P<0.05$) recovery of L. monocytogenes in increased sample sizes in turkey, but not roast beef or bologna ($P>0.05$), when compared to 25 gram sample sizes.

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APPENDIX: TABLE AND FIGURES

Table 1: Differentiation of *Listeria* Species[a]

	Rhamnose	Xylose	Mannitol	Hemolysis
L. monoctyogenes	+[b]	-[c]	-	+
L. ivanovii	-	+	-	+
L. innocua	V [d]	-	-	-
L. welshimeri	-	+	-	-
L. seeligeri	-	+	-	+
L. grayi	V	-	+	-

a: All *Listeria* species are motile
b: Positive
c: Negative
d: Variable

Table 2: Sensitivity and Specificity of Selective Plating Media for Detecting *L. monocytogenes* in Luncheon Meat

	True Positives[a]	False Positives[b]	True Negatives[c]	False Negative[d]	Sensitivity[e]	Specificity[f]
MOX	6	25	1524	0	100%	98%
Rapid L' Mono	6	0	1549	0	100%	100%
CHROMagar *Listeria*	6	4	1545	0	100%	99.7%

a: Positive sample, detection
b: Negative sample, detection
c: Negative sample, no detection
d: Positive sample, no detection
e: (True positive/ true positive + false positive) X 100
f: (True negative/ true negative + false negative) X 100

Table 3: Comparison of MPN of *L. monocytogenes* in UVM-1 Enrichment Broth

Replication	Inoculation	MPN/g for Dilution	
		1:4	1:10
Ham1	10 CFU/g[a]	17.9	30.9
Ham2	100 CFU/g[b]	106.8	92.9

a: No significant difference (P>0.05)
b: No significant difference (P>0.05)

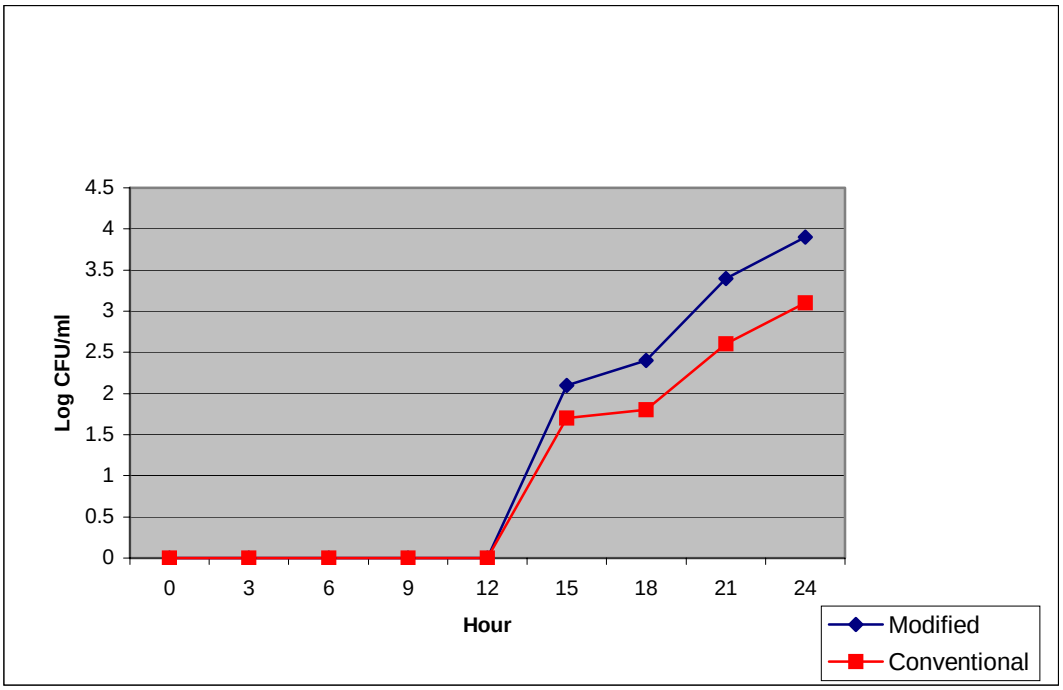

Figure 1: Growth of *L. monocytogenes* in UVM-1 Enrichment Broth Comparing Conventional (1:10) and Modified (1:4) Dilutions

PART III
SURVEY OF TENNESSEE GROCERY STORES FOR THE OCCURRENCE OF *LISTERIA MONOCYTOGENES*

ABSTRACT

The current USDA and FDA policy regarding *L. monocytogenes* in RTE foods is zero tolerance. Food processors have implemented food safety and sanitation programs to insure compliance, however such programs are not as strictly defined or enforced in the retail segment. 2000 deli meat samples collected throughout Tennessee from retail grocery stores were analyzed for the presence of *L. monocytogenes* using the USDA microbiological method with slight modifications and additional plating media. The overall occurrence of *L. monocytogenes* was 0.8%; occurrence was 1.5% in deli sliced and 0.2% in manufactured packaged luncheon meat. Beef products represented 59% of the positive samples. The majority of the levels of contamination were less than 0.3 MPN/g, and three samples had greater than 100 MPN/g. Results from this study showed the impact of contamination of luncheon meat in the deli department and developed baseline data of *L. monocytogenes* in the retail environment in high-risk RTE foods.

INTRODUCTION

Murray *et al*. (1926) first described *L. monocytogenes* (*Bacterium monocytogenes)* as the causative agent of disease in rabbits. It was hypothesized that feed was source of the organism. In light of its recovery from environmental samples*, L. monocytogenes* was initially considered a soil-borne bacterium. It was not until the 1980s that it was considered a foodborne pathogen. This discovery came when the organism was linked with numerous outbreaks that occurred from eating implicated ready-to-eat (RTE) foods

(Gombas *et al.* 2003, Shank *et al*. 1996). Since that time, the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) established a policy of zero tolerance for *L. monocytogenes* in RTE foods (Shank *et al.* 1996). Both agencies consider the food items to be adulterated, unfit for human consumption, if the pathogen is detected during routine testing.

Hazard Analysis Critical Control Point (HACCP) and Good Manufacturing Practices (GMPs) programs, in addition to sanitation programs, were put in place to reduce the likelihood of problematic bacteria such as *L. monocytogenes* occurring in food processing plants. Despite the zero tolerance policy, cases of listeriosis linked to consumption of contaminated RTE foods continue to occur. It is widely believed that RTE foods, including luncheon meats, are contaminated during post processing steps, such as slicing and packaging, due to the ability of *L. monocytogenes*, even under stress, to survive in low numbers in isolated niches (Kozak *et al.* 1996). As a result, the USDA (2003) published its final rule requiring federally inspected processors to increase their efforts to reduce the incidence of *L. monocytogenes* by choosing three alternatives to reduce the likelihood of post processing contamination:

- **Alternative 1. Use a post-lethality treatment that reduces or eliminates *L. monocytogenes* AND an antimicrobial agent or process that suppresses or limits *L. monocytogenes* growth throughout the product shelf life.**

- **Alternative 2. Use either a post-lethality treatment that reduces or eliminates *L. monocytogenes* OR an antimicrobial agent or process that suppresses or limits growth *L. monocytogenes* throughout shelf life.**
- **Alternative 3. Use only sanitation measures to prevent *L. monocytogenes* contamination.**

Even though new cases of listeriosis are decreasing, the Centers for Disease Control and Prevention (CDC) FoodNet program reports that *L. monocytogenes* illnesses continue to have the highest hospitalization rate, highest mortality rate, while accounting for less than 1% of the total infections reported in 2002 (CDC 2004).

Studies investigating the occurrence of *L. monocytogenes* in RTE food items are well documented. Gombas *et al*. (2003) surveyed *L. monocytogenes* in eight classifications of RTE foods. Collectively, the overall prevalence was 1.82%. Fresh soft cheeses had the lowest percentage of positive samples (0.17%), while seafood salads had the highest percentage (4.70%). A total of 9,199 samples of luncheon meat were analyzed, but only 82 (0.89%) were positive for *L. monocytogenes.* The level of contamination in this particular study ranged from 0.04 to greater that 10^5 CFU/g, respectively. Wilson (1995) analyzed 8360 RTE food items for the presence of *Listeria* species and found a total of 410 (approximately 5%) of the samples were positive. Of that 5%, the majority of the species (49%) were identified as *L. monocytogenes.* Gillespie *et*

al. (2000) tested RTE sliced meats from catering facilities for the presence of pathogenic bacteria, including *L. monocytogenes,* in the United Kingdom. In total, 3494 samples were collected and examined from over 2500 facilities. *L. monocytogenes* was isolated from 13 of 3494 samples (0.4%). The luncheon meat was considered unacceptable if the levels of L. monocytogenes were greater 10^2CFU/g. Of the 13 positive samples, five were deemed unacceptable, while the remaining samples were shown to have less than 100 CFU/g.

While a number of studies have examined RTE food products, few studies have exclusively surveyed RTE luncheon meats. Even fewer studies have compared the microbial integrity of luncheon meats that are purchased from the deli departments with manufactured packaged luncheon meats available in the refrigerated section of the grocery store. While it is assumed that extensive handling of opened luncheon meat chubs would occur, data concerning the impact of this assumption is limited. Gombas et al. (2003) found that the occurrence of *L. monocytogenes* in luncheon meats, deli salads, and seafood salads prepared in the store was significantly higher ($P < 0.05$) than their manufactured packaged counterparts. However, Gillespie (2000) found opposite results ($P < 0.001$) when comparing prevalence rates. In both studies, the investigators were not initially comparing the prevalence rates between manufactured packaged and deli sliced luncheon meats.

In order to better recognize the point of contamination of luncheon meats, this study was designed to compare the occurrence and level of contamination of *L. monocytogenes* in luncheon meats that were freshly sliced to order from deli

departments with manufacturer-packaged luncheon meats. In addition, data collected from this study were evaluated to determine if the size of the store, type of luncheon meat, presence of curing agents, and sanitation practices had a significant impact on the occurrence of *L. monocytogenes* in ready-to-eat deli style meats.

MATERIALS AND METHODS

Sampling Site Location

Tennessee, along with 10 other states, is an active site of the CDC foodborne diseases active surveillance network (FoodNet). Data from FoodNet provides the most accurate estimate of the incidence of listeriosis within each active site. In 2004, the incidence of foodborne listeriosis in Tennessee was 2.6 per 100,000 population (CDC 2005)

Selection of Sampling Locations within Tennessee Counties

The population density within Tennessee counties determined the sampling locations for the study. Population data sets were taken from the 2000 US census (www.census.gov). For example, 20% of the population of Tennessee resides in Shelby County (Memphis). As such, this study was designed so that 20% of the total samples would be collected in Shelby county, and so on. In order to sufficiently collect samples from lesser-populated counties while minimizing traveling costs, counties containing smaller populations were grouped together to provide adequate sample numbers. The order in which the counties were sampled was based on a random number table previously generated.

Selection of Grocery Stores within Tennessee Counties

Lists of grocery stores located in Tennessee were created with the use of information gathered from the Library of Congress and the reference USA website (www.referenceUSA.com). For each county, the list of grocery stores was divided into list A and list B. List A stores were considered major supermarkets that were ranked in the top 20 grocery businesses in the United States and likely to carry prepackaged luncheon meat and have a deli department. List B stores were smaller non-chain stores localized within a region or county. Both types of stores were contacted prior to sampling in order to confirm address, hours of operation, and store amenities. The stores on the list were numbered, and the random number table was used to select stores for each collection week. Each store was visited only once.

Sample Selection

Approximately 2000 luncheon meat samples per month were collected over a 10-month period. Based on shopping demographics (Gombas *et al*., 2003), 25% of the samples were collected from list B stores. Approximately 75% of the samples were collected from list A stores. Approximately 50% of the samples purchased were prepackaged and 50% were sliced to order from the deli department. Splitting the location of sample collection within a grocery store allowed the observation of potential cross contamination within the deli department during slicing. Supplier did not match luncheon meats between deli and prepackaged product in the display case since selections varied by store. Luncheon meats that were collected for microbial analysis were poultry- (~50%),

pork- (~25%), and beef- (~25%) containing products. Approximately half of the poultry samples were cured, while the remainder was uncured. For this study, cured luncheon meat was defined as containing nitrates or nitrites. Luncheon meats that did not contain nitrites or nitrates were considered uncured.

Collection of Luncheon Meat Samples

Approximately 50 samples were collected each week in order to maintain the sampling schedule of 10 months. Prior to each sampling week, collectors were provided with specific instructions regarding sampling locations, the type of samples to collect from each store, and the number of samples to collect from the deli and meat departments. The collector was responsible for determining the directions to the sampling locations, store hours, and amenities. They were allowed the option of purchasing luncheon meat that was low-fat, low-sodium, fat free, smoked, not smoked, etc., as long as it was specified within their collection sheets and the collection specified criteria of cured or uncured. Samples that contained additional herbs or spices on the surface were not allowed to be collected. If specific meat types were not able to be collected, alternative selections were purchased.

Sample collectors were instructed to make purchases from at least 2 A stores and 2 B stores until the weekly specifications were met, as long as the samples were evenly collected from each store. The GPS coordinates, address, meat types, USDA identification number, antimicrobials, store and product temperatures, and time were logged in addition to our sanitation score of the deli department.

Sanitation Scoring of Deli Department

The sanitation of the deli department and its personnel was observed and scored using our “mystery shopper” format. The questionnaire was divided into five sections: personnel cleanliness, product condition, display case cleanliness, facilities and equipment, and overall deli department premises. These practices could be observed on the consumer side of the deli counter. Within each section, the sample collector answered several questions “yes or well done”, “done”, or “no”. If the majority of the questions within a section were answered “yes or well done” the section was given a score of 2. If the majority of the answers were “no” the section was given a score of 0. The scores from each section were added to provide a total score. A copy of the questionnaire is located at the end of this section.

Sample Storage

Samples were placed in insulated coolers containing frozen ice packs and were analyzed within 24 hours. Temperature was monitored and product was discarded if temperature exceeded 10°C before analysis. Samples were transferred to labeled WhirlPak bags after receipt at the laboratory. The USDA microbiological method for isolation of *L.monocytogenes* from meat and poultry products was employed for this project, using additional steps and minor modifications as described below.

Sample Screening for *L. monocytogenes*

A test portion of 125g of lunchmeat was aseptically transferred to a stomacher bag and blended in a stomacher blender with 200 ml of *Listeria* enrichment broth-University of Vermont formulation (UVM I Acumedia Manufacturers, Inc. Baltimore, MD) for two minutes. An additional 175 ml of UVM-1 broth was added to create a 1:4 dilution. The homogenate was incubated for 24 h at 32°C. An aliquot of 0.1 ml was transferred to 10 ml of Fraser broth (FB Acumedia Manufacturers, Inc. Baltimore, MD) and incubated up to 48 hours at 35°C. After incubation, FB tubes were examined for the presence of hydrolyzed esculin indicated by blackened appearance. Positive tubes were streaked on to Modifed Oxford agar (MOX Acumedia Manufacturers, Inc. Baltimore, MD) and CHROMagar™ *listeria* agar (CHROM Beta Dickinson, Franklin Lakes, NJ), and Rapid L'Mono (RLM BioRad, Hercules, Ca) agars, and incubated accordingly for up to 48 hours. Typical *L. monocytogenes* colonies appearing on the agars were confirmed by motility, hemolysis, and biochemical confirmation. Typical colonies appearing on MOX plates were spot inoculated onto RLM for species identification. Additionally, typical *L. monocyotogenes* isolates from MOX plates were screened for genetic confirmation using the Gene-Trak assay (Neogen Corporation, Lansing, MI).

Biochemical Confirmation

Typical *L. monocytogenes* colonies on the agars were picked and transferred to trypticase soy broth (Difco™, Sparks, MD) containing 0.6% yeast extract (TSB-YE) and incubated for 24 h at 30°C. Table 1 provides the

biochemical reactions of all *Listeria* species which is located in the appendix. After incubation, the presence of tumbling rod shaped bacteria was determined utilizing phase contrast microscopy. Positive samples underwent carbohydrate fermentation testing utilizing 0.5% purple broth solutions of mannitol, rhamnose and xylose (Difco™, Beckton Dickson, Sparks, MD.). In addition, trypiticase soy agar blood plates (TSA-B) (Remel, Inc., Lenexa, KS.) were stabbed and incubated at 35°C for 36 h. After incubation, TSA-B plates were examined for the presence of lysed blood cells while the carbohydrate tubes were examined for rhamnose fermentation. Typical cultures were genetically confirmed using GeneQuence™ *Listeria monocytogenes*-specific DNA probe (Neogen Corporation, East Lansing MI.). Positive cultures were stored at -80°C in brain heart infusion broth containing 50% glycerol (Difco™, Beckton Dickson, Sparks, MD.).

Enumeration of *L. monocytogenes*

Upon presumptive confirmation of *L. monocytogenes* on the GeneTrak and chromogenic agars, 125 g of presumptive positive lunchmeat samples were aseptically removed from the original sample and immediately placed into 250 ml of UVM I enrichment broth. The samples were stomached for two minutes and 875 ml of UVM I enrichment broth was added to raise the volume to 1125 ml (1:10). The homogenate was mixed by hand for 30 seconds. A nine-tube MPN assay was performed. Three aliquots (10, 1, and 0.1 ml) of the sample homogenate representing 0.1, 0.01, and 0.001 g of the original sample were dispensed into the three sets of tubes. The tubes were incubated at 32°C for 24

h and 0.1 ml from each tube was transferred to 10 ml of FB and incubated for 48 hours at 35°C. After incubation, tubes were examined for the presence of hydrolyzed esculin as indicated by blackened appearance. The MPN was calculated using the tables provided by the USDA (USDA-FSIS 2002). Blackened tubes at the highest dilution were streaked onto RLM to determine presence of *Listeria* monocytogenes. Typical colonies were subjected to additional confirmatory biochemical testing as described above.

Statistical Analysis

Data was analyzed using SPSS 13.0 (2004) statistical software package for Windows in order to compare the means. Significance of differences was set at $P<0.05$.

RESULTS

Approximately 2000 luncheon meat samples were analyzed over a 10-month period for the presence of *L. monocytogenes*. A total of 17 samples (0.8%) were positive for *L. monocytogenes*. All tables and figures are located in the appendix. In six of those samples, an isolate did not remain viable. Table 2 characterizes the positive samples as to type of store in which purchased, the type of meat product, origin of product, slicing order and sanitation score of store where the meat product was purchased. The overall occurrence of *L. monocytogenes* was 1.8% in deli sliced luncheon meat, which was significantly higher ($p<0.05$) than the incidence of 0.2% in manufacturer packaged deli meat.

Table 3 shows the breakdown of positive samples by meat type. Deli meat consisting of beef had the highest percentage of positive samples while

poultry-based deli meat had the lowest percentage. No *L. monocytogenes* positives were found in mixed meat samples. The presence of nitrates or nitrites did not have an impact (P> 0.05) on the number of positive samples.

Significantly higher (P<0.05) numbers of positive samples were collected from B stores as compared to A stores. The sanitation scores of the deli departments in the A and B grocery stores are presented in figure 1. Mean sanitation score in A stores was 6.5 (out of a possible 10), and the mean scores for B stores were 4 (out of a possible 10). The median sanitation scores for both A (34%) and B (22%) grocery stores were 5.0. These scores should not be confused with a state health department inspection score, which is on a scale of 1 to 100; the sanitation scores used in this study are based only upon the sanitation worksheet described in the materials and methods that was designed to be used without physically entering the delicatessen employee areas.

The population of *L. monocytogenes* in luncheon meat ranged from less that 0.3 to greater than 100 MPN/g (figure 2); (41%) had a population of less than 0.3 MPN/g. Three samples had populations greater than 100 MPN/g.

DISCUSSION

The overall occurrence of *L. monocytogenes* in approximately 2000 RTE luncheon meat samples was 0.8%. This result was quite similar to that found in other studies from the United States and Europe. Gombas *et al.* (2003) found that 0.89% of luncheon meat samples from Maryland and California were positive for *L. monocytogenes* and that, overall, 1.82% of 31,000 samples of a variety of ready-to-eat deli foods including vegetable, meat and seafood products were

positive for *L. monocytogenes*. Seafood salads and smoked seafood products had the highest rates of positive *L. monocytogenes* (4.7% and 4.3%, respectively). Luncheon meat samples had an occurrence of 0.8%. Levine *et al.* (2001) analyzed over 31,000 RTE meats over a 10-year span and found a prevalence rate of 2.8%. In luncheon meats, *L. monocytogenes* was detected in 5.16% of the luncheon meat samples tested (approximately 2300). Wilson (1995) recovered 199 *L. monocytogenes* isolates (2.4%) from over 8300 RTE foods in England. In all classifications of RTE foods analyzed, *L. monocytogenes* was the pathogen recovered most often; however, specific data were not supplied. Another British study by Gillespie *et al.* (2000) isolated *L. monocytogenes* from 0.4% (13 isolates) of 3494 samples analyzed. In Belgium, Uyttendaele et al. (1999) recovered 167 isolates of *L. monocytogenes* (4.90%) from 3405 cooked meat samples.

Data from this study show that the occurrence of the *L. monocytogenes* in deli sliced lunchmeat (1.8%) was significantly higher ($P<0.05$) than manufacturers packaged lunchmeat (0.2%). Gombas *et al*. (2003) also found a similar trend in that 2.7% of the deli-sliced luncheon meats were positive for *L. monocytogenes*, while 0.4% ($P<0.05$) of the manufactured packaged lunchmeat was contaminated with *L. monocytogenes.* Uyttendaele *et al.* (1999) also noted that significantly higher ($P<0.05$) recoveries of *L. monocytogenes* occurred in cooked meat products that were sliced (6.65%) as compared with whole muscle products (3.96%) purchased from Belgium retail markets. In contrast, Gillespie *et al.* (2000) noted that significantly higher samples of “pre-sliced” (manufacturer

packaged) cooked meat contained *L. monocytogenes* than “fresh-sliced” cooked meat. However, specific data were not cited which makes it difficult to determine the factors affecting the stated incidence of *L. monocytogenes*.

Numerous studies have investigated the occurrence of *L. monocytogenes* in a variety of RTE foods, including luncheon meat. Differences in results could be attributed to methodology, a particular nation’s government policy pertaining to *L. monocytogenes* in RTE foods, sanitation programs and overall experimental design of various studies. In a number of studies performed in other countries, a sample was not considered “positive” unless the microbial population surpassed a certain standard for *L. monocytogenes* (Wilson 1995, Gillespie *et al.* 2000). The results of those studies potentially under-estimated the hazard associated with *L. monocytogenes* in ready-to-eat foods. These differences among the studies show that, due to the low incidence of *L. monocytogenes* in RTE foods, it is necessary to analyze a large number of samples and develop a statistically valid sampling plan to obtain data needed for useful risk assessment studies (Gombas *et al*. 2003, Levine *et al.* 2001, Wilson 1995, Uyttendaele *et al.* 1999, and Gillespie *et al*. 2000).

Under the conditions of this study, samples positive for *L. monocytogenes* occurred significantly more frequently ($P<0.05$) in B stores compared to A stores. The overall sanitation score of A stores was 6.5, while B stores had an average sanitation score of 4. It was noted that larger retail establishments had significantly higher ($P<0.001$) sanitation scores than smaller retail establishments. Therefore, it is clear that the greater financial resources and

increased focus of large grocery chains on sanitation contributes to a deli, which is cleaner and better maintained. Our results are interesting in light of the FDA report (2000) that cited 74% of the delis inspected were in compliance. The most common infractions were poor personnel hygiene and improper holding times. According to the Tennessee Department of Agriculture (2002), rules and regulations regarding grocery stores health and sanitation practices are listed in the state's Food and Drug Division under retail food store sanitation (0080-4-9). Employees are required to clean food contact equipment at least once per day or when there is a change between raw and ready-to-eat products, but are encouraged to clean during "any disruption in operation." All retail stores are inspected twice per year by the state, however larger national chain grocery stores are likely to be inspected more often by internal and other 3rd party inspectors in order to insure and monitor compliance. In addition, it is equally likely that smaller businesses do not get the same food safety expertise and training as larger businesses (Gillespie *et al.* 2000). In a review of articles that describe foodborne disease outbreaks linked to retail food preparation, poor hygiene by food workers, improper glove use and working while ill were the leading causes of foodborne outbreaks (CFSAN-FDA 1999). Interestingly, the overall sanitation score of grocery delicatessens having a positive *L. monocytogenes* was 8.3 (out of a possible 10), which was significantly higher ($p<0.05$) than A or B stores overall. It is evident that a clean appearance and environment in a deli does not always mean that a food contact surface is sanitized. Gillespie *et al.* (2000) made similar observations. This finding is

problematic since we assumed that the appearance of unclean environments in grocery stores might lead to increased occurrence of *L. monocytogenes.* The solution to this problem will not be simply teaching employees to “clean” better but to also teach them the importance of “sanitation” which is a more difficult concept for most people to understand.

The presence of nitrates or nitrites did not have an impact (P> 0.05) on the number of positive samples. Meat processing steps in conjunction with additional antimicrobials in the meat formulation and post processing lethality steps contribute to the elimination of *L. monocytogenes* on the interior of RTE meat products. However, post-processing contamination is evidently still a problem both at the processing plant and at the deli. The low incidence of only 0.2% positive *L. monocytogenes* RTE meat and poultry samples from USDA inspected manufacturing plants can probably be attributed to the implementation of the “Listeria Rule” in late 2003. Nitrates and nitrites in meat products contribute to flavor, color, and preventing the germination of *Clostridium botulinum* spores (Jay 2000). While some has questioned the safety of nitrites, it is believed that nitrites can contribute to the inhibition of *L. monocytogenes* under certain, but not all, conditions (Tompkin 2005). However, their presence has no effect on the incidence of *L. monocytogenes* in RTE meat and poultry based on the results of this study. The efficiency of nitrites as antimicrobials has been shown to vary at different pH, temperatures, and in interaction of other antimicrobials and bacteria (Tompkin 2005).

The population of *L. monocytogenes* in luncheon meat ranged from less that 0.3 MPN/ml to greater than 100 MPN/g; 41% of the positive samples had a population of less than 0.3 MPN/g. Three samples had populations greater than 100 MPN/g. Many studies have determined the prevalence of *L. monocytogenes* in RTE foods; however due to the 'zero tolerance' policy the contamination level was rarely determined. Our results are similar to Gombas *et al.* (2003) for a variety of RTE products including meat and poultry: 402 of their 577 samples were <0.3MPN/g in various RTE foods. However, they reported at least one sample with a population of *L. monocytogenes* as high as 10^6 CFU/g. The population of *L. monocytogenes* in franks and their packaging did not exceed 27.6 MPN/pkg in a 1994 study (Wang and Muriana 1994). Gillespie *et al.* (2000) only found five sliced meat samples to harbor levels of L. monocytogenes that were considered "unacceptable" (>100 CFU/g), while the other eight samples contained a population that is tolerated by British regulations.

The results of this study show that progress is being made by grocery stores and the RTE meat and poultry industry in reducing overall levels of *L. monocytogenes*; however, the increased 5 to 6-fold incidence of *L. monocytogenes* in RTE meat and poultry sliced at the grocery delicatessen is cause for concern and needs to be addressed by the retail industry. Individuals who are immune compromised should be advised to cook luncheon meats to destroy *L. monocytogenes* before consumption, especially if they are purchased in a deli that slices to order. Until more information is available, it is

recommended that immune compromised individuals not consume sandwiches purchased in deli shops that slice deli meats to order because of the potential for increased levels of *L. monocytogenes* on meats sliced in a retail environment.

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APPENDIX: TABLES AND FIGURES

Table1: Biochemical variation of *Listeria* spp.

	Motility	Rhamnose	Xylose	Mannitol	Hemolysis
L. monocytogenes	+[a]	+	-[b]	-	+
L. ivanovii	+	-	+	-	+
L. innocua	+	V[c]	-	-	-
L. welshimeri	+	-	+	-	-
L. seeligeri	+	-	+	-	+
L. grayi	+	V	-	+	-

a: Positive
b: Negative
c: Variable

Table 2: Characterization of Luncheon Meats Containing *L. monocytogenes*

Sample	Store[a]	Sample Origin[b]	Meat Origin[c]	Cured[d]	Sanitation[e]	Slicing Order[f]	Population[g]
7554	B	D	beef	U	9	5	<0.3
7558	A	P	beef	C	n/a	n/a	<0.3
7574	A	D	beef	U	9	9	2.3
7579	A	P	pork	C	n/a	n/a	<0.3
7584	B	D	beef	C	6	1	<0.3
7621	A	D	pork	C	9	6	<0.3
7636	B	D	poultry	U	10	1	> 110
7638	B	D	pork	C	10	3	> 110
7639	B	D	pork	C	10	4	> 110
7640	B	D	beef	C	10	5	23
7641	B	D	beef	C	10	6	15
8268	B	D	beef	U	4	10	0.92
8269	B	D	beef	U	4	11	0.92
9297	A	P	beef	U	n/a	n/a	<0.3
9357	A	D	poultry	C	9	4	<0.3
9358	A	D	pork	C	9	5	2.3

a: “A” store = major grocery chain, “B” store= local grocery store
b: “D” = lunch meat purchased from deli, “P”= manufactured packaged lunchmeat
c: Type of meat: beef, pork, poultry, mixed
d: “C”=Cured, “U”=Uncured
e: Sanitation score of deli when applicable
f: Order in which they were sliced
g: MPN/g

Table 3: Prevalence (%) of *L. monocytogenes* in Deli Meats Collected Throughout Tennessee[a]

		Beef		Poultry		Pork		Mixed	
		Cured	**Uncured**	**Cured**	**Uncured**	**Cured**	**Uncured**	**Cured**	**Uncured**
A store	**Deli-sliced**	2/88 (2.2%)	1/94 (1.1%)	1/156 (0.6%)	0/179 -	2/139 (1.4%)	0/28 -	0/6 -	0/28 -
	Packaged	0/59 -	1/77 (1.3%)	0/201 -	0/159 -	1/156 (0.6%)	0/7 -	0/65 -	0/11 -
B store	**Deli-sliced**	3/30 (10%)	3/26 (11.5%)	0/45 -	1/79 (1.3%)	2/86 (2.3%)	0/7 -	0/24 -	0/2 -
	Packaged	0/33 -	0/18 -	0/76 -	0/29 -	0/59 -	0/6 -	0/22 -	0/0 -
Total		5/210 (2.4%)	5/215 (2.4%)	1/478 (0.2%)	1/446 (0.2%)	5/440 (1.1%)	0/48 -	0/117 -	0/41 -

a: 1995 samples analyzed

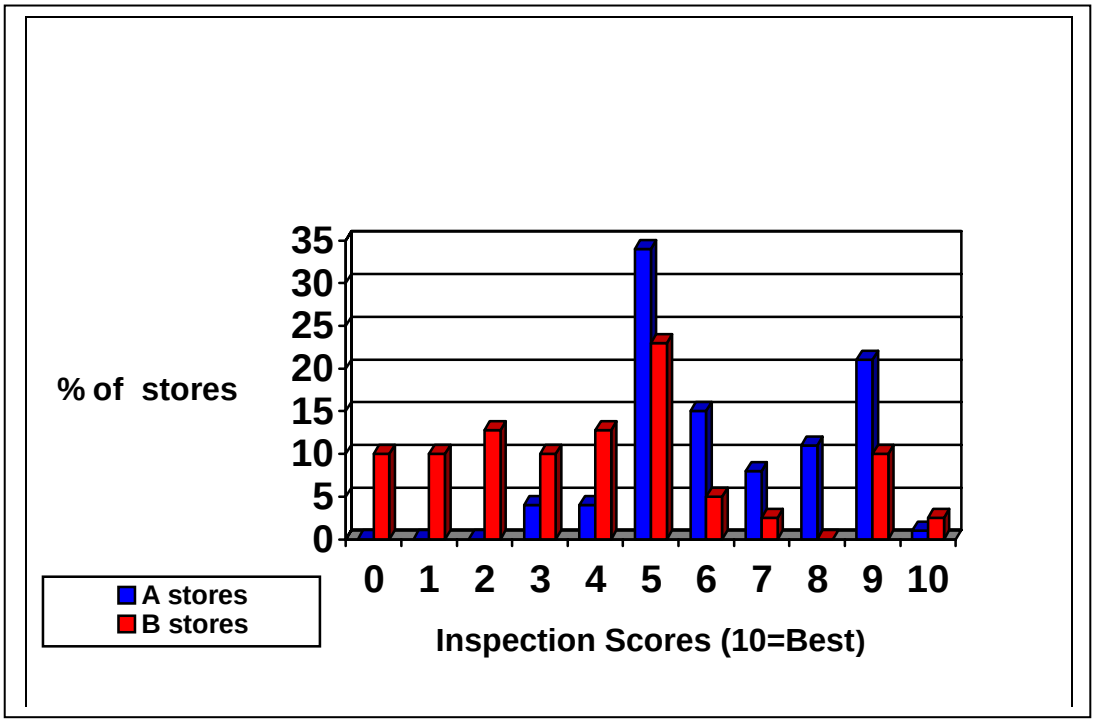

Figure 1: Sanitation Inspection Scores for A and B Grocery Store Deli Departments

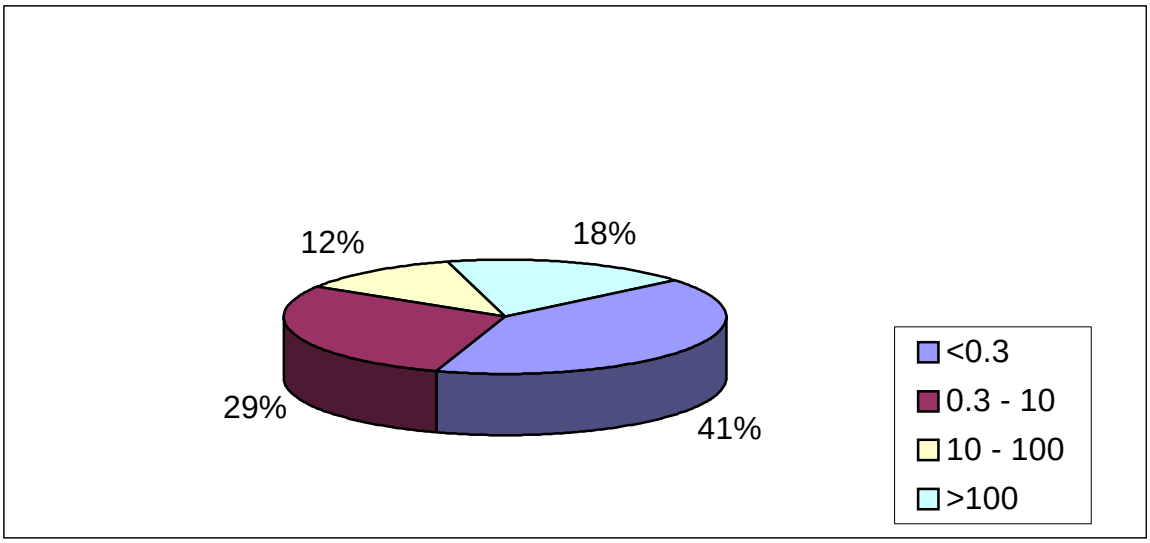

Figure 2: Population (MPN/g) of *L. monocytogenes* in Luncheon Meats

SANITATION SCORE SHEET

Personnel	Yes or Well Done	Done	No	Score
Employee wearing aprons Apron Clean Employee Wearing Hairnet				0 Poor
Hair Completely Covered with Hairnet				1 Good
Gloves Used for Handling Product				
Gloves Used Properly				2 Excellent
Gloves are Fresh				
Rings Worn				
Nail Polish Worn				
Long Nails (Longer than end of finger)				
Product				0 Poor
Orderly Arrangement				
New Product Opened For Slicing				1 Good
Approximately ½ of Chub or Breast Remaining				
Approximately ¼ of Chub or Breast Remaining				2 Excellent
Product Touches Scale during Weighing (off holding paper)				
Display Case				0 Poor
Cleanliness of Display Case				
Condensation or Drip on Product				1 Good
No Customer Contact with Case Product Pre-Packaged Product Separate from Deli				2 Excellent
Facilities				
Cleaning Chemicals Near Food				
Food Prep Area Separated from Customer Space				
Other Foods Prepared in Slicing Area (i.e. Sandwich, salads)				0 Poor
Hand Washing Facilities Present				1 Good
Ware Washing Facilities Visible				
Slicing Machine Clean (food contact areas)				2 Excellent
Slicing Machine Clean (non contact food areas)				
Slicing Machine Cleaned or Sanitized After Each Product				
Knife Bath Present and Clean				
Scales Clean				
Scales Cleaned After Each Use				
Trash Covered				
Floors Clean (no paper, trash or visible soil)				
Premises				
Deli Area Appears Clean				
Well Lighted				0 Poor
Off Odors detected				
Insects, rodents or baits in view				1 Good
Standing Water on Floor or Work Surfaces				
Appropriate Indoor Temperature (68 to 78 F) Humidity (if available)				2 Excellent
				SUM Column
Overall Deli Score (0 to 10)				

VITA

Jacob (Jake) Henry Stevens was born on February 13, 1980 to a hippy and a farm girl, Guy and Tedye Stevens. In June of 1998, he graduated from McMinn Central High School. That fall he entered college at Hiwassee College in Madisonville, TN. To the best he can recall, he graduated with an Associates of Science degree in Agriculture in 2000. He transferred to the University of Tennessee, Knoxville where he pursued his Bachelors degree in Food Science and Technology. He graduated in December of 2002. Jake took some time off to plan a wedding and catch up on sleep. In the summer of 2003, he began his pursuit for a Masters degree in Food Science and he married his longtime girlfriend Abby Wallace. He graduated with a 3.6 GPA in May of 2006. During he entire academic career he worked in part time jobs while maintaining at least a 3.0 GPA