Risk Assessment for *Listeria monocytogenes* in Ready-to-eat Meat and Poultry Products

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ABSTRACT

Various control methods used in the meat and poultry processing environment to mitigate listeriosis were evaluated using a dynamic in-plant Monte Carlo model. These control methods included food contact surface testing, sanitation, post-processing lethality treatment, and product formulation with microbial growth inhibitors. The dynamic in-plant model served as an input into the risk assessment model developed by the FDA and FSIS in 2003 which predicts the number of deaths and illnesses resulting from the use of each control method. The use of growth inhibitors combined with a post-processing lethality step was estimated to save over 200 more lives than the FSIS proposed minimum sampling standard.

An analysis of data collected by the National Alliance for Food Safety and Security (NAFSS) found that retail-sliced deli meats have a greater prevalence and concentration of *L. monocytogenes* than prepackaged deli meats. Cross contamination at the retail level is suspected due to clustering of sample positives by store and the influence of sampling time of day on the prevalence of *L. monocytogenes*.

The comparative risk of *Listeria monocytogenes* in retail sliced versus prepackaged deli meats was evaluated using a modified version of the 2003 FDA-FSIS risk assessment model which considered slicing location and the use of growth inhibitors. The comparative risk ratio for the number of deaths from retail-sliced versus prepackaged deli meats was found to be 9.1 and retail-sliced product with a growth inhibitor was found to be at greater risk for listeriosis than prepackaged product without growth inhibitor.

Table of Contents

List of Figures

List of Tables

Chapter 1. Introduction

Listeriosis is a serious public health issue due to its severity of infection and high case fatality rate. According to the Centers for Disease Control and Prevention (CDC), there are an estimated 2,500 cases of listeriosis in the United States each year, resulting in 500 deaths *(20)*. Persons with compromised immune systems, pregnant women, neonates, and the elderly are at the greatest risk of listeriosis. Listeriosis is caused by infection with the foodborne pathogen *Listeria monocytogenes*. Numerous listeriosis outbreaks have been linked to ready-to-eat foods *(7)*. Ready-to-eat foods are products which are in edible form and require no additional preparation to achieve food safety *(11)*. Ready-to-eat foods may become contaminated with *L. monocytogenes* due to cross contamination or physical contact with contaminated raw foods. Out of 23 ready-to-eat food categories studied, meat and poultry products were found to pose the greatest risk for listeriosis *(7)*. Although the incidence of listeriosis has seen a steady decline from 1996 to 2003 *(29)*, trends observed at Foodborne Diseases Active Surveillance Network (FoodNet) sites indicate that the incidence has since leveled off *(23)*. The CDC set a target incidence rate of 2.5 cases per 100,000 population for the year 2005, however, in 2007, this goal has not been met indicating that additional measures must be taken in order to meet this goal by 2010 *(4)*. The objectives of this work are (i) to evaluate various industry practices and procedures within meat and poultry processing facilities on their ability to mitigate *L. monocytogenes* contamination and (ii) to investigate and compare the risk of listeriosis from deli meat sliced and packaged at processing establishments versus those sliced and packaged at a retail slicing location.

Previous research suggests that slicing location of deli meats may have a significant effect on *L. monocytogenes* prevalence in ready-to-eat product. In a study conducted by Gombas *et al.(14)*, ready-to-eat deli meats sliced and packaged at the retail level were found to have a higher prevalence of *L. monocytogenes* than deli meats sliced and packaged at a processing facility. Since the focus of this study was to conduct a survey of *L. monocytogenes* across a number of ready-to-eat foods, it did not exclusively examine deli meats. Therefore, this finding was based on limited data and further analysis was necessary to explain this difference. In 2006, the National Alliance for Food Safety and Security (NAFSS) completed a more comprehensive study with the intention of evaluating the relative risk of listeriosis from deli meat sliced and

packaged at processing facilities versus those sliced and packaged at retail *(6)* This paper uses the dataset collected from the NAFSS study to perform a comparative analysis of the relative risk of listeriosis from deli meats sliced at retail versus prepackaged. Additionally, the relative risk of listeriosis for product formulated with and without growth inhibitors was considered. Basic assumptions of shelf life of ready-to-eat deli meats and typical consumer storage times were validated and their influence on the outcome of the study were assessed.

Gombas *et al.* surveyed *Listeria monocytogenes* in eight categories of ready-to-eat foods. The purpose of this study was to generate data for calculating the risk of listeriosis across a number of food groups. The result was the recognition of trends in the prevalence of *L. monocytogenes* in deli meats. A prevalence of 0.4% was estimated for luncheon meats packaged by the manufacturer versus 2.7% for luncheon meats packaged "in-store." These numbers were based on a total of 9,199 samples collected from two FoodNet sites. Approximately half of the samples were collected from a Maryland site and half were collected from a northern California site. One limitation to this study was that the overall prevalence of *L. monocytogenes* observed at the two FoodNet sites were inexplicably different. The prevalence of *L. monocytogenes* at the Maryland and the northern California sites were 1.17% and 0.61% respectively. While the results of this study indicated that slicing location of deli meats has an effect on the prevalence of *L. monocytogenes* in retail deli meats, the unexplained difference between overall prevalence of *L. monocytogenes* at the two FoodNet sites suggested that further research was necessary to confirm these findings. Moreover, identification of the factors contributing to the elevated prevalence of *L. monocytogenes* in retail-sliced deli meats was necessary in understanding this difference.

Transmission of *L. monocytogenes* in retail delis may be one factor contributing to the elevated risk of listeriosis from retail-sliced products compared to prepackaged products. Retail delis are most commonly out of compliance with the FDA Food Code for improper holding times and temperatures of product, poor personal hygiene of workers handling product, and a lack of adequate safeguards against contamination *(8)*. Since retail-sliced deli meats are sliced and handled immediately prior to purchase by the consumer, exposure to these factors may increase the risk of contamination with *L. monocytogenes*.

Previous studies have found that contamination via deli meat processing equipment is a common pathway for *L. monocytogenes* transmission. Lunden *et al. (19)* conducted an

experiment by relocating a dicing machine to three different plants and tracking the movement of persistent *L. monocytogenes* strains. It was found that despite regular cleaning and disinfection of the machine, adherence to stainless steel allowed persistent *L. monocytogenes* strains to be transferred from one plant to the next. Vorst *et al. (30)* investigated the transfer of *L. monocytogenes* during the slicing of turkey, bologna, and salami. This study considered the transfer of *L. monocytogenes* from an inoculated slicer blade to uninoculated product and from inoculated product to uninoculated product via the slicer. The transfer of *L. monocytogenes* from an inoculated slicer blade to uninoculated product occurred for all three product types. Transfer of *L. monocytogenes* from inoculated product to uninoculated product via the slicer was exhibited at *L. monocytogenes* levels of 10^8 CFU/cm^2. For lower levels, *L. monocytogenes* was only found to be transferred at detectable levels for certain products and the transfer from one product type to another was found to be dependent on the order of slicing. The results of this study indicated that *L. monocytogenes* may be spread through the use of a mechanical slicer on contaminated meat and poultry product. When slicing occurs within a processing plant, this contamination may be mitigated through the use of post-processing treatments, however, retail-sliced deli meats are purchased immediately after slicing, therefore any contamination originating at the slicer is passed on the consumer.

The detection of *L. monocytogenes* in ready-to-eat products has been found to be effected by consumer refrigerated storage times and deli meat sample sizes. Lin *et al. (18)* conducted a study including an analysis on the fate of *L. monocytogenes* during refrigerated storage and the effect of sample size on the efficacy of the BAX-PCR and U.S. Department of Agriculture – Food Safety and Inspection Service enrichment culture assays in detecting *L. monocytogenes*. It was found that using larger cell numbers of *Listeria* for inoculation resulted in a greater number of samples positive for *L. monocytogenes* and increasing the sample size taken improved the detection of *L. monocytogenes*. Also, growth of *L. monocytogenes* occurred during the storage of ready-to-eat deli meats even when kept at a constant 4°C. A study by Wallace *et al. (31)* specifically focused on the recovery rate of *L. monocytogenes* during extended refrigerated storage. Some plants with frankfurters testing negative for *L. monocytogenes* during the first 5 days of storage had a significant percent of positive samples from frankfurters taken from the same lot that were stored for 30 days. There was no statistical difference between the recovery rate of *L. monocytogenes* for packages stored at 4° or 10°C. This study indicated the importance

of detecting *Listeria* at low levels before it is able to grow to dangerous levels and the importance of carefully monitoring consumer storage times. Also, observed clustering of positive samples at individual plants indicated that cross contamination was occurring in plants contaminated with *L. monocytogenes*.

To help reduce and control the growth of *Listeria*, many processing plants formulate meat and poultry products with microbial growth inhibitors. In the United States sodium or potassium lactates combined with sodium diacetate are the most common antimicrobial agents in ready-to-eat meat processing facilities *(13)*. Other antimicrobials include sodium or potassium acetate, and sodium diacetate used singly. In 2000, the U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) set the permissible level of the sodium lactate at 3% and allowed the use of 0.25% sodium acetate and sodium diacetate as an antimicrobial agent in cured meat products. A study conducted by Bedie *et al*. *(2)* compared the effectiveness of antimicrobial agents on frankfurters stored at 4°C. At 3% sodium lactate there was no significant *L. monocytogenes* growth until day 90. In comparison, 0.25% sodium acetate permitted significant growth of *L. monocytogenes* at 35 days. In frankfurters not formulated with antimicrobials, *L. monocytogenes* levels rose from 3.2 - 3.4 log CFU/cm^2 to over 6 log CFU/cm^2 in only 20 days. The results of this study indicated that antimicrobials are effective in suppressing the growth of *L. monocytogenes* during refrigerated storage and controlling post-processing contamination of *L. monocytogenes* in ready-to-eat meat products , however even the use of an antimicrobial agent may not prevent *L. monocytogenes* for the targeted retail shelf life of 75 to 90 days *(32)*.

The following chapters present three studies that build upon the previous research concerning *L. monocytogenes* contamination in ready-to-eat deli meat products. The first compares the effect of microbial growth inhibitors to post-processing lethality and traditional testing and sanitation regimes in mitigating *L. monocytogenes* contamination. The second analyzes the presence and level of *L. monocytogenes* based on slicing location as well as store type (large chain or independent grocer) and time of day (AM or PM) and the final study calculates the comparative risk of retail-sliced versus prepackaged deli meats. The concluding chapter summarizes the findings and conclusions from each of these three studies.

In-Plant Dynamic Model

1.1 Introduction

Listeria monocytogenes is a foodborne pathogen that is able to grow at refrigeration temperatures and is resistant to many controls used for other foodborne pathogens *(11)*. It is found on foods, in household refrigerators, and within food processing environments. It can be present in ready-to-eat (RTE) foods due to post-processing contamination *(20)*. *Listeria monocytogenes* contamination is a critical public health issue, owing to the severity of infection and high case fatality rate of its associated disease listeriosis *(20)*. In 2003, the Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS) completed a risk assessment identifying which RTE foods pose the greatest risk of listeriosis *(7)*. Of the 23 RTE food categories evaluated, deli meats were found to pose the highest per annum risk of illness and death. To reduce the prevalence of *L. monocytogenes* in RTE meat and poultry, product testing and sanitation are conventional control methods utilized by processing plants. Post-processing lethality and the use of growth inhibitors are other methods of control.

The objectives of this work were to develop a model to:

i) Determine the effectiveness of various food contact surface testing and sanitation regimes on mitigating *L. monocytogenes* contamination in finished RTE product

ii) Determine the effectiveness of other interventions (e.g., post-processing lethality or the use of growth inhibitors) in mitigating *L. monocytogenes* contamination in finished RTE product.

To address these objectives, a dynamic in-plant Monte Carlo model (referred to as the in-plant model) was developed to quantitatively characterize the relationship between *Listeria* species in the in-plant environment and *L. monocytogenes* in deli meats at retail. The output of the in-plant model was the concentration of *L. monocytogenes* on deli meat at retail which was input to the 2003 FDA-FSIS exposure assessment model for deli meats *(7)*. The FDA-FSIS exposure assessment model is coupled with the dose-response model to provide estimates of the subsequent risk of illness or death from consuming RTE products across three population groups: elderly, intermediate, and neonatal. These two connected models – the in-plant model and the FDA-FSIS exposure assessment and FDA-FSIS dose-response relationship – comprise the overall FSIS *Listeria* risk assessment model. The FSIS *Listeria* risk assessment model was

used to evaluate the effectiveness of various control interventions in mitigating *L. monocytogenes* contamination of RTE product and reducing the subsequent risk of illness or death from listeriosis.

1.2 Material and Methods

1.2.1 Model Overview.

The in-plant model uses Monte Carlo sampling to predict the concentration of *L. monocytogenes* in each lot of RTE product across time resulting in a dynamic model. In this model, a lot was defined as the product produced in an 8-hour period. Many of the parameters used in the model are stochastic random variables meaning that different values are selected for each lot produced. The exposure assessment for deli meats also uses Monte Carlo sampling.

The inputs for the in-plant model are modeled as variability distributions. The number and deposition of *Listeria* organisms are tracked for both food contact surface area and the product across time using a mass balance approach. The *L. Monocytogenes* concentration for RTE deli meat at retail modeled by the in-plant model is input to the exposure assessment model and dose-response model to estimate the risk of illness or death on a per serving and per annum basis for *L. monocytogenes* on RTE meat and poultry products. The estimated number of illnesses and deaths are ultimately modeled as functions of *Listeria* species testing and sanitation frequency of food contact surfaces, post-processing lethality, and the use of growth inhibitors. Deli meats were selected for this model based on the 2003 FDA-FSIS risk ranking analysis that found this food category to pose the greatest risk of illness and death among consumers *(7)*.

1.2.2 Model Limitations.

The data available within the published literature dealing with *Listeria* in the processing plant environment was limited. The limited data, the time available for model development, and the intended use of the model dictated the following:

i) Food contact surfaces are the only source of *Listeria* species/*L. monocytogenes* considered by the model

ii) Food contact surfaces have no spatial component within the plant (e.g., slicer, conveyor belt, etc.).

iii) *Listeria* species are considered to be evenly distributed across food contact surfaces

iv) *L. monocytogenes* is considered to be evenly distributed within the product lot

v) The RTE product lot is the smallest unit for which model results are available

vi) Testing and sanitation affect the distribution of *Listeria* at retail, but do not change the timing, duration, or concentration of *L. monocytogenes* transferred during a contamination event.

1.2.3 In-plant Dynamic Model.

A schematic overview of the conceptual model is provided in Figure 1 below. The model assumes that a *Listeria* reservoir exists in the plant that is capable of contaminating the food contact surface. This reservoir can be harborage sites such as floor drains or air conditioning ducts, or other surfaces/equipment in the plant *(19)*.

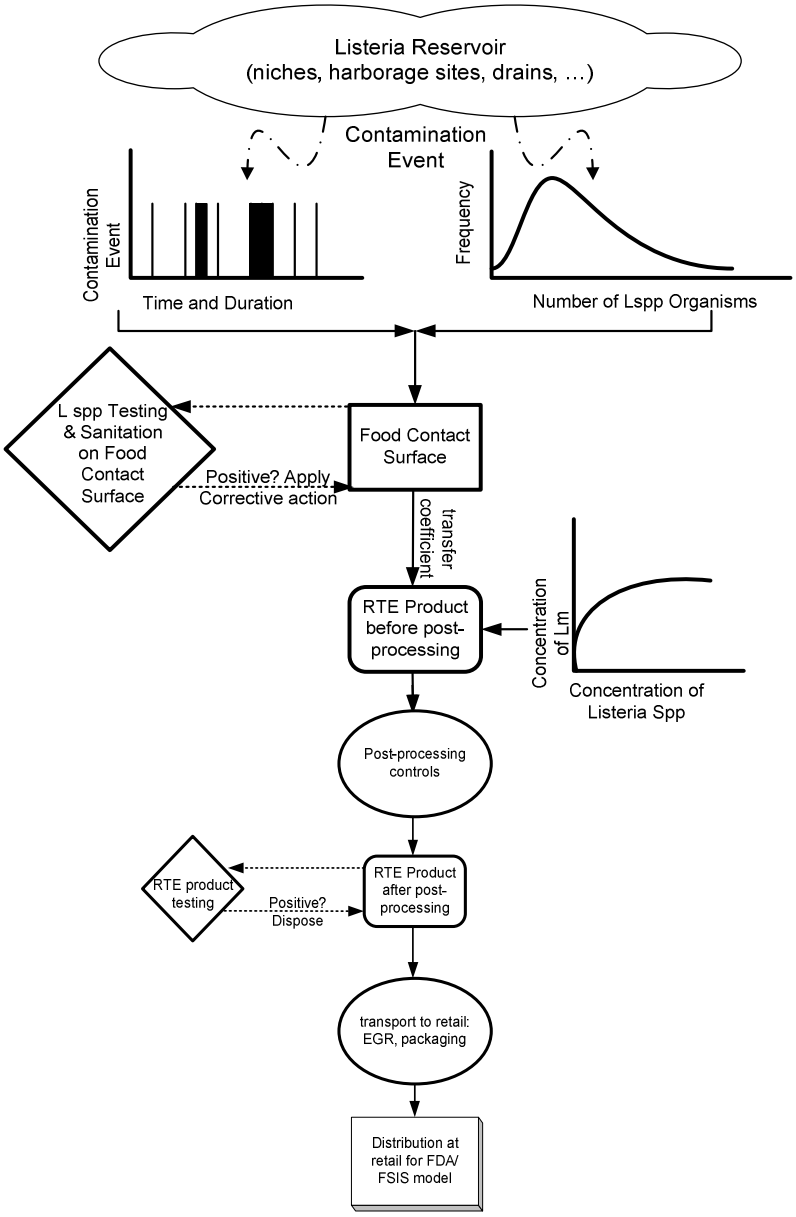


Figure 1. Conceptual model showing the flow of *Listeria* from the original contamination event through sanitation measures, post processing, packaging, and on to retail distribution. See reference *(11)*.

The in-plant model supposes that *Listeria* species move from this reservoir onto the food contact surface during what is termed a contamination event. The key parameters defining a contamination event are the time between initialization of events (i.e., How often is a food

contact surface contaminated?); the duration of the event (i.e., How long does it last?); and the amount of *Listeria* species transferred from the in-plant reservoir to the food contact surface.

1.2.4 Contamination Event Parameters

The frequency of a contamination event was estimated based on time series *Listeria* species prevalence data taken from an FSIS in-depth verification conducted in a plant that was associated with an *L. monocytogenes* outbreak in humans *(16)*. The data were analyzed using survival analysis and distribution fitting using NCSS statistical software *(15)*. Based on this analysis, the data were found to best fit the lognormal distribution. The mean time between contamination events was found to be approximately 20 days ± 29 days.

The duration of a contamination event was estimated based on sequential weekly *Listeria* species testing results from Tompkin *(26)*. These data provided the number of consecutive weeks that *Listeria* species positives persisted during the weekly testing, allowing the duration of a contamination event to be estimated. For ease of interpretation, consistency, and based on the maximum likelihood fit as determined using survival analysis and distribution fitting, these data were also fit to a lognormal distribution for model simulation. The mean contamination event duration was found to be approximately 9 days ± 20 days.

Once the frequency and duration of a contamination event were estimated, the amount of *Listeria* species transferred from the in-plant reservoir to the food contact surface needed to be determined. As there was no reported literature available to estimate the *Listeria* spp. transferred from a harborage site to a food contact surface during a contamination event, the parameters were calibrated so that the simulated distribution of *Listeria* spp. concentration at retail under baseline conditions matched the observed FDA-FSIS risk ranking model's input for *L. monocytogenes* contamination at retail. The parameter vales for the baseline conditions are given in the Base Value column of each variable table provided. These parameters were changed as necessary to simulate the desired control scenarios. The mean *Listeria* spp. transferred was calibrated to a mean log value of -6 cfu/cm^2/shift (one lot per shift) with a standard deviation of 3.5 cfu/cm^2.

The amount of *Listeria* species then transferred from the food contact surface to the RTE product was estimated based on a number of factors including the transfer coefficient for *Listeria* species and the effectiveness of in-plant sanitation procedures. The transfer coefficient (TC) ranged from 0 to 1 and indicated the fraction of *Listeria* species transferred from the food contact

surface to the product lot being processed. A transfer coefficient of 1 indicated that all the *Listeria* species on the food contact surface were transferred to the lot. A transfer coefficient of 0 indicated that the *Listeria* species transferred from the harborage site remained on the food contact surface. The mean transfer coefficient of 0.72 was assumed based on the work of Midelet and Carpentier *(21)*. This is equivalent to a mean log transfer coefficient of -0.14. A standard deviation of 1 log was assumed based on the studies conducted by Montville *et al. (22)* and Chen *et al. (5)*. During a contamination event, the in-plant model modifies the concentration of *Listeria* species on the food contact surface by a stochastic amount for each RTE lot simulated to account for the transfer of organisms from the harborage site to the food contact surface.

1.2.5 Model Parameters

Sanitation effectiveness measures the proportion of bacteria on the food contact surface that are removed through sanitation procedures. The model assumes the effectiveness of sanitation between lots is 50% and the effectiveness of sanitation measures at the end of the day is 75%. Therefore, total effectiveness of daily routine cleaning is actually 1-[(1-50%)*(1-75%)]=87.5%, or just less than a one $\log_{10}$ reduction in the amount of contamination remaining on food contact surfaces. The sanitation effectiveness was evaluated for each lot as follows,:

$$s_j = \begin{cases} s_{wipe} & \text{if 1st lot of day} \\ s_{sop} & \text{if 2nd lot of day} \\ s_{enhan} & \text{if } LS_{j\text{-}s_{lag}} \text{ tested positive, and enhanced sanitation option selected} \end{cases}$$

where the parameters and their base values are listed in Table 1.

Table 1. Variables and base values for sanitation of food contact surface.

Variable	Definition	Type	Base Value
s_j	sanitation effectiveness for RTE lot j	calculated	NA
s_{wipe}	between-lot sanitation effectiveness (dimensionless)	fixed, input	0.50
s_{sop}	end of day sanitation effectiveness (dimensionless)	fixed, input	0.75
s_{enhan}	enhanced sanitation effectiveness if a previous FCS was tested, found positive, and the enhanced sanitation option is selected (dimensionless)	fixed, input	0.95
LS_j	*Listeria* spp concentration on food contact surface at end of lot j (cfu/cm^2)	stochastic, calculated	NA
s_{lag}	S_{lag}=FCS report lag in days x number of lots produced per day (lot units, i.e. time)	fixed, input	6 (3 days x 2 lots per day)

Based on the transfer coefficient and sanitation effectiveness, the *Listeria* species concentration on the food contact surface was calculated as:

$$LS_j = \left(LS_{j-1} + \delta(j)\right) \quad \left(1 - TC_j\right) \quad \left(1 - s_j\right)$$

where the parameters and their base values are listed in Table 2.

Table 2. Variables and base values for *Listeria* concentration on food contact surface.

Variable	Definition	Type	Base Value
TC_j	transfer coefficient for lot j that explains the fraction of *Listeria* transferred from food contact surfaces to RTE product (dimensionless)	stochastic, input	LN(-0.14, 1), truncated to between 0 and 1
$\delta(j)$	*Listeria* spp. concentration added to the food contact surface if a contamination event is ongoing (cfu/cm^2) $\delta(j) = \begin{cases} 0 & \text{if not during contamination event} \\ RN \sim LN(-6,3.5) & \text{if during contamination event} \end{cases}$	stochastic, input	LN(-6, 3.5)

Once the number of *Listeria* species present on the food contact surface was calculated, the model calculated the *Listeria* species concentration per gram of product in each lot. The lot size varied based on the size of the processing plant. Three different processing plant sizes were modeled in this research; large, small, and very small. The size of each plant was classified according to Hazard Analysis and Critical Control Point (HACCP) guidelines where a large plant is defined as having 500 or more employees, a small plant defined as having 10 or more, but fewer than 500 employees, and a very small plant defined as having less than 10 employees *(24)*. The fraction of the deli meat food supply produced by large, small and very small plants and the pounds per shift per line for each plant size were estimated. A survey among RTE processors of deli meats as reported by FSIS found that for deli meats, about 48% of the food supply is produced by large plants, 48% by small plants, and the remaining 4% by very small plants *(11)*. The estimated average production volume in pounds of deli meats per line per shift is shown in Table 3.

Table 3. Lot (per line per shift) weight by plant size.

Plant size	**Lot weight (lbs)**	**Lot standard deviation (lbs)**
Large	19371	14000
Small	7100	10600
Very Small	2800	9500

Lot weights (i.e., pounds of deli meat per line per shift) were varied stochastically from lot to lot. These distributions were assumed to be normal. Simulated lot weights less than 1000 pounds were rounded up to 1000 pounds.

While the survey found that the average mass of a lot of RTE product varied by plant size, there was no evidence of a difference in the occurrence of *L. monocytogenes* in RTE product by plant size. To account for the variation in lot mass, the model adjusted the food contact surface area by plant size.

Next, the *Listeria* species concentration was converted to a concentration of *L. monocytogenes* using a *L. monocytogenes* to *Listeria* species ratio. This ratio was estimated from available data on the prevalence of *L. monocytogenes* to *Listeria* species. The data indicated whether or not a food contact surface was positive for *L. monocytogenes* when a

surface was found positive for *Listeria* species. These prevalence data were available from the published literature *(26)* and some unpublished industry data provided to FSIS *(33)*. The mean ratio of *Listeria* species/*L. monocytogenes* was found to be 52% and the standard deviation was 26%. Using this *Listeria* species/*L. monocytogenes* concentration ratio, the *L. monocytogenes* concentration in the RTE lot was calculated as:

$$LM_j = \left(LS_{j-1} + \delta(j)\right) \times TC_j \times \frac{A_j}{M_j} \times R_j$$

where the parameters and their base values are listed in Table 4.

Table 4. Variables and base values for the concentration of *L. monocytogenes* in a RTE product lot produced in the plant.

Variable	Definition	Type	Base Value
LM_j	*L. monocytogenes* concentration in RTE product lot j, cfu/g	stochastic, calculated	NA
A_j*	food contact surface area at lot j , stochastic (* only varies for new contamination event), cm^2	stochastic, input	U(100000, 1000000)
M_j	mass of lot j, lb, internally converted to g	stochastic, input	varies by plant size large: N(19371, 14000) small: N(7100, 10600) very small: N(2800, 9500)
R_j	*L. monocytogenes* / *Listeria* spp ratio for lot j (dimensionless)	stochastic, input	N(0.52, 0.26), truncated to between 0 and 1

The model also considered the effect of post-processing lethality and growth inhibitors in determining the *L. monocytogenes* concentration at retail. Post-processing lethality treatment reduces the concentration of *L. monocytogenes* in the product and growth inhibitors limit the growth of *L. monocytogenes* during the distribution of product from the plant to retail. The concentration of *L. monocytogenes* based on the use of post-processing lethality is determined as follows:

$$LMPP_j = \begin{cases} LM_j & \text{if } RN_j \geq FPP_k \\ LM_j * (1 - PP_k) & \text{if } RN_j < FPP_k \end{cases}$$

where the parameters and their base values are listed in Table 5.

Table 5. Variables and base values for the concentration of *L. monocytogenes* in a RTE product lot with consideration of post-processing interventions.

Variable	Definition	Type	Base Value
$LMPP_j$	*L. monocytogenes* concentration in RTE lot j after post processing interventions (cfu/g)	stochastic, calculated	NA
PP_k	Post processing intervention effectiveness for plant size k (dimensionless)	Stochastic, input	0 $U(PP_{min}, PP_{max})$ when applied
FPP_k	Fraction of lots for plant size k that undergo post processing interventions (dimensionless)	Fixed, Input	0
RN_j	Uniform random number used to test if lot j should undergo post processing	Stochastic, calculated	U(0,1)

Different plant sizes were allowed to have different minimum and maximum post-processing and growth inhibiting effectiveness. Which lots undergo either control intervention was decided using a simple binomial test based on the fraction of lots appropriate for the given plant size. Post-processing and growth inhibitor were not modeled for the base run.

The effect of growth inhibitors on the concentration of *L. monocytogenes* was accounted for by adjusting the *L. monocytogenes* growth factor. The growth of *L. monocytogenes* during shipment from the plant to retail was assumed to be 1.0 log units (i.e, a growth factor of 1 which effectively multiplies the cfu's by 10) for all product lots and this growth factor was adjusted for those lots using a growth inhibitor. The *L. monocytogenes* concentration based on the use of growth inhibitors is determined as follows:

$$LMGI_j = \begin{cases} LMPP_j * 10^{GF} & \text{if } RN_j \geq FGI_k \\ LMPP_j * 10^{GF+\log 10(1-GI)} & \text{if } RN_j < FGI_k \end{cases}$$

where the parameters and their base values are listed in Table 6.

Table 6. Variables and base values for modeling growth of *L. monocytogenes* in product.

Variable	Definition	Type	Base Value
$LMGI_j$	*L. monocytogenes* concentration in lot j after growth and growth inhibition during transport to retail (cfu/g)	Stochastic, calculated	NA
GF	Growth factor applied to all lots	Fixed, input	1
GI	Growth inhibition factor (a decimal reduction factor constrained as 0< GI <1)	Stochastic, input	0 $UN(GI_{min}, GI_{max})$ when applied
FGI_k	Fraction of lots for plant size k that undergo growth inhibition (dimensionless)	Fixed, Input	0

The impact of post-processing lethality treatment and growth inhibitors was evaluated by running the model using different scenarios to include using post-processing lethality and growth inhibitors in combination, using each intervention separately, or not using an intervention at all.

Following these control interventions, the lot would then be tested for *L. monocytogenes*, either because of routine lot testing or because an earlier food contact surface tested positive for *Listeria* species. The lot testing response is lagged by the time it takes to analyze a food contact surface sample for *Listeria* species and obtain results of this test. This lag time was assumed to be 3 days. The model also assumed that product lots of RTE product that test positive for *L. monocytogenes* are removed from the food supply.

The final step in the model was to select the lots that appear at retail from among the lots produced by each plant size: large, small, and very small. The model generates the requested number of lots for each plant size, then selects a continuous run to combine for the retail distribution. The number of lots in the run was determined by the fraction of production for each

plant size. The *L. monocytogenes* concentration after combining lots from different plant sizes was determined by:

$$LMComb_i = \begin{cases} LMGI_k^{large} & \forall\ k = start, FP_{large} * N_{Sim} \quad \cup \\ LMGI_k^{small} & \forall\ k = start, FP_{small} * N_{Sim} \quad \cup \\ LMGI_k^{very\ small} & \forall\ k = start, FP_{verysmall} * N_{Sim} \end{cases}$$

where the parameters and their base values are listed in Table 7. The union symbol convention is used here to indicate that the lots simulated for each plant size were combined to arrive at the resulting distribution.

Table 7. Variables and base values for modeling retail concentration of *L. monocytogenes* in a product lot.

Variable	Definition	Type	Base Value
$LMComb_i$	*L. monocytogenes* concentration in lot i after combining lots from different plant sizes (cfu/g)	Stochastic, calculated	NA
start	Starting lot number for run	Fixed, built-in	100
FP_k	Fraction of pounds produced by each plant size k (dimensionless)	Fixed, input	Large = 0.48 Small = 0.48 Very small = 0.04
N_{Sim}	Number of lots to simulate for each plant size	Fixed, input	1000000

For the first lot produced, it was assumed that the food contact surface *Listeria* concentration was 0 cfu/gram. To prevent this initial value from biasing the final results, the first 100 lots simulated for each plant size were excluded. This seeds the starting food contact surface concentration.

The final retail distribution is based upon the combined distribution, but filtered depending on whether or not the lot was tested and the corresponding result of the test. Any lot that was not tested and any lot that was tested and found negative passes on to retail. Any lot

that was tested and found positive is removed. The *L. monocytogenes* concentration at retail is calculated as

$$LM\text{Retail}_i = LMComb_i \mid_{\text{i not tested}} \cup LMComb_i \mid_{\text{i tested negative}}$$

The union convention used here indicates the the lots not tested and those lots testing negative were combined to arrive at the resulting retail distribution. The resulting distribution of *L. monocytogenes* concentrations on RTE product at retail serves as an input for the updated FDA-FSIS risk ranking model to estimate the public health impacts in terms of the estimated number of illnesses and deaths due to listeriosis.

1.2.6 Food Contact Surface and RTE Product Testing.

The testing procedure for *L. monocytogenes* in a lot was calculated by first generating a Poisson random number using a population mean as mean cfu's within the sample (sample mass, SM_j, g × concentration, LM_j, cfu/g):

$$LM_{sample\ j} = Poisson\left(SM_j \times LM_j\right)$$

where the sample mass for this study was approximately 125 g.

The RTE lot sample is judged positive by:

$$LMR_{sample\ j} = \begin{cases} positive \text{ if } \mathrm{LM}_{\text{sample}} > 0 \text{ and } 1 - \left(1 - \mathrm{pDLM}\right)^{\mathrm{LM}_{\text{sample}}} < \mathrm{U}\left(0,1\right)_j \\ negative \qquad \text{otherwise} \end{cases}$$

where the parameters and their base values are listed in Table 8.

Table 8. Variables and base values for testing for *L. monocytogenes* in product.

Variable	Definition	Type	Base Value
$LM_{sample\ j}$	total *L. monocytogenes* cfu in test sample j (cfu)	stochastic, calculated	NA
pDLM	probability of detecting 1 *L. monocytogenes* cfu in test if present (dimensionless)	fixed, input	0.75
$U(0,1)_j$	uniform random number between 0 and 1 (dimensionless)	stochastic, calculated	NA
$LMR_{sample\ j}$	*L. monocytogenes* test result for lot j (positive or negative)	stochastic, calculated	NA

The testing procedure for food contact surfaces was calculated by generating a Poisson random number using a population mean as mean cfu's on the contact surface tested (contact surface area, A_j, cm^2 × concentration, LS_j, cfu/cm^2):

$$LS_{sample\ j} = Poisson\left(A_j \times LS_j\right)$$

The FCS sample is judged positive by

$$LSR_{sample\ j} = \begin{cases} positive \text{ if } \mathrm{LS}_{\mathrm{sample\ j}} > 0 \text{ and } 1 - \left(1 - \mathrm{pDLS}\right)^{\mathrm{LM}_{\mathrm{sample\ j}}} < \mathrm{U}\left(0,1\right)_j \\ negative \qquad \text{otherwise} \end{cases}$$

where the parameters and their base values are listed in Table 9.

Table 9. Variables and base values for testing for *Listeria* on food contact surface.

Variable	Definition	Type	Base Value
$LS_{sample\ j}$	total *Listeria* species cfu in test sample j (cfu)	stochastic, calculated	NA
pDLS	probability of detecting 1 *Listeria* species cfu in test if present (dimensionless)	fixed, input	0.75
$U(0,1)_j$	uniform random number between 0 and 1 (dimensionless)	stochastic, calculated	NA
$LSR_{sample\ j}$	LS test result for lot j (positive or negative)	stochastic, calculated	NA

For both contact surface testing and product testing, the modeled concentration of the organism was multiplied by the sample size to estimate the mean of a Poisson distribution. For food contact surfaces, the concentration is measured in cfu/cm^2 and the sample size is measured in cm^2. For RTE product, the concentration is measured in cfu/gram, and the sample size in grams. A random number was generated from this distribution to represent the number of cfu's in the sample itself.

Once the number of organisms in the sample was known, the probability that a test to detect the presence of the pathogen would yield a positive result could be determined by using a binomial distribution:

$$p(positive\ test) = 1 - (1 - pDLS)^{LM_{sample}}$$

where pDLS is the probability of detecting 1 cfu in the sample, and LM_{sample} is the number of cfu's in the sample from the Poisson calculation. The pDLS probability is based on the detection limit and microbiological test sensitivity and is an input parameter to the risk assessment model.

1.3 Results and Discussion

1.3.1 Effect on *Listeria* concentrations at retail.

The FSIS *Listeria* risk assessment was designed to determine the effectiveness of various food contact surface testing and sanitation scenarios (e.g., vary the frequency of testing by plant size – large, small, and very small plants) as well as other interventions (e.g., post-processing lethality or the use of growth inhibitors) on mitigating *L. monocytogenes* contamination in finished RTE product.

Figure 2 shows three quantile (i.e., the 80^{th}, 99^{th}, and 99.99^{th} percentiles) concentrations of *L. monocytogenes* in deli meats at retail for the scenarios analyzed. Most of the scenarios are given as triplet numbers, e.g. 4-2-1, and represent the number of monthly food contact surface samples per line for large, small, and very small plants. The "60-60-60" triplet represents testing the food contact surface for every lot that is produced, because the model assumes that each line produces 60 lots per month. The "60-60-60 Lot" scenario represents testing every lot produced for *L. monocytogenes*, rather than a food contact surface for *Listeria* species. The FDA scenario shows the concentration at retail predicted by the FDA-FSIS model *(7)*. "PP" represents post-processing intervention/control, assuming that 100% of the industry incorporates some form of post-processing that is 90-95% effective. The "GIP" represents that 100% of the industry incorporates growth inhibiting packaging or product reformulation that is 90-95% effective. Finally, the "PP&GIP" scenario represents a combination of the previous two scenarios: 100% of the industry incorporates both post-processing and some form of growth inhibition, each of which is 90-95% effective.

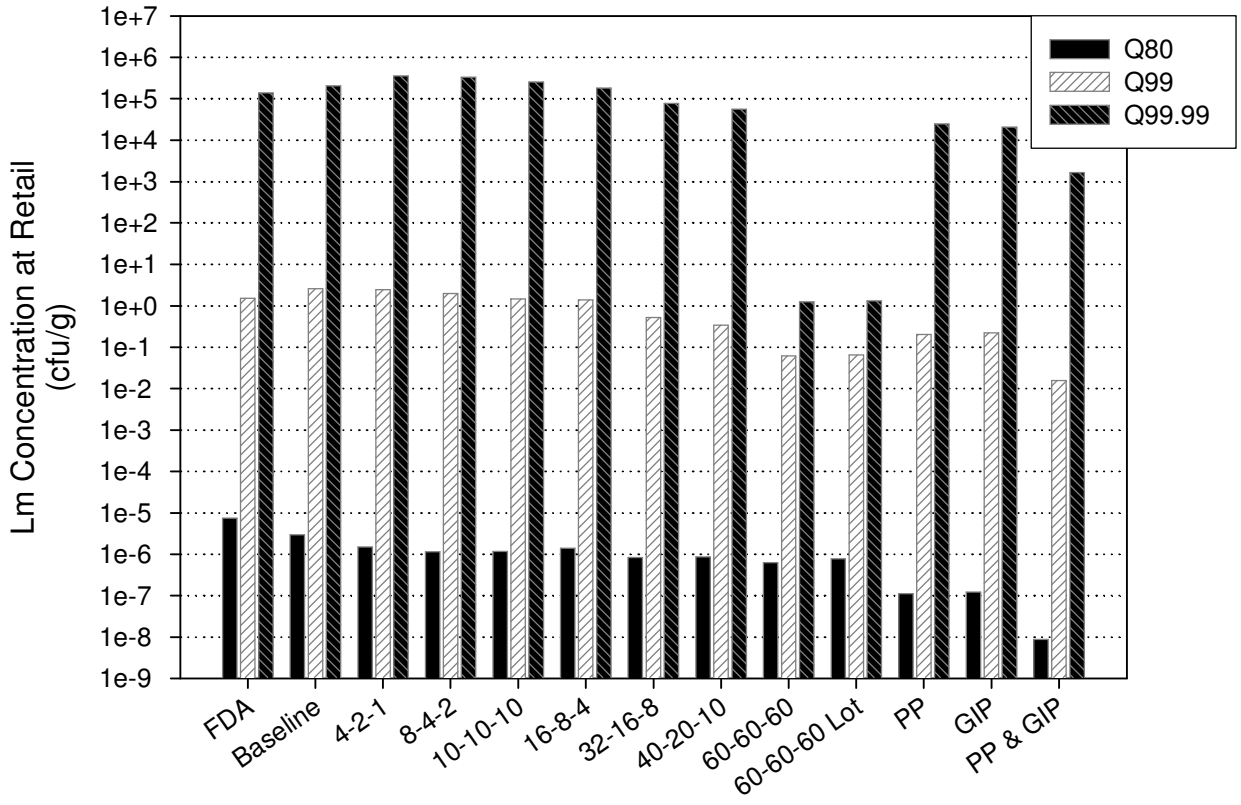


Figure 2. Quantiles of *L. monocytogenes* at retail for various scenarios tested.

The data generally show a decline in the *L. monocytogenes* concentration in RTE product at retail as the food contact surface testing and sanitation effort increases. The decline is apparent in the 99.99th percent quantile, however there is little change in the 80th percentile across the food contact surface testing and sanitation scenarios. This pattern suggests that testing and sanitation are effective at detecting (and ultimately leading to the removal of) high concentrations of *L. monocytogenes,* but may not detect low concentrations.

Post-processing lethality and growth inhibitors each have lower 80th percent quantiles than 60-60-60 testing (i.e., testing each lot of RTE product). Most importantly, there is the greatest decrease in the 80th percent quantile when post-processing lethality and growth inhibitors are combined meaning that this combination is the most effective at eliminating the low concentrations of *L. monocytogenes*.

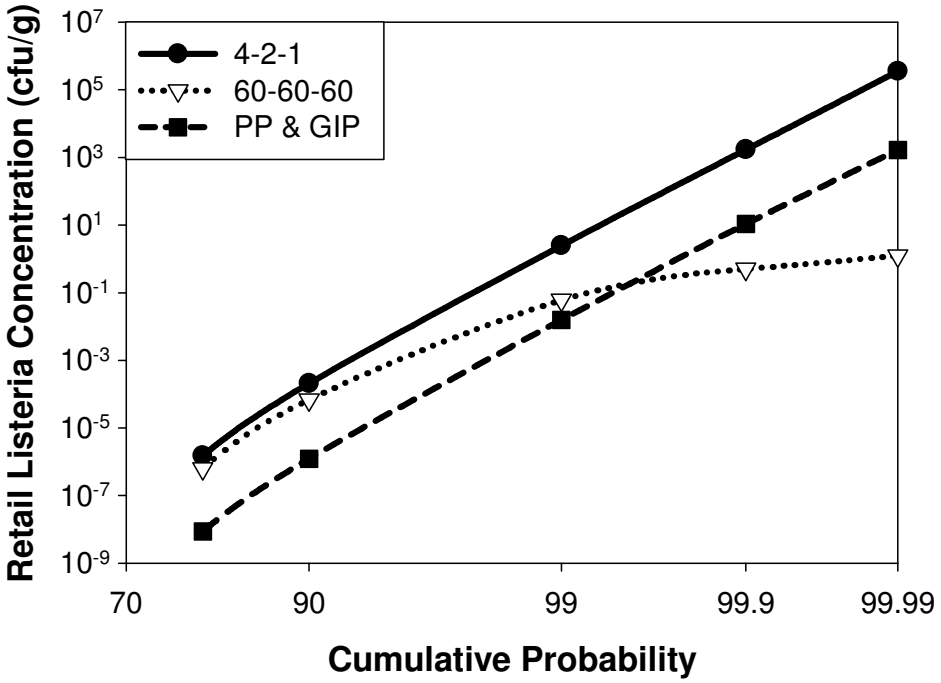


Figure 3. Retail *Listeria* concentrations for sampling versus post-processing and growth inhibitors.

Figure 3 compares the cumulative probability of detecting *L. monocytogenes* in RTE product over a range of retail *Listeria* concentrations via three control methods for a large processing plant: the FSIS minimum sampling level, testing every lot of RTE product, and using a combination of post-processing lethality and growth inhibitors. As seen in the quantile plot, sampling and testing each product in the lot greatly reduces the higher *Listeria* concentrations when compared to the minimum sampling requirement, but at the lower concentrations (i.e. below the detection limit) increased sampling is ineffective. The detection limit is determined by the sample size or food contact surface area tested; therefore to improve sampling effectiveness larger samples would be necessary. The use of a combination of post processing lethality and growth inhibitor decreases the entire probability distribution of *L. monocytogenes* to include even the lowest concentrations. Eliminating the low *Listeria* concentrations helps prevent regrowth of *Listeria* on the product therefore reducing the concentration observed at consumption and subsequently reducing the number of illnesses and deaths.

The vertical distance between the 4 samples/ month and the post-processing lethality and antimicrobial distributions is controlled by the effectiveness and degree of use within the industry for the lethality and growth inhibiting controls. A higher effectiveness than the 1.5 – 2 log reduction modeled in this study would lower the location of the line while maintaining a similar slope. Many currently available post-processing lethality technologies are capable of 5 log or higher reduction*(27)*.

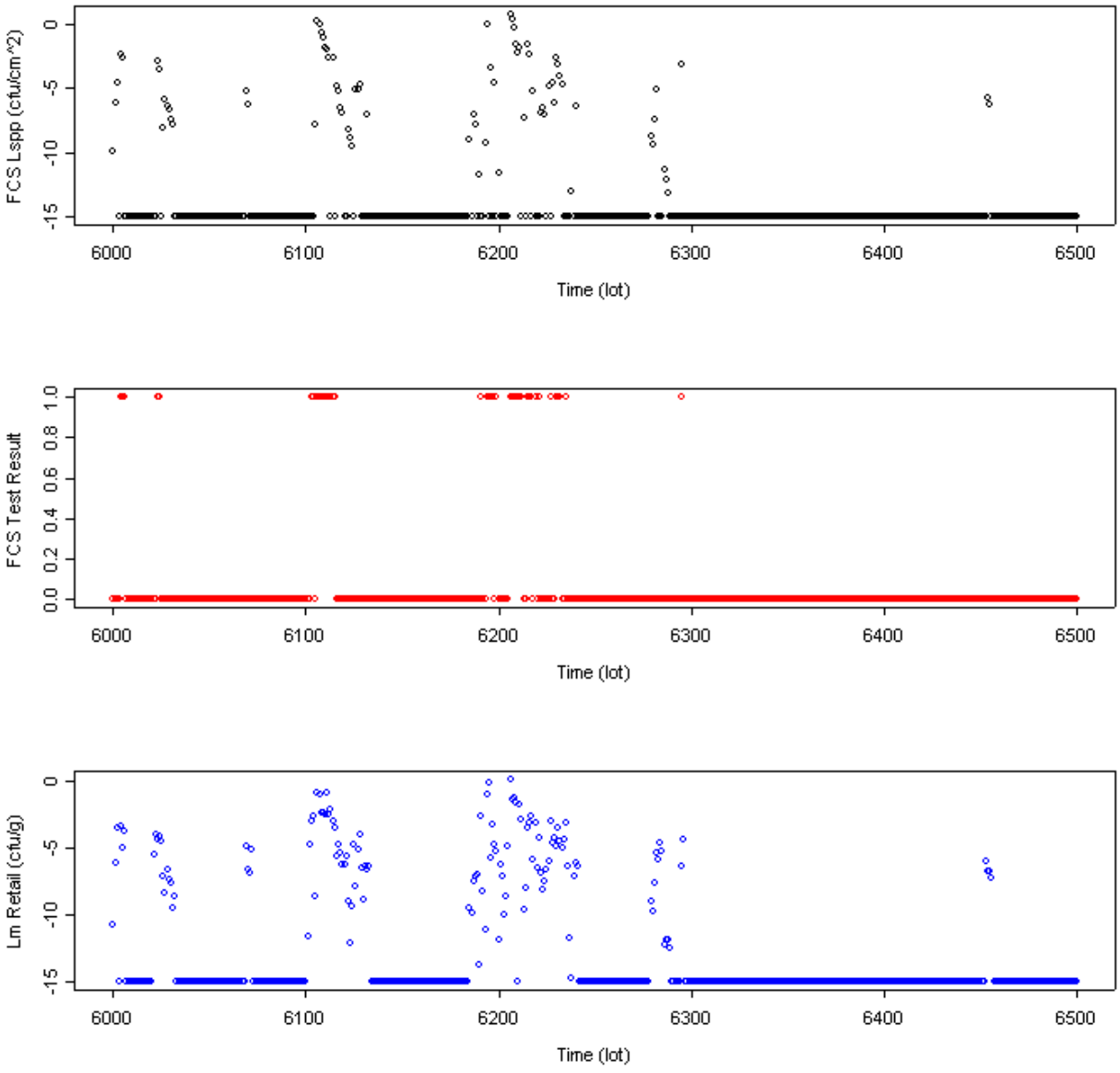


Figure 4. Example temporal clustering during contamination events.

In Figure 4 the concentration of *Listeria* species on a food contact surface was plotted simultaneously with food contact surface testing results and *L. monocytogenes* concentrations at retail for a given time period. The positive food contact surface results were temporally clustered during contamination events and correlated with the *L. monocytogenes* and *Listeria* species concentrations. This shows that even if sanitation and testing are the only control interventions used, their effectiveness in mitigating *L. monocytogenes* contamination may be enhanced if temporal clustering is occurring. This clustering allows for food safety improvements if response to a positive food contact surface result is rapid and may help decrease the duration and severity of a contamination event.

1.3.2 Public Health Effects.

The *L. monocytogenes* distributions at retail predicted by the in-plant model for the various scenarios were fed into the FDA-FSIS model to determine the effect on consumer exposure and the dose-response in terms of the number of deaths and illnesses resulting from listeriosis. The dose-response portion of the FDA-FSIS model was calibrated to 310 deaths per year among the elderly *(11)*.

Figure 5 depicts the estimated median numbers of lives saved among the elderly for each the scenarios tested. For the proposed minimum food contact surface testing (i.e., the 4-2-1 scenario *(9)*) the estimated median number of deaths among the elderly was reduced by approximately 20 per year. Post processing and growth inhibitors used in combination was estimated to prevent over 180 elderly deaths, a 60% reduction from the number of elderly deaths estimated in the baseline model.

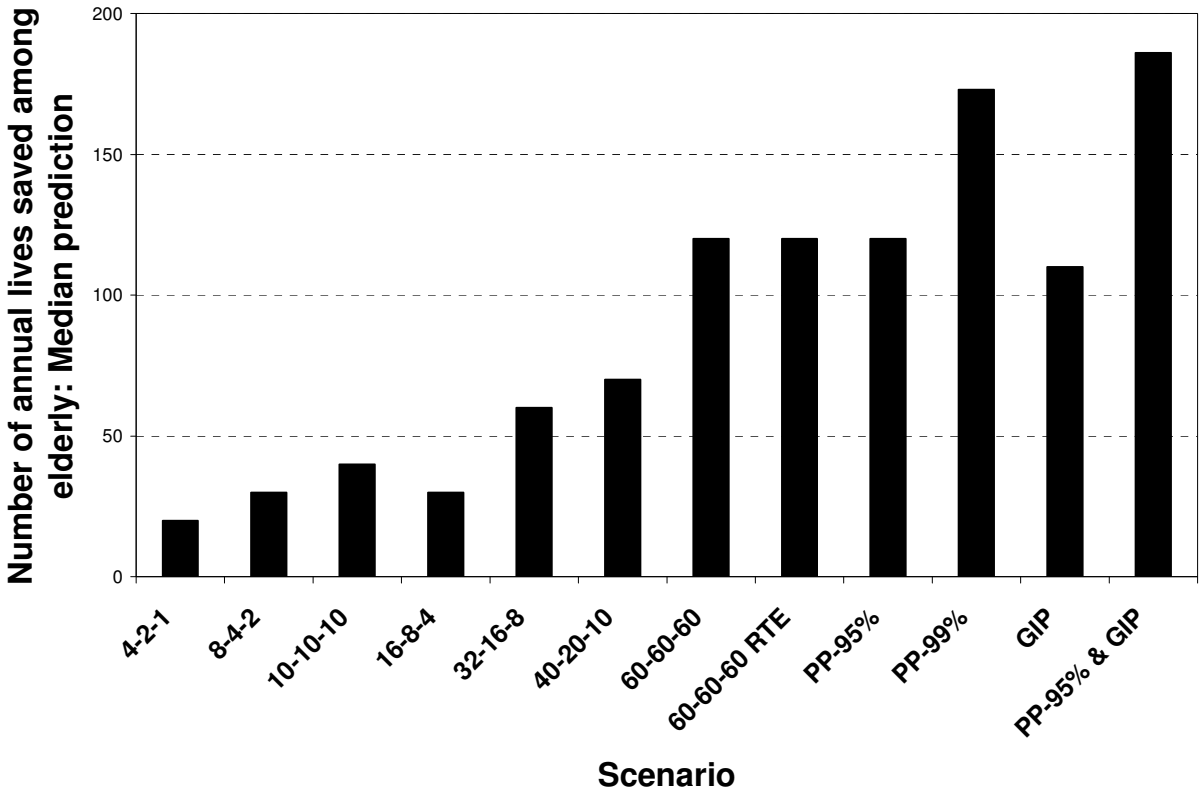


Figure 5. Estimated number of deaths among the elderly for the various scenarios tested.

Based on a monotonic Kendall tau statistical test for trend, the increase in the number of lives saved with increasing frequency of testing is statistically significant at the 99% significance level. (tau=0.88, p=0.0028). Nevertheless, the combination of [consistent and universal] post processing and growth inhibition saves 66 more lives among the elderly than sampling every product in each lot. This enforces the finding that post-processing lethality treatments used in combination with antimicrobial growth inhibitors are more effective than product sampling alone.

1.3.3 Public health policy.

As a result of the FSIS *Listeria* risk assessment, the USDA created Interim Final Rule 9 CFR 430 *(10)* to regulate ready-to-eat food processors. The rule allows processors to choose from 3 alternatives:

1. use both a growth inhibiting agent and a post-processing lethality

2. use either a growth inhibiting agent or a post-processing lethality step

3. use neither a growth inhibitor nor a post-processing lethality step.

Plants implementing fewer controls and adopting alternatives 2 or 3 are required to have higher sampling frequencies than those implementing alternative 1. Since the establishment of the Interim Final Rule, tracking of food processors indicates a voluntary shift to those categories using additional controls and the incidence of listeriosis has declined *(4)*. Figure 6 demonstrates the shift towards alternatives 1 and 2. Based on this analysis, this shift may be preventing a number of listeriosis deaths.

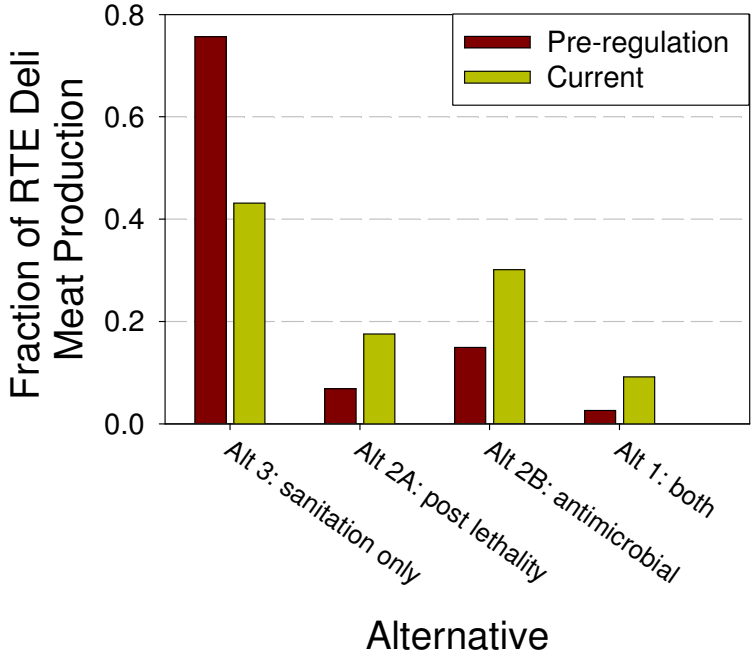


Figure 6. Fraction of RTE meat production by Interim Final Rule alternative.

1.4 Conclusions

The FSIS *Listeria* risk assessment model results indicated that the proposed minimal frequency of testing and sanitation of food contact surfaces, as presented in the FSIS proposed rule *(9)*, will result in a small reduction in the levels of *L. monocytogenes* on deli meats at retail, but greater frequency of food contact surface testing and sanitation is estimated to lead to a proportionally lower risk of listeriosis. The use of a combination of interventions (e.g., post-processing lethality and the use of growth inhibitors) is more effective in mitigating potential

contamination of RTE meat and poultry product with *L. monocytogenes* than sampling or any single intervention used alone. Subsequently, the use of a combination of interventions best reduces the risk of illness or death due to listeriosis. Also, when relying on sampling alone to maintain food safety, a timely response to a positive food contact surface may help reduce the duration and severity of a contamination event due to the temporal clustering of food contact surface positives.

The FSIS *Listeria* risk assessment model provides a method for comparing the relative effectiveness of various control interventions. This is valuable information which has been used to help guide public policy in an effort to reduce the incidence of listeriosis. In the future, this model may be used to compare additional management scenarios or demonstrate the effect of the scenarios presented in this risk assessment for a variety of other RTE products, such as frankfurters which pose a moderate health risk and have also been associated with a number of listeriosis outbreaks.

Chapter 2. *Listeria monocytogenes* Prevalence and Level in Ready-to-eat Meat and Poultry deli meat

2.1 Introduction

The presence and level of *L. monocytogenes* in ready-to-eat (RTE) meat and poultry products was determined using data from a study conducted by the National Alliance for Food Safety and Security (NAFFS) *(6)*. The data collected in this study were also used in calculating the comparative risk ratio for listeriosis in retail-sliced versus prepackaged ready-to-eat meat and poultry products.

2.2 Materials and Methods

2.2.1 Data Collection.

The sampling group comprised four designated sites in the Foodborne Disease Active Surveillance Network (FoodNet). These were Northern California (CA), Georgia (GA), Minnesota (MN), and Tennessee (TN). Sampling was weighted by the populations in counties *(3)* so that exposure could be estimated. Approximately 75% of shopping is done at major supermarket chains and 25% is done at other grocers, such as independent retailers *(14)*. The number of samples collected from supermarkets versus independent retailers was weighted accordingly. Also, approximately 50% of consumers purchase RTE meat products that are sliced at delicatessens with the remainder purchasing sliced prepackaged products *(1)*. The relative number of samples between prepackaged and retail-sliced was therefore kept approximately equal as part of the sampling design. Sample data were encoded by the researchers to prevent identification of the store.

Approximately 2,000 samples (125 grams each) were analyzed from each of the four designated sites, with approximately equal numbers of retail-sliced and prepackaged samples, and a small number of intact chubs or logs. Chubs data not included in this analysis. The sampling protocol was designed to allow for statistically valid comparisons among sites, RTE products type, and retail-sliced versus prepackaged, assuming an $\alpha = 0.05$ and a 90% power of detecting a difference of 2% in the comparison of binomial proportions.

The following product types were sampled: cured poultry, uncured poultry, pork, and beef. Approximately 1,000 samples of each product type were analyzed to support conclusions at the desired level of certainty. Use of any growth inhibitors was noted at the time of sample

collection. Specific instructions were provided for sample collectors, including the product category, the number of samples of each type of product to be obtained, size of the sample to be purchased, and how to choose, collect, hold and transport the sample.

Sample collection was standardized to maintain consistency. Sampling and laboratory analyses followed standard laboratory practices. These included temperature monitoring during shipment, chain of custody documentation, aseptic transfer and handling within the laboratory, and initiating analyses within 24 hours of receipt of sample. The laboratories were instructed to discard any sample with package damage such that the microbiological integrity of the sample was not compromised. Samples not meeting quality control requirements were noted and discarded. The FSIS standard laboratory method for *L. monocytogenes* detection was implemented by the laboratories for use in this study. All samples were tested for the presence of *L. monocytogenes* by inoculation in UVM broth followed by Fraser broth then modified Oxford (MOX) agar. Original samples were saved in case the sample was positive so that the concentration of *L. monocytogenes* could be quantified in cfu's per gram. Positive samples were quantified using a FSIS protocol 9-tube Most Probable Number (MPN) method with a reported detection limit of 0.3 MPN/gram.

Samples were assigned codes and the following product information recorded: sampling location (FoodNet site along with producer information, retailer's name, and location of purchase), date of receipt at the laboratory, whether the sample appeared to be packaged in-store or prepackaged, and the use-by or sell-by date. Any store information or identifiers were removed prior to transfer to FSIS.

2.2.2 Statistical Analyses

Statistical analyses were performed using Number Cruncher Statistical Systems (NCSS) 2001 *(15)* and R version 2.6.1 *(25)*. For statistical tests, p values less then 0.05 were considered statistically significant, and p values between 0.05 and 0.10 were considered marginally significant.

Data were analyzed in a variety of ways. The prevalence of *L. monocytogenes* among retail-sliced and prepackaged samples were analyzed by sampling site, product type, store type, time of day (morning or afternoon), and quarter of the year using tests of proportions. The null hypothesis for this test was that all the prevalence for both product types were equal. The alternative hypothesis was that the prevalence differed. This statistical test assumed

independence among the samples, although this assumption is not likely met for these data. Because multiple samples were collected at the same store, multiple positive *L. monocytogenes* findings were likely correlated because of cross-contamination and poor hygienic conditions at the store. Statistical tests with correlated positive samples claim to find statistically significant results more commonly than intended.

Tests of proportions were also conducted at the retail store level. A store was considered positive for retail-sliced or prepackaged product if any of the samples for that category were found positive for *L. monocytogenes*. Stores are more likely to be independent than the individual data, but there are problems with using this approach. Store identifiers (even arbitrary labels) were removed from data provided prior to submittal to FSIS as part of the data encoding and blinding process. Store visits had to be estimated based on date and time of sampling collection. Sample collection times were not provided for samples from Minnesota, therefore the number of stores available was much smaller than the number of samples. Also, statistical tests based on only a few hundred samples have limited statistical power and are unlikely to detect small differences in prevalence at reasonable levels of confidence. Finally, this approach does not directly incorporate the number of samples collected at each store.

Another approach used was a logistic regression to predict the store prevalence for retail-sliced and prepackaged product as a function of a number of indicator variables: where the product was sliced, the store type, and the time of day the sample was collected. This approach is not subject to the correlation problem because it is based on store prevalence. The regression was weighted by the number of samples taken at the store, and evaluated more than one explanatory variable simultaneously.

2.3 Results

2.3.1 Prevalence and Number of Samples

Fifty-seven samples were found to be positive for *L. monocytogenes* resulting in an overall prevalence of 0.76%. Two of these positives were found in chub samples, six were found in prepackaged samples, and the remaining 49 positives were found in retail-sliced samples. The number of prepackaged and retail-sliced samples across the four FoodNet sites is shown in Table 8.

Table 8. Prevalence of positive product samples[1] and stores visited based on sampling site.

Category	Sampling Site			
	CA	**GA**	**MN**	**TN**
Product samples	0.74% (10/1360)	0.60% (12/2000)	0.95% (16/1685)	0.85% (17/1995)
Stores[2]	6.98% (6/86)	4.93% (7/142)	n/a[3]	10.23% (9/88)

[1]Product samples include both retail-sliced and prepackaged RTE meat and poultry product.

[2] Store visit estimated based on similar sampling date and time. No sample times were provided for MN, so estimate of stores sampled was not available.

Slightly fewer product samples were taken in CA than other sites. More stores were sampled in GA than other sites. In addition to prepackaged and retail-sliced product samples, 105 and 300 additional chub samples were collected in MN and TN respectively. Assuming independence, a test of proportions indicated no statistically significant difference for the prevalence within product samples among the four sites ($p = 0.75$). Neither was there any statistical difference for the store prevalence across the sites ($p = 0.31$). This allowed for pooling of the data for purposes of discussing total prevalence. The number and prevalence for retail-sliced and prepackaged samples by quarter of the year is shown in Table 9. More product samples and more stores were visited in the 3rd quarter than in other quarters. Assuming independence, a test of proportions indicated a statistically significant difference for the prevalence within product samples ($p = 0.01$) but not store prevalence ($p = 0.31$).

Table 9. Prevalence of positive product samples[1] and stores visited based on quarter of year.

Category	Quarter of Year			
	1st	**2nd**	**3rd**	**4th**
Product samples	0.16% (2/1275)	0.74% (13/1746)	1.15% (28/2430)	0.76% (12/1589)
Stores[2]	2.63% (2/76)	7.37% (7/95)	5.34% (7/131)	10.00% (6/60)

[1]Product samples include both retail-sliced and prepackaged RTE meat and poultry product.

[2]Store visit estimated based on similar sampling date and time. No sample times were provided for MN, so product samples include MN but stores sampled do not.

The prevalence of positive retail-sliced and prepackaged samples by time of day is shown in Table 10. Slightly more product samples and stores were sampled in the afternoon. Assuming independence, a test of proportions indicated a statistically significant difference for the prevalence within product samples ($p = 0.04$) but not store prevalence ($p = 0.75$).

Table 10. Prevalence of positive product samples[1] and stores visited based on time of day (AM versus PM).

Category	Time of Day	
	AM	**PM**
Product samples[2]	0.51% (13/2540)	1.04% (32/3060)
Stores[2]	5.42% (9/166)	6.81% (13/191)

[1]Product samples include both retail-sliced and prepackaged RTE meat and poultry product.

[2]Store visit estimated based on similar sampling date and time. No sample times were provided for MN, so neither product samples nor stores sampled include MN.

The more interesting time of day analysis looked solely at retail-sliced product as shown in Table 11. Retail-sliced product samples collected in the afternoon were more than twice as likely to test positive for *L. monocytogenes* – 1.92% versus 0.92%. Assuming independence, this difference was statistically significant ($p = 0.04$). While store prevalence was also higher in the afternoon (7.83% versus 5.80%), this difference was not statistically significant ($p = 0.64$).

Table 11. Prevalence of positive retail-sliced product and stores visited based on time of day (AM versus PM).

Category	Time of Day	
	AM	**PM**
Number of product samples[1]	0.92% (12/1307)	1.92% (31/1612)
Estimated number of stores sampled[1]	5.80% (8/138)	7.83% (13/166)

[1]Store visit estimated based on similar sampling date and time. No sample times were provided for MN, so neither product samples nor stores sampled include MN.

The number and prevalence for retail-sliced and prepackaged samples is shown in Table 12. As designed, more product samples were collected at major grocery chains. Assuming independence, a test of proportions found a marginal statistically significant difference for the prevalence within product samples ($p = 0.07$) but not store prevalence ($p = 0.82$).

Table 12. Prevalence of positive product samples[1] and stores visited based on store type.

Category	Store Type	
	Major Chain Grocer	**Other Grocer**
Number of product samples[2]	0.64% (31/4801)	1.10% (24/2186)
Estimated number of stores sampled[2]	5.58% (11/197)	6.71% (11/164)

[1]Product samples include retail-sliced, prepackaged, and chub RTE meat and poultry product.

[2]Store visit estimated based on similar sampling date and time. No sample times were provided for MN, so product samples include MN but stores sampled do not.

Product samples were collected from prepackaged product, from product sliced at retail delis, and a limited number from intact chubs collected at retail. The number of RTE product samples by location of slicing is shown in Figure 7. A total of 3,518 retail-sliced samples, 3,522 prepackaged samples, and 405 chub samples were collected.

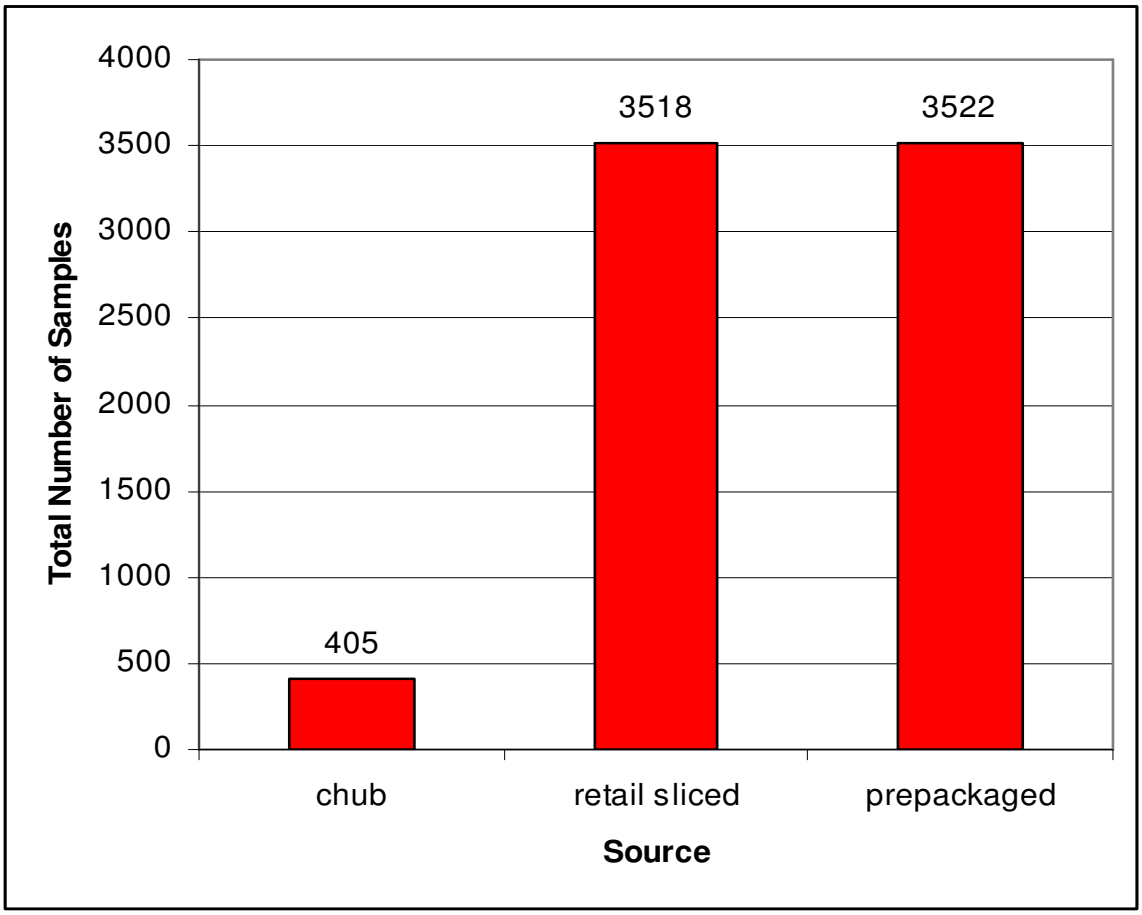


Figure 7. Number of RTE samples by location of slicing.

The data also indicate that deli meat sliced at retail is more likely to be contaminated than prepackaged deli meat (1.39% versus 0.17%). The results are shown in Figure 8. Assuming independence, a test of proportions between retail and prepackaged prevalence indicated retail-sliced deli meat had a statistically significant higher prevalence ($p < 0.0001$).

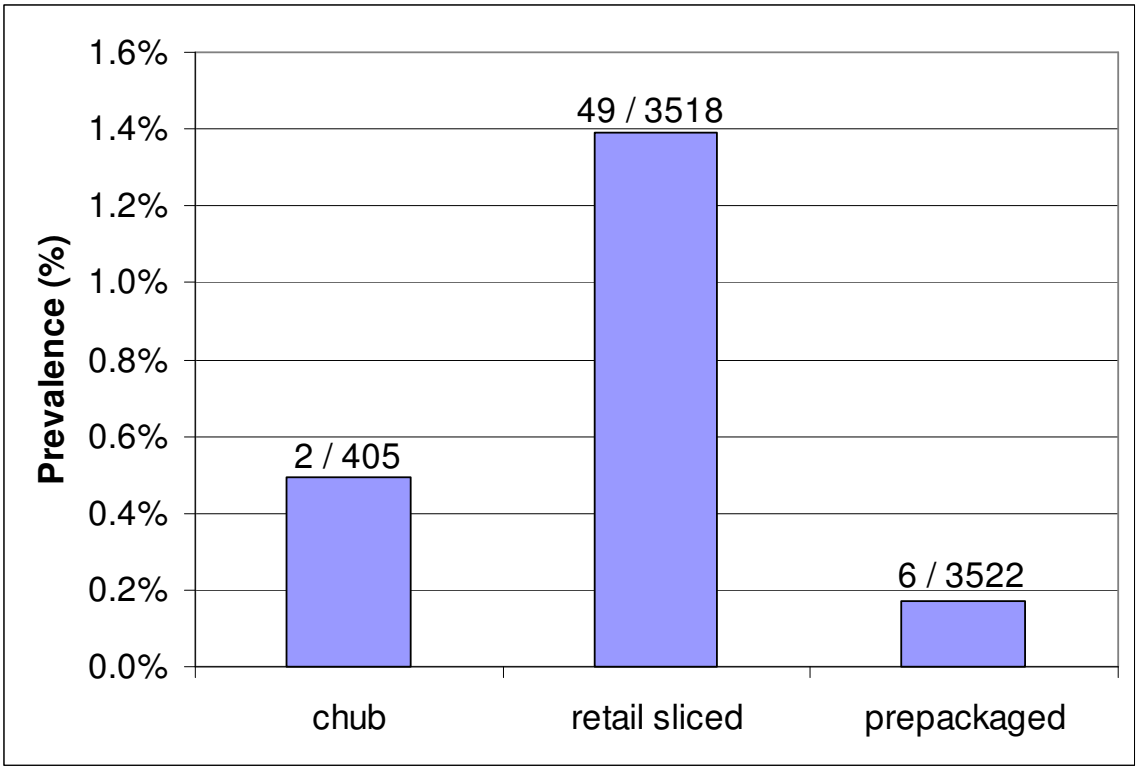


Figure 8. Prevalence of *L. monocytogenes* in deli meat by location of slicing.

The site and slicing location results for sliced deli meat only are shown in Table 13. Chub results are not included. The striking difference in prevalence between retail-sliced versus prepackaged is evident at all sites. Differences among the sites are relatively minor.

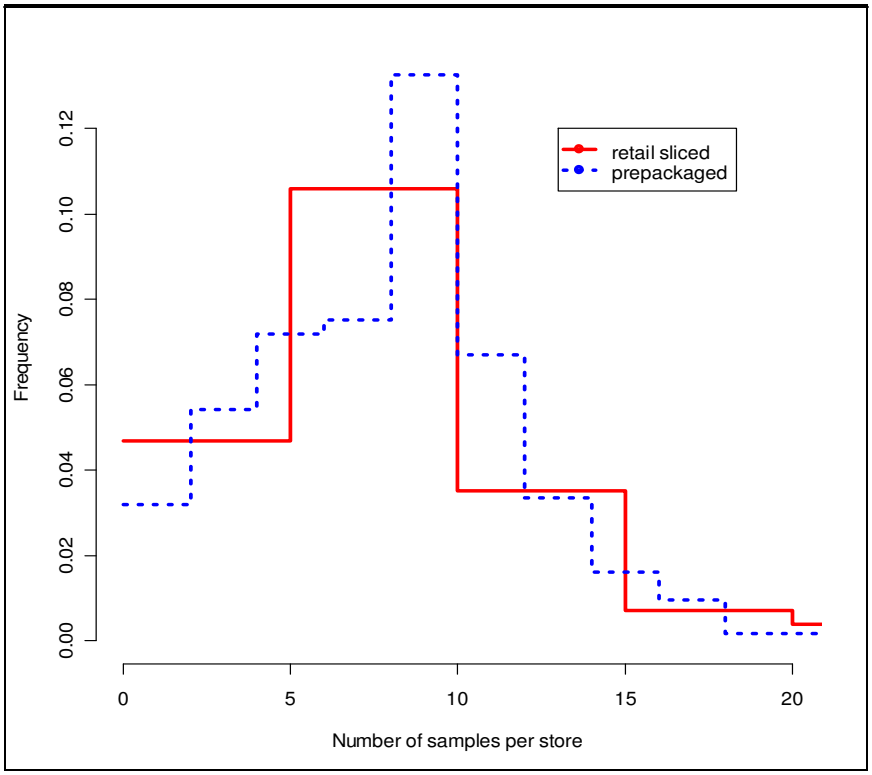
Table 13. Prevalence of *L. monocytogenes* in retail-sliced and prepackaged deli meat by site.

		Site				
		CA	**GA**	**MN**	**TN**	**Overall**
Processing	Retail-sliced	1.3% (12/929)	1.4% (10/731)	1.4% (12/841)	1.5% (15/1017)	1.4% (49/3518)
	Prepackaged	0.0% (0/1071)	0.0% (0/629)	0.5% (4/844)	0.2% (2/978)	0.2% (6/3522)
	Overall	0.6% (12/2000)	0.7% (10/1360)	0.9% (16/1685)	0.9% (17/1995)	0.8% (55/7040)

Note: The number of positive samples and the total number of samples are shown in parentheses. Chub data are not included.

For the 362 stores identified across the three sites available (CA, GA, TN) retail-sliced deli meat was sampled at 308 stores and prepackaged deli meat was sampled at 313 stores. For most stores, both types of deli meat was collected – 259 of these stores had both retail-sliced and prepackaged samples collected, 49 had only retail-sliced samples collected, and 54 had only prepackaged sliced samples collected. The testing results showed that only one store had positives samples for both retail-sliced and prepackaged deli meat. An additional 20 of the stores had positive retailed-sliced samples, and one store had positive prepackaged deli meat only.

Histograms of the number of retail-sliced and prepackaged deli meat samples taken at each store are shown in Figure 9. For retail-sliced deli meat, the number of deli meat samples per store ranged from 1 to 30, with a median of 8. The 25th and 75th% quantiles were 6 and 10 respectively. For prepackaged deli meat, the number of deli meat samples ranged from 1 to 24, with a median of 9. The 25th and 75th% quantiles were 6 and 11, respectively.

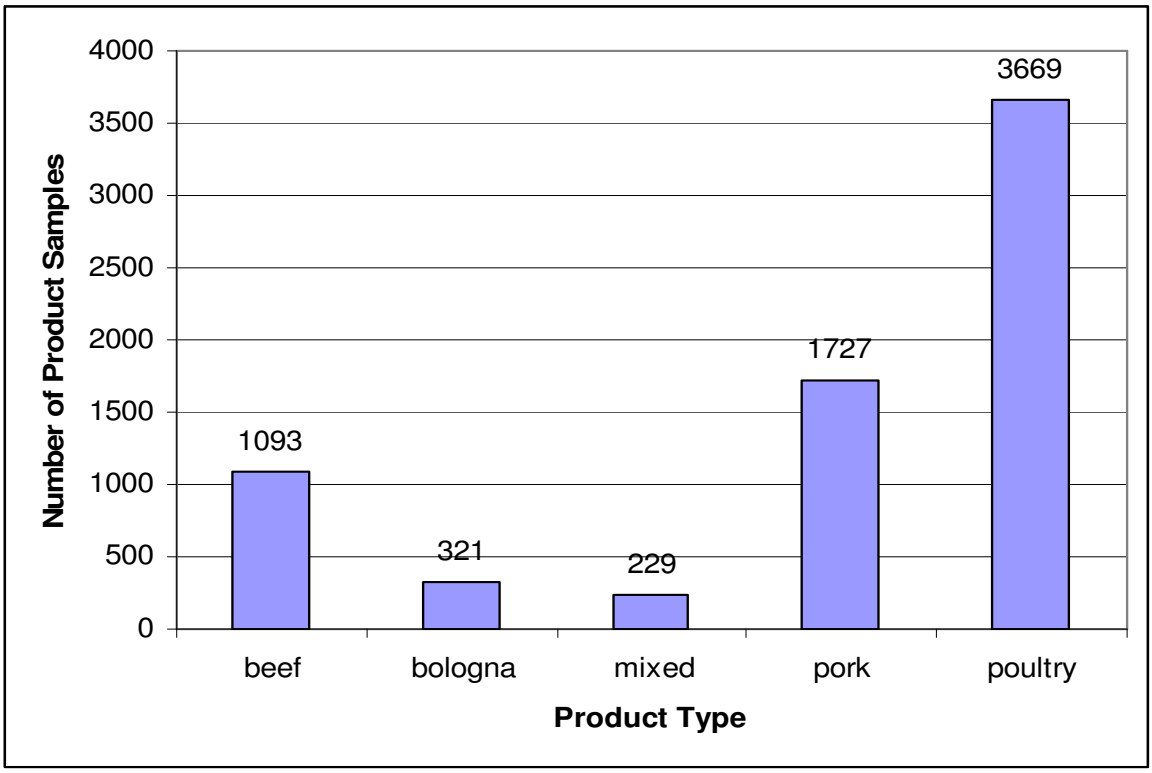


Note: MN data are not included because stores could not be identified.

Figure 9. Number of deli meat samples collected per store.

Some differences existed among the different sites for labeling types of deli meats. After correcting for obvious misspellings and accounting for multiple orderings, the types of deli meats listed in the data were: beef, beef/chicken/pork, beef/chicken/turkey, beef/pork, beef/pork/turkey, bologna, chicken, chicken/pork, chicken/turkey/pork, ham, mixed, pork, pork/turkey, poultry, poultry (chicken), poultry (chicken/pork), poultry (chicken/pork/beef), poultry (turkey), poultry (turkey/pork), and roast beef.

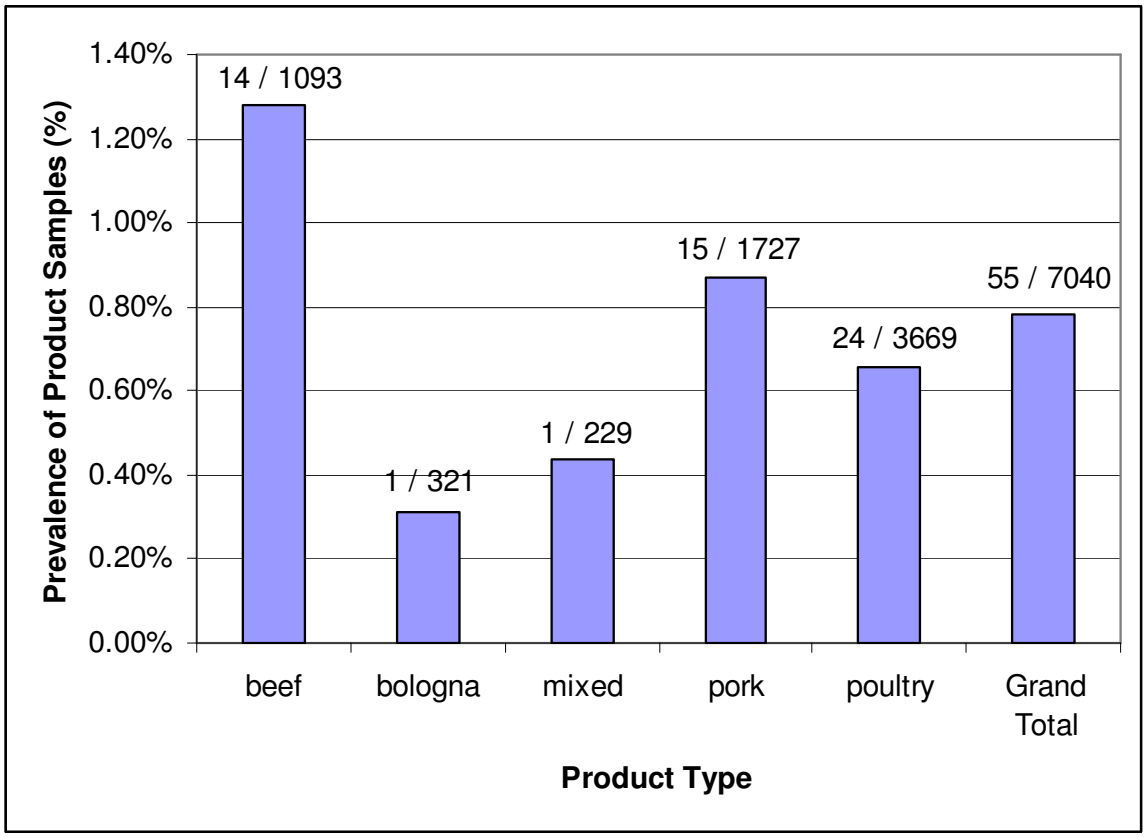
Many categories of deli meat types had very few samples. For purposes of this analysis, these categories were combined into 5: beef, bologna, pork, poultry, or mixed. Deli meat labeled as "bologna" was classified into different product types. If labeled by the sampler as "beef bologna," it was categorized as beef. If labeled with mixed components, it was categorized as mixed. If labeled simply as bologna, it was categorized as bologna. Deli meat listed as poultry but containing mixed components was categorized as mixed. For example, the samples labeled "poultry (chicken/pork)" were categorized as mixed. Based on this categorization, the counts by product type are given in Figure 10.



Note: Chub data are not included. One sample (not shown) did not include any listing for deli meat type.

Figure 10. Number of RTE samples by deli meat type.

The prevalence of *L. monocytogenes* across the different deli meat types is shown in Figure 11. Although it appears that beef has a slightly higher prevalence, the differences were not statistically significant based on a test of proportions ($p = 0.22$) among the five different deli meat types (beef, bologna, mixed, pork, poultry). The corresponding *L. monocytogenes* prevalence for beef, bologna, mixed meat, pork, poultry deli meats were 1.28%, 0.31%, 0.44%, 0.87%, and 0.65%, respectively. There does not appear to be any difference in the prevalence of *L. monocytogenes* based on whether the deli meat was cured or uncured. A similar test was conducted for retail-sliced only deli meat samples with similar results. Overall, there was no statistically significant difference in the prevalence of *L. monocytogenes* among the different deli meat types ($p = 0.43$)



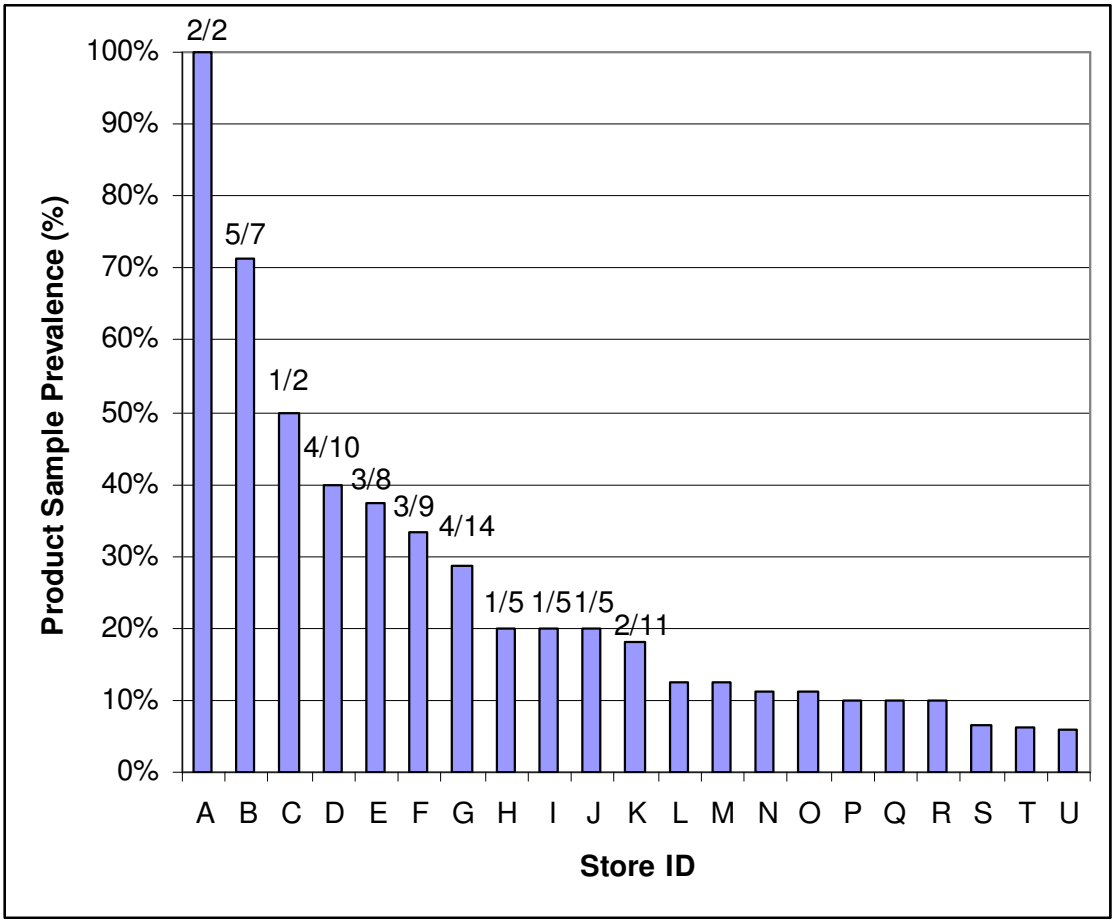
Note: Chub data not included.

Figure 11. Prevalence of *L. monocytogenes* in RTE deli meats by deli meat type.

Samplers were asked to identify if the sample included an antimicrobial formulation. Of the 7,446 samples, 51 were identified as using an antimicrobial agent, 1,008 did not use an

antimicrobial agent, and 6,387 were blank. Antimicrobial agents listed included potassium lactate, sodium diacetate, sodium erythorbate, calcium lactate, sodium phosphate, sodium benzoate, ascorbic acid, sodium citrate, and citric acid. Of the 57 samples positive for *L. monocytogenes*, 6 listed sodium erythorbate use, 1 listed sodium lactate/sodium diacetate use, and 50 were blank. Because of the large number of blanks, this antimicrobial formulation data was not used as part of the risk assessment described below. Instead, USDA data on current industry practices were used to estimate the fraction of product with antimicrobial usage.

There is an indication that positive retail-sliced samples were clustered by store when positive *L. monocytogenes* results were found. Figure 12 illustrates the deli meat sample prevalence among the 21 stores with at least one positive result for retail-sliced deli meats. Three of these stores had 50% or greater prevalence, and six of these stores had greater than 30% prevalence. Of the 308 identified stores sampled for retail-sliced deli meat, 37 *L. monocytogenes* positive deli meat samples were found among 22 stores. The remaining positive samples were from MN, where individual stores could not be identified. Six of these stores accounted for 21 of the 37 positive samples found. Thus, it appears that a few retail stores accounted for most of the positive deli meat samples found. The clustering of positives among a small number of stores is indicative of cross contamination at the retail establishment. It is also the reason that the independence assumption of the test of proportions for deli meat samples is likely not completely valid.



Note: The estimated store visit was based on similar sampling date and time. No sample times were provided for MN; thus, MN data not included. Thirty-seven total deli meat samples are shown.

Figure 12. Prevalence of *L. monocytogenes* in RTE deli meats samples sliced at retail by store.

2.4 Logistic Regression

To overcome the limitations with the test of proportions used above (non-independence for deli meat samples and small sample size for store samples), a logistic regression was performed. Logistic regression is appropriate when the dependent variable represents a proportion of positive results such as the deli meat prevalence for retail-sliced deli meat at an individual store. The assumptions for standard linear regression are not valid not here: the dependent variable is bounded to fall between 0 and 1, the errors are not normally distributed, and the regression must be weighted by the sample size used to calculate the prevalence. Logistic regression transforms the prevalence to a scale more suitable for regression. The analysis was performed in R using the generalized linear model (glm). In the language of R, a binomial family was specified which used the logit transformation as the link function.

The prevalence of retail-sliced and prepackaged deli meat was calculated separately for each store. This prevalence was regressed against several indicator variables: slicing location (retail-sliced versus prepackaged), time of day, and store type. Retail-sliced and prepackaged prevalences from the same store were treated as independent. Given that only one store had both processing types found positive, this seemed a reasonable approach. The number of samples of each type was used to weight the regression. Thus, store prevalences with only one sample received less weight than store samples with 30 samples. The logistic regression approach also had the advantage that all three explanatory variables were included simultaneously. The regression function was

$$\text{logit}(\text{prevalence}) = \beta_0 + \beta_1 \cdot \text{processing type} + \beta_2 \cdot \text{store type} + \beta_3 \cdot \text{time of day}$$

where: logit() = the logit transformation function; prevalence = the deli meat sample prevalence for each store and slicing location(retail-sliced versus prepackaged); slicing location= 0/1 indicator variable with 0 for prepackaged and 1 for retail-sliced; store type = 0/1 indicator variable with 0 for type A stores (major grocery chains) and 1 for type B stores (other grocery stores); and time of day = 0/1 indicator variable with 0 for AM and 1 for PM.

The number of data points used in the regression was 613. This is less than twice the number of individual stores sampled (2*362=724) because not all stores had both retail-sliced and prepackaged samples collected.

The results for the parameter estimates are given in Table 14. The variables slicing location and store type are statistically significant. The time of day the sample was collected is marginally significant.

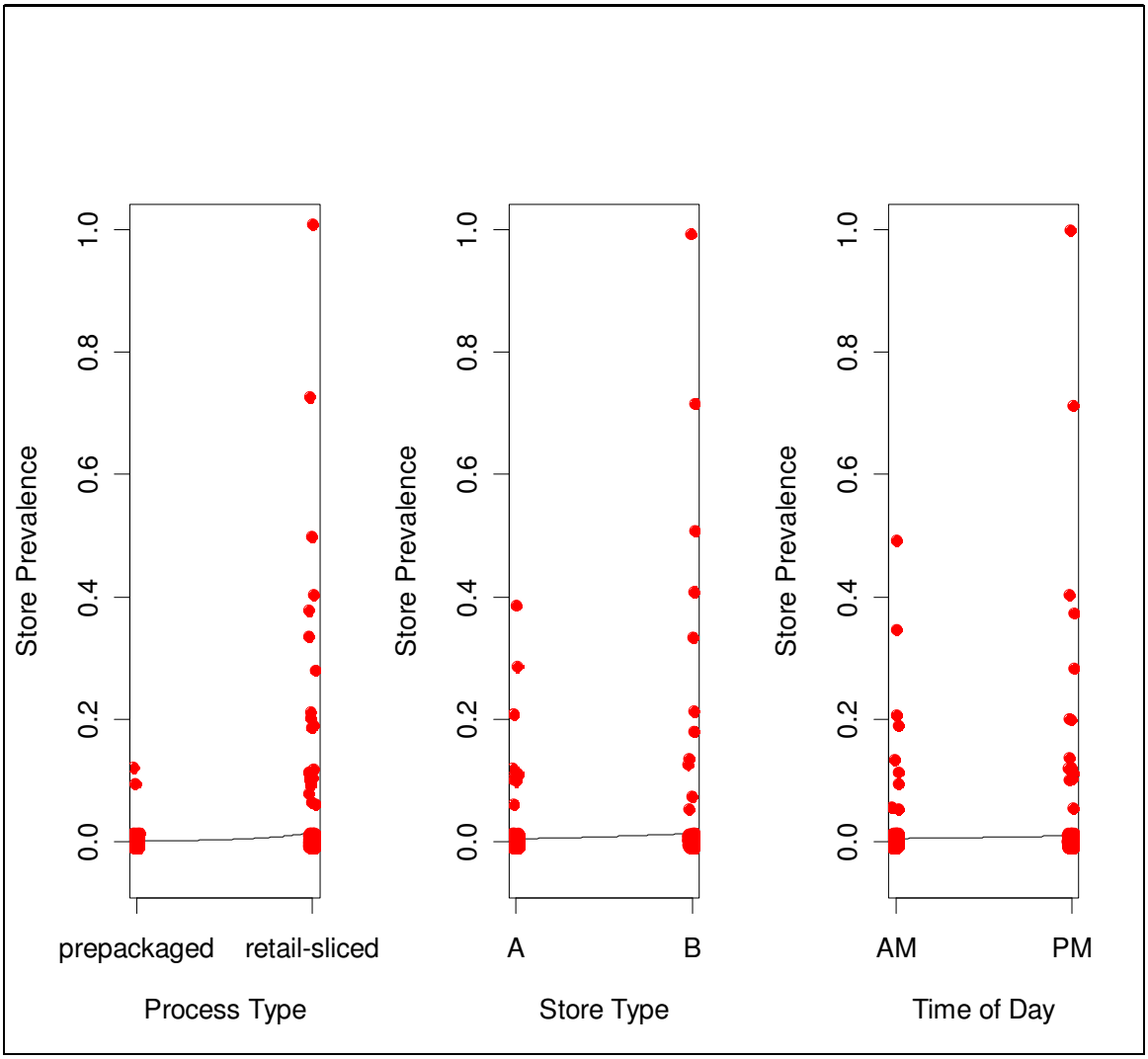
Table 14. Results of logistic regression for store prevalence as function of slicing location, store type, and time of day indicator variables.

Parameter	Estimate	Standard Error	Z value	p
Intercept	-7.96	0.76	-10.39	<0.0001
Processing type	2.90	0.73	4.00	<0.0001
Store type	0.99	0.33	3.03	0.002
Time of day	0.59	0.35	1.68	0.093

Note: Data for MN not included. N=613.

As expected from examining the data, whether the sample was prepackaged versus retail-sliced was strongly statistically significant. This is consistent with the test of proportions for deli meat samples. The result for time of day is consistent with the deli meat sample test of proportions for time of day. Both results indicate marginal statistical significance.

Figure 13 illustrates the results using logistic regressions based on one explanatory variable at a time. Because the vast majority of points had 0 prevalence and only two values (0/1) were used for the explanatory variables, a small random number (the statistical term for this is jitter) was added to the (x,y) coordinate for each point in order to better illustrate the density of points at 0 prevalences.



Note: MN data not included.

Figure 13. Graphical display of logistic regression results using deli meat sample prevalence at individuals stores as the dependent variable.

2.5 Comparison of Findings of the National Alliance for Food Safety and Security with those of the Food Processors Association

A comparison of NAFSS retail contamination findings with those of the National Food Processors Association (now Food Products Association) *(14)* is enlightening, although keep in mind that sample collection methods, sample sizes and analytic methods differed and these can all affect the results. The total number of deli meat samples was roughly equivalent: Gombas *et al.* sampled approximately 9,000 deli meat samples compared to about 7,000 (excluding chubs) for this research. The split between retail-sliced and prepackaged was somewhat different however. Approximately 77% of the samples from Gombas *et al.* were prepackaged, versus

approximately 50% for this work. USDA/FSIS data suggest that approximately 47% of RTE deli meat is sliced at the processing plant and prepackaged *(1)*.

Gombas *et al.* found retail-sliced and prepackaged prevalences of 2.7% and 0.4% respectively using a sample size of 25 g. This research found prevalences lower by about a factor of 2: 1.4% and 0.2% respectively using a sample size of 125 g. The prevalence found in this study is half that of the prevalence from the previous study despite a much larger sample size. This may indicate improvements in deli meat handling, increased use of post-processing lethality and antimicrobial growth inhibitor, or other improvements at the processing plant or retail that occurred between the times the studies were conducted.

The earlier research found a difference in prevalence between their two sampled sites. Table 15 below shows the derived results. Compare these data to the corresponding Table 18 above for the more recent data. Whereas this work found a consistent prevalence across all sites and a significant difference between retail-sliced versus prepackaged, the earlier work found no difference in slicing location at one site and a statistically significant difference at another.

Table 15. Prevalence of *L. monocytogenes* in sliced deli meat by site and slicing location from the Food Products Association *(14)*.

		Site		
		CA	**MD**	**Overall**
Processing[1]	Retail-sliced	0.70%	4.2%	2.7%
	Prepackaged	0.55%	0.19%	0.4%
	Overall	0.6% (28/4600)	1.2% (54/4599)	0.9% (82/9199)

[1]The number of positive samples and the total number of samples are shown in parentheses where available.

Gombas *et al*. also found that the prevalence was higher for retail-sliced deli meat, but that the levels of *L. monocytogenes* within positive samples were actually higher for prepackaged deli meat. This current work found consistently that both the prevalence and levels were higher for retail-sliced deli meat compared to prepackaged. The enumeration data collected in this study are provided in Table 16. All prepackaged positive samples were found to be at or below the enumeration limit whereas retail-sliced concentrations ranged to greater than 110 MPN/gram.

Table 16. Level of *L. monocytogenes* in deli meats at retail.

Retail-sliced			Prepackaged	
No. Samples[1]	***L. monocytogenes* level (MPN/gram)[1]**		**No. Samples**	***L. monocytogenes* level (MPN/gram)**
3,469	≤0.008		3,516	≤ 0.008
1	Between 0.008 and 0.3		1	Between 0.008 and 0.3
29	0.3		5	0.3
3	0.92			
1	0.93			
1	0.94			
3	2.3			
1	15.3			
1	24			
1	46			
3	≥ 110			

[1] *L. monocytogenes* levels were not given for five positive retail-sliced deli meat samples.

2.6 Conclusions

Table 17 summarizes the results of all the statistical testing. RTE deli meat is more contaminated with *L. monocytogenes*, both in terms of prevalence and level, when sliced at retail than when prepackaged. The marginal statistical link between positive results and time of day as well as the clustering according to the store where the sample was collected is an indication that cross contamination within retail establishments is occurring. There was no significant difference in prevalence of *L. monocytogenes* among the various four FoodNet sites.

Table 17. Overall results of statistical tests for prevalence of *L. monocytogenes* on RTE meat and poultry deli meats by location, season, time of day for slicing at retail, and by deli meat type.

Variable	Statistical Test[1]		
	Deli meat samples[2]	**Stores[3]**	**Logistic regression[4]**
Geographic location	N (p=0.75)	N (p=0.31)	
Quarter of year	Y (p=0.01)	N (p=0.31)	
Time of Day	Y (p=0.04)	N (p=0.75)	M (p=0.093)
Time of day (retail-sliced only)	Y (p=0.04)	N (p=0.64)	
Store Type	M (p=0.07)	N (p=0.82)	Y (p=0.002)
Prepackaged versus retail-sliced	Y (p<0.0001)		Y (p<0.0001)
Deli meat Type	N (p=0.22)		
Deli meat Type (retail-sliced only)	N (p=0.43)		

[1] Chub data were not included in any of the analyses. Statistical test results were considered statistically significant if $\alpha < 0.05$ and marginal if $0.05 \leq \alpha \leq 0.10$. A "Y" indicates the differences were statistically significant; an N" indicates that they were not; an "M" indicates that the differences were marginally significant.

[2] Deli meat samples were assumed independent for the purposes of the test of proportions. [3] A store was considered positive if at least one of the deli meat samples collected at the store was positive for *L. monocytogenes*.

[4] All three explanatory variables were included simultaneously.

Chapter 3. Comparative Risk of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products

3.1 Introduction

Infection with *Listeria monocytogenes* is a serious foodborne public health problem, owing to its severity of infection and high case fatality rate (Mead et al 1999). In 2000, the Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN) and the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) initiated a quantitative assessment of the relative risk to public health from *Listeria monocytogenes* among twenty-three categories of ready-to-eat (RTE) foods in the United States *(7)*. The assessment found that deli meats pose the greatest risk of listeriosis, and subsequent death, among all ready-to-eat foods. Deli meats were estimated to cause approximately 1,600 cases of listeriosis each year, resulting in approximately 300 annual deaths. Subsequently, the FDA and FSIS performed a preliminary analysis of retail deli meats using the *L. monocytogenes* contamination data collected by Gombas *et al. (14)* to estimate the relative risk of listeriosis from deli meat sliced and packaged in FSIS-inspected processing establishments (prepackaged) versus those sliced and packaged at retail facilities (retail-sliced). Results suggested that deli meat sliced and packaged at retail posed the greater risk, accounting for approximately 80% of all listeriosis cases from deli meat.

However, because the study of Gombas *et al. (14)*, on which this analysis was based, included *L. monocytogenes* prevalence data from just two sites – one in northern California and one in Maryland – the data and findings of the analysis were limited. Additionally, the overall prevalence of *L. monocytogenes* at each site differed. Therefore, to gather more representative data for *L. monocytogenes* in retail RTE meat and poultry products, a study was conducted by researchers with the National Alliance for Food Safety and Security (NAFSS) - a consortium of 25 research universities *(6)*. Samples of prepackaged and retail-sliced RTE meat and poultry products were collected from four sites: Georgia, Minnesota, Maryland, and northern California. Prevalence and enumeration data for *L. monocytogenes*, as well as information on growth inhibitor use, were collected from each of the four sites.

Using the *L. monocytogenes* data from the NAFFS study, the objectives of this research were (i) to develop a risk assessment model incorporating growth inhibitor use and *L.*

monocytogenes concentrations based on slicing location to estimate the number of deaths and illnesses resulting from listeriosis and (ii) to determine the comparative risk of deli meat sliced and packaged at processing establishments versus those sliced and packaged at retail.

3.2 Materials and Methods

3.2.1 *L. monocytogenes* sampling.

Data for concentration of *L. monocytogenes* in prepackaged and retail-sliced meant and poultry deli products were generated by Draughon *et al (6)*. Samples were collected from four designated sites in the Foodborne Disease Active Surveillance Network (FoodNet). These sites were Northern California (CA), Georgia (GA), Minnesota (MN), and Tennessee (TN). Food samples were collected at these FoodNet sites to facilitate relating exposure data and actual cases of illness. Approximately 2,000 samples (125 grams each) were analyzed from each of the four designated sites. According to consumer survey data, 50% of deli meat products are retail-sliced and the remaining half are prepackaged. Therefore, the samples were collected accordingly. Three categories of products were tested: products sliced and packaged in a processing establishment and prepackaged for retail sale, products sliced and packaged at a retail delicatessen, and intact deli meat not yet sliced at retail.

3.2.2 Statistical Analysis.

The data generated by Draughon et al *(6)* was analyzed using Number Cruncher Statistical Systems (NCSS) 2001 *(15)* and R version 2.6.1 *(25)*.

3.2.3 Risk Assessment Modeling.

This risk assessment was conducted using a modified version of the FDA-FSIS *Listeria* model *(7)*. The model consists of two sub-models: the exposure assessment model (Figure 14) and the dose-response model (Figure 15). The exposure assessment model starts with the retail *L. monocytogenes* distribution, and predicts the distribution at consumption. The dose-response model uses the distribution at consumption together with an age-specific dose-response function to estimate number of deaths for three age groups: neonatal, intermediate, and elderly. Neonates included fetuses and newborns from 16 weeks after fertilization to 30 days after birth, the intermediate population were those older than 30 days and less than 60 years old, and the elderly were defined as being 60 years of age or older. Illnesses are then calculated based on age-specific illness to mortality ratios: 12.7 for neonatal, 11.3 for intermediate, and 3.7 for elderly.

The model contains information on 23 food categories including deli meat, frankfurters, smoked seafood, and soft cheeses.

The observed *L. monocytogenes* concentrations at retail were fitted to probability distributions for retail-sliced and prepackaged deli meats. The probability distributions served as inputs to the exposure assessment model. The deli meat category of the exposure model was divided into four categories: prepackaged deli meat with growth inhibitor, prepackaged deli meat without growth inhibitor, retail-sliced deli meat with growth inhibitor, and retail-sliced deli meat without growth inhibitor. The exposure assessment model was also modified to account for the different *L. monocytogenes* growth rates for product with and without growth inhibitors. Finally, the dose response model for each age group was modified by splitting the deli meat category into the four new categories.

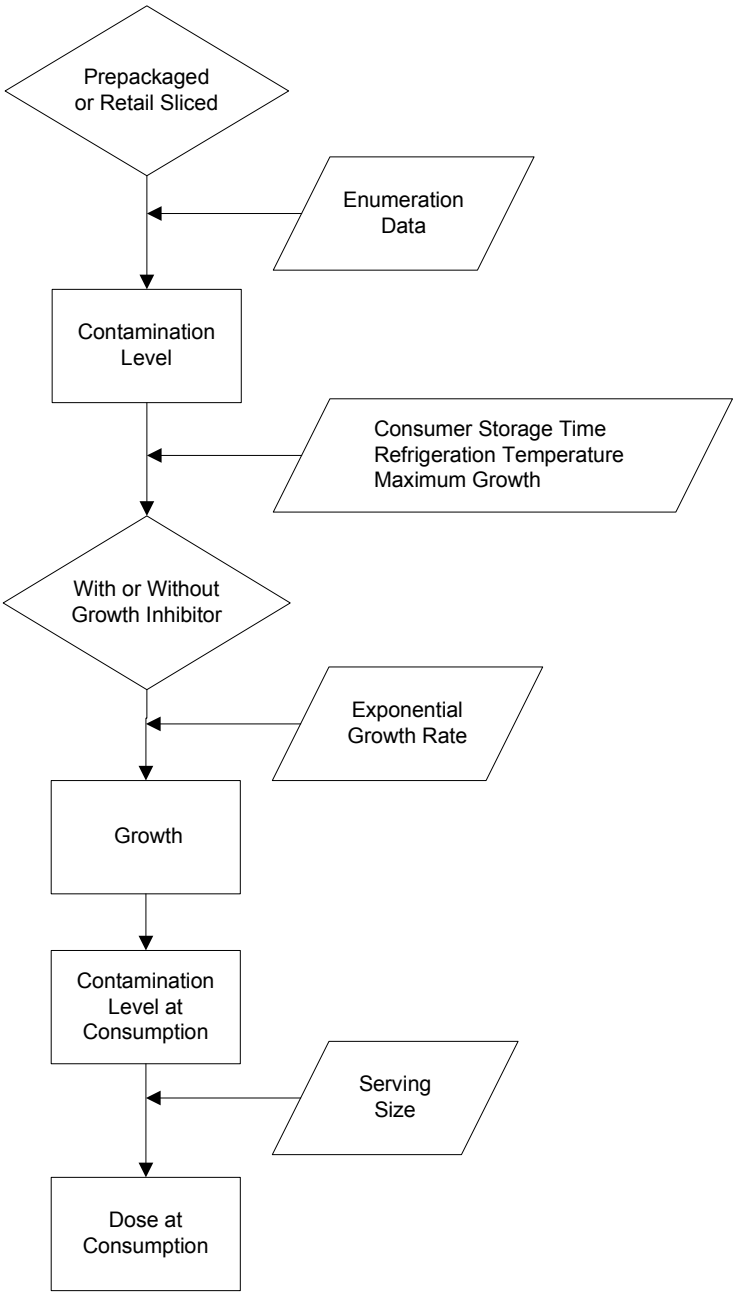


Figure 14. Flowchart of the exposure assessment model. Adapted from FDA-FSIS, 2003 *(7)*.

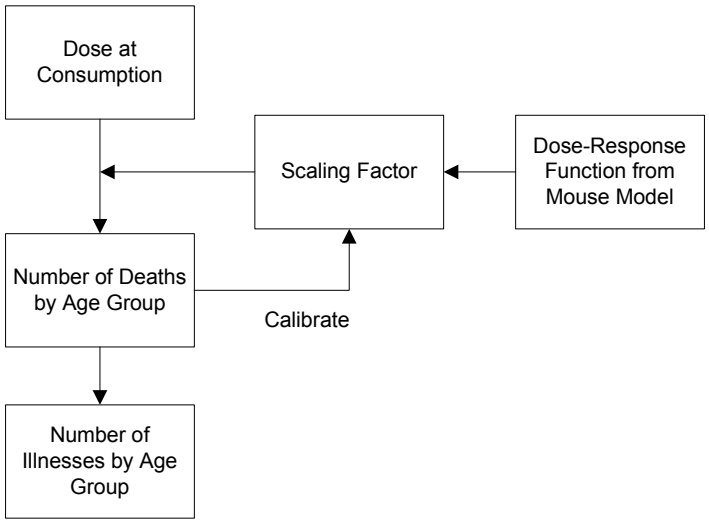


Figure 15. Flowchart of the dose-response model. Adapted from FDA-FSIS, 2003 *(7)*.

3.2.4 Use of Growth Inhibitors.

Microbial growth inhibitors may be used as a post-processing control to retard the growth of *L. monocytogenes* in RTE meat and poultry products *(13)*. The use of growth inhibitors was a key factor considered by the model. In 2003 the USDA passed the Interim Final Rule 9 CFR 430 to regulate ready-to-eat food processors. The rule allows processors to choose from 3 alternatives:

4. use both a growth inhibiting agent and a post-processing lethality
5. use either a growth inhibiting agent or a post-processing lethality step
6. use neither a growth inhibitor nor a post-processing lethality step.

Alternative 2 is divided into two options, to distinguish if only post-processing lethality or only growth inhibitor was used. The fraction of production under each alternative was estimated before and after the Interim Final Rule *(12)* is shown in Table 18. It is estimated growth inhibitors were used in 17.5% (2.6% + 14.9%) of RTE meat and poultry prior to the implementation of the Interim Final Rule. It is estimated growth inhibitors are used in 39.3% (9.2% + 30.1%) of product currently. The growth rate for *L. monocytogenes* was estimated using the data from prior to the Interim Final Rule, therefore, the pre-Interim Final Rule data was used in the model. The percentage of product using growth inhibitor was assumed the same for both prepackaged and retail-sliced product.

Table 18. Estimated fraction of production among the various alternatives before and after implementation of Interim Final Rule 9 CFR 430.

Alternative	Pre Interim Final Rule	Post Interim Final Rule
1. Both growth inhibitor and post-processing lethality	2.6%	9.2%
2A. Post-processing lethality alone	6.8%	17.6%
2B. Growth inhibitor alone	14.9%	30.1%
3. Neither growth inhibitor nor post-processing lethality	75.7%	43.1%

3.2.5 Exponential Growth Rate.

To model growth of *L. monocytogenes* in deli meats, growth rates for RTE meat and poultry with and without growth inhibitor were determined. The exponential growth rate of *L. monocytogenes* was used in the exposure model to simulate growth from retail to consumption. The growth rate was treated a stochastic input parameter. It was adjusted for stochastic storage time and temperature and a correlation between the two. The growth rate was calculated using data from 15 published articles with 23 reported growth rates across a range of deli meat products (see reference *(7)*). This growth rate was adjusted to account for the use of growth inhibitor.

To qualify as using a growth inhibitor under the Interim Final Rule 9 CFR 430, the growth of *L. monocytogenes* may not exceed two logs over the shelf life of the product. Exponential growth rates for *L. monocytogenes* were calculated for RTE meat and poultry with and without growth inhibitors using data from the 2003 FDA-FSIS *(7)* risk assessment and the estimated fraction of deli meat in each alternative prior to the implementation of the Interim Final Rule. The FDA-FSIS risk assessment model estimated that the overall mean exponential growth rate for deli meat at 5^{o} C was 0.282 $\log_{10}$ cfu/g/day. The Interim Final Rule is vague with regard to shelf life and temperature. If the Interim Final Rule 9 CFR 430 standard is interpreted to be 2 $\log_{10}$ growth over 14 days at 5^{o}C, the maximum allowable exponential growth rate is 2 $\log_{10}$ cfu/g/14 days = 0.143 $\log_{10}$ cfu/g/d. Based on these assumptions, the exponential growth rate (EGR) for product with growth inhibitor (GI) was estimated using a weighted log linear equation:

$$f_{GI} \text{ x } EGR_{with} + (1 - f_{GI}) \text{ x } EGR_{without} = EGR_{FDA}$$

$$0.206 \text{ x } 0.143 \log_{10} \text{cfu/g/d} + 0.794 \text{ x } EGR_{without} = 0.282 \log_{10} \text{cfu/g/day}$$

$$EGR_{without} = 0.318 \log_{10} \text{cfu/g/day}$$

The calculated mean exponential growth rate was input into the exposure model and the predicted concentrations of *L. monocytogenes* at consumption were used in the dose-response model.

3.2.6 Distribution Fitting.

The *L. monocytogenes* concentrations at retail were fitted to probability distributions as inputs to exposure assessment model. Even though a large number of samples was collected, there were relatively few positives. Therefore, the distribution fits must be considered only approximate. The prepackaged product, for example, had only six *L. monocytogenes*-positive findings out the 3,522 prepackaged samples tested. All six positive samples were reported as below the reported enumeration limit of 0.3 MPN/g. Forty-nine retail-sliced samples out of 3,513 were found to be positive for *L. monocytogenes*.

The survival analysis module of the NCSS software package *(15)* was used to fit an appropriate statistical model to retail-sliced and prepackaged RTE meat and poultry products separately. Negative samples were assumed to have a concentration less than or equal to the *L. monocytogenes* detection limit of 0.008 MPN/g (i.e. ≤ 1 MPN/125 g). To be conservative, all but one of the observed positive concentrations listed as ≤ 0.3 MPN/g were treated as = 0.3 MPN/g, and the remaining 1 sample was treated as an interval measurement between 0.008 MPN/g and 0.3 MPN/g. The level of *L. monocytogenes* and censor inputs for the survival analysis are provided in Table 19.

Table 19. Survival analysis input for statistical distribution fitting for the level of *L. monocytogenes* in deli meats at retail.

Retail-sliced				Prepackaged		
No. Samples[1]	***L. monocytogenes* level (MPN/gram)[1]**	**Censor Type[2]**		**No. Samples**	***L. monocytogenes* level (MPN/gram)**	**Censor Type[2]**
3,469	≤0.008	L		3,516	≤ 0.008	L
1	Between 0.008 and 0.3	I		1	Between 0.008 and 0.3	I
29	0.3	F		5	0.3	F
3	0.92	F				
1	0.93	F				
1	0.94	F				
3	2.3	F				
1	15.3	F				
1	24	F				
1	46	F				
3	≥ 110	R				

[1] *L. monocytogenes* levels were not given for five positive retail-sliced deli meat samples. These data were thus not used in the distribution fitting.

[2] Censor type refers to the censoring used by the survival analysis fit. L indicates left censoring (actual value is less than observed); I indicates interval censoring (actual value is between two known values). F indicates actual value is observed level. R indicates right censoring (actual value is greater than observed).

The parameters for the retail and prepackaged *L. monocytogenes* distributions were determined by least-squares regression and fit to the corresponding probability plot. Based on the results of the survival analysis, the lognormal distribution was selected as the most appropriate distribution. The lognormal provided an adequate fit to the retail-sliced distribution and is the conventional distribution used for fitting environmental contaminant data such as bacterial concentrations. (See, for example, van Belle, 2002 *(28)*).

The fitted cumulative density plots and observed data points are shown in Figure 16. The fit for the retail-sliced product appears adequate. The fit for the prepackaged product may be adequate, but is very uncertain because only two data points are available.

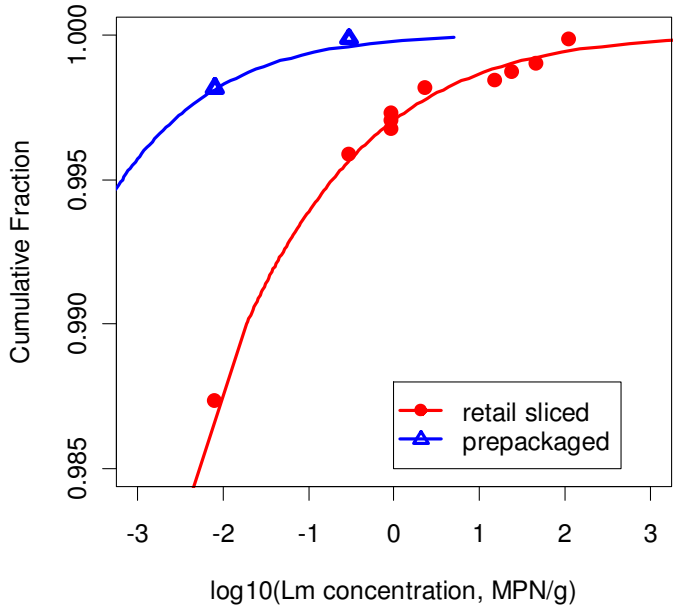


Figure 16. Fitted cumulative density plots for retail- and plant-sliced data.

3.2.7 Exposure Assessment Modeling.

Four separate exposure assessment models were run: (i) prepackaged with growth inhibitor; (ii) prepackaged without growth inhibitor; (iii) retail-sliced with growth inhibitor; and (iv) retail-sliced without growth inhibitor. The starting retail *L. monocytogenes* concentration differed between prepackaged and retail-sliced RTE meat and poultry. *L. monocytogenes* growth rates differed depending on the use of growth inhibitors. The storage time and temperature distributions were left unchanged from the FDA-FSIS *(7)* model and the same time and temperature distributions were used for both prepackaged and retail-sliced product. Anecdotal evidence suggests that product sliced at retail is consumed more quickly than prepackaged product. To investigate this difference, consumer storage times were adjusted in the exposure assessment model during the sensitivity analysis.

3.2.8 Dose Response Modeling.

In each of the three age-specific dose-response models (neonatal, intermediate, and elderly), the deli meat category was split into the four separate categories (prepackaged with growth inhibitor, prepackaged without growth inhibitor, retail-sliced with growth inhibitor, and retail-sliced without growth inhibitor). The *L. monocytogenes* distributions at consumption from the exposure assessment model were input to each of the age-specific dose-response models. The fraction of servings for each of the four deli meat categories was estimated from the USDA July 2007 Form 10,240-1 database *(1)*. These data are shown in Table 20 as a fraction of total production.

Table 20. Fraction of deli meat production by slicing location and growth inhibitor use during July 2007.

Alternative	**Prepackaged (sliced at plant)**	**Retail-Sliced**	**Total**
With growth inhibitor	0.322	0.267	0.589
Without growth inhibitor	0.144	0.267	0.411
Total	0.466	0.534	1.000

Because there have been no human clinical trials with *L. monocytogenes*, the dose-response curve is generated by relating the effects observed in mice to the effects of *L. monocytogenes* in humans using an appropriate scaling factor. Using a calibrated mode to run the dose-response model, a scaling factor was used in 4,000 simulations to adjust the dose-response curve from the mouse model to meet a specified number of deaths in humans. For this analysis, the target number of deaths was taken from the 2003 FDA-FSIS *(7)* risk assessment, i.e. 307 elderly deaths, 67 intermediate deaths, and 16 neonatal deaths, across all 26 food groups. (Recall the original model used 23 food groups, but that deli meat was now split into four groups for this analysis.)

A bootstrap analysis was used to evaluate if the estimated mean number of deaths resulting from retail-sliced RTE meat and poultry was different from that for prepackaged. Four thousand samples (with replacement) were drawn from the 4,000 simulations of each specified scenario. The mean of each of these samples was then calculated. This process was repeated

100,000 times to generate a distribution of means. The mean and 95% confidence interval from this distribution was then obtained.

3.2.9 Sensitivity Analysis

To investigate the effect of consumer storage time and shelf life assumptions on the outcome of the model, a sensitively analysis was conducted. Consumer storage times used in the exposure assessment model were taken from a consumer survey conducted by the American Meat Institute (AMI) *(17)*. According to the AMI survey, approximately 40% of ready-to-eat product is stored for up to 2 days, 90% of product is stored up to 9 days, and 99% of product is stored for 26 days or less *(7)*. The survey did not distinguish between retail-sliced and prepackaged product; therefore, the same storage time distribution was used for both the retail and prepackaged exposure assessment models. Consumers may store retail-sliced deli meats for shorter periods than prepackaged deli meats. Thus, to assess the effect of a reduced consumer storage time, the storage time distribution in the retail exposure model was adjusted by arbitrary factors of 0.25, 0.50, and 0.75. The model assumed a shelf life of 14 days for ready-to-eat deli meat products. To assess the effect of the shelf life assumption, a 10 day and 21 day shelf life were also considered.

3.3 Results

The estimated mean numbers of deaths per year and the 95% confidence interval about the means among the three age groups for the four deli meat categories are summarized in Table 21. The estimated mean number of deaths from products with growth inhibitor was 28, while the estimated mean number of deaths from product without growth inhibitor was 111. Retail-sliced product, which started with a higher concentration of *L. monocytogenes* at retail compared to prepackaged product, saw the greatest reduction in the estimated mean number of deaths with the use of growth inhibitors.

Table 21. Estimated mean number of deaths per year and 95% confidence interval about the mean among three populations stratified by age and four deli meat categories.

Food Category	**Elderly**	**Intermediate**	**Neonatal**	**Total**
Prepackaged with growth inhibitor	3.4	0.8	0.2	4.4
	(3.3, 3.5)	(0.8, 0.8)	(0.2, 0.2)	(4.3, 4.5)
Prepackaged without growth inhibitor	7.2	1.8	0.5	9.4
	(7.0, 7.3)	(1.7, 1.8)	(0.4, 0.5)	(9.1, 9.6)
Retail-sliced with growth inhibitor	18	4.4	1.1	23.5
	(17.5, 18.4)	(4.3, 4.6)	(1.1, 1.2)	(23.0, 24.1)
Retail-sliced without growth inhibitor	78	18.9	5.1	102
	(76.5, 79.6)	(18.5, 19.3)	(5.0, 5.2)	(100.1, 104.0)
Prepackaged total	10.5	2.6	0.7	13.8
	(10.3, 10.8)	(2.5, 2.6)	(0.7, 0.7)	(13.5, 14.1)
Retail-sliced total	96	23.3	6.2	125.6
	(94.3, 97.7)	(22.9, 23.7)	(6.1, 6.3)	(123.4, 127.7)
With growth inhibitor total	21.3	5.3	1.4	27.9
	(20.8, 21.8)	(5.1, 5.4)	(1.3, 1.4)	(27.3, 28.6)
Without growth inhibitor total	85.2	20.7	5.6	111.4
	(83.6, 86.8)	(20.3, 21.0)	(5.5, 5.6)	(109.4, 113.4)
Total	106.5	25.9	6.9	139.3
	(104.7, 108.3)	(25.5, 26.3)	(6.8, 7.0)	(137.1, 141.6)

The estimated mean number of deaths per year associated with prepackaged product was 13.8, while the estimated mean number of deaths per year associated with retail-sliced product was 125.5. Ten percent of the estimated annual deaths (13.8/139.3=9.89%) were attributable to prepackaged product, while the remaining 90% were attributable to retail-sliced product (125.6/139.3=90.11%). Since 50% of ready-to-eat deli meat products are retail-sliced and the remaining half are prepackaged, the relative risk for ready-to-eat product retail-sliced product versus prepackaged product is thus 125.6/13.8=9.1.

A similar analysis was conducted for illnesses. The FDA-FSIS *(7)* model assumed a constant illness to death ratio by age group of 12.7, 11.3, and 3.7 for neonatal, intermediate, and elderly age groups, respectively. Because these ratios are fixed, the relative risk of illness from retail-sliced product versus prepackaged product is similar to that of death. A mean of 76.8 illnesses was attributed to prepackaged product and a mean of 698.0 illnesses for retail-sliced product, for a relative risk ratio of 9.1. The difference in the mean number of deaths between prepackaged and retail-sliced deli meats was 111.8 with a 95% confidence interval of 109.6 to 114.0. The difference in means is statistically significant at 95% confidence.

A recursive partitioning and regression tree was generated in R *(25)* to determine which factor (age, slicing location, or growth inhibitor use) had the greatest effect on the number of resulting deaths (Figure 17). The first division in the tree indicates that age is the most important factor and that the elderly are more likely to die from listeriosis than either the neonatal or intermediate population. Following the tree along the elderly branch, the next division is by slicing location. The tree indicates that retail-sliced product is at greater risk for causing listeriosis than prepackaged product. Finally, the retail-sliced product is divided according the growth inhibitor use.

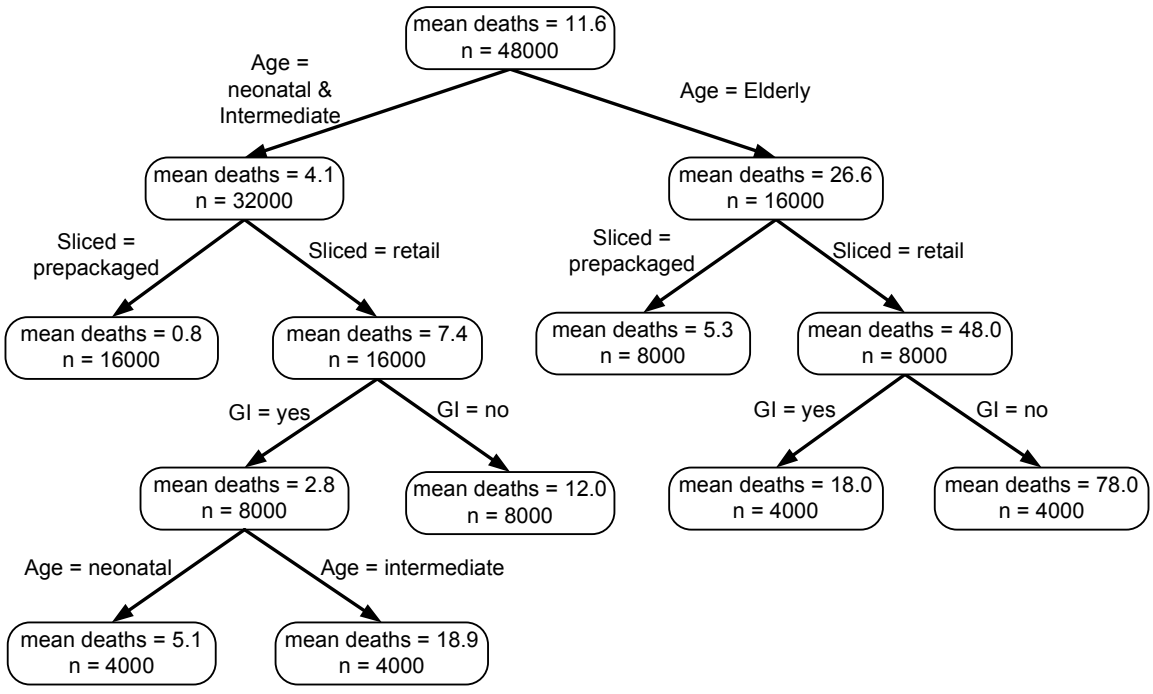
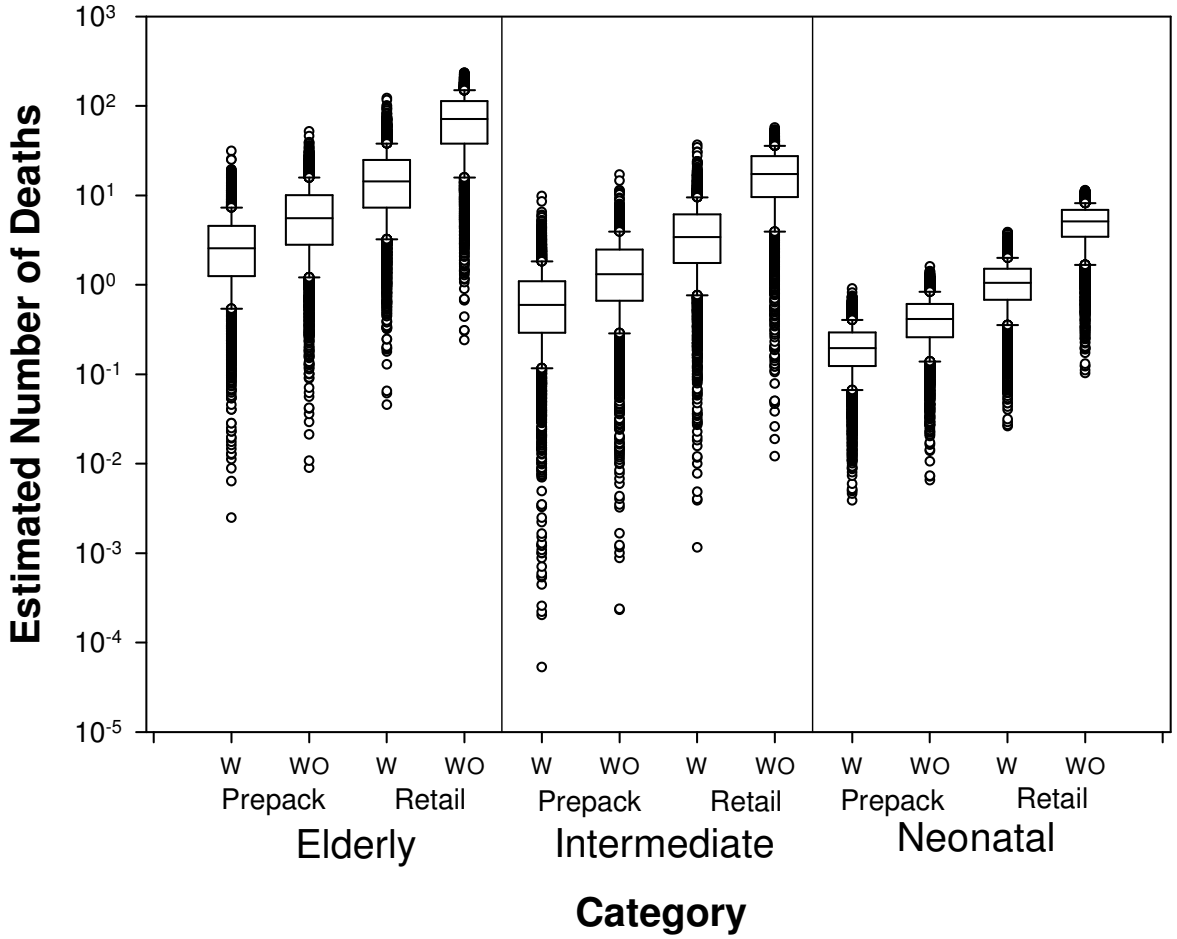


Figure 17. Recursive partitioning and regression tree.

Using the data from all 4,000 simulations, box plots were generated for each deli meat category by age group (Figure 18). The box plots reemphasize the effect of age on the risk of death from listeriosis, with the elderly population having the highest number of deaths for each of the deli meat categories. Within each age group, growth inhibitor reduced the number of deaths; however, the box plots show that even with the use of growth inhibitor, retail-sliced deli meats result in a greater risk of death due to listeriosis than prepackaged meats.



*Prepack = prepackaged, Retail = retail-sliced, W = with growth inhibitor, WO = without growth inhibitor

Figure 18. Box plots for each deli meat category by age group.

As seen in the box plots, each of the four deli meat categories follows a similar trend, with the elderly age group at the highest risk for death by listeriosis. An interaction plot for the

elderly age group was created to compare the effect of growth inhibitor use and product slicing location on the mean number of deaths. There is a significant difference between the mean number of deaths resulting from retail-sliced product when compared to prepackaged product (Figure 19a). While the use of growth inhibitor greatly decreased the mean number of deaths resulting from retail sliced product, prepackaged product without growth inhibitor results in fewer deaths than retail sliced product with growth inhibitor (Figure 19b).

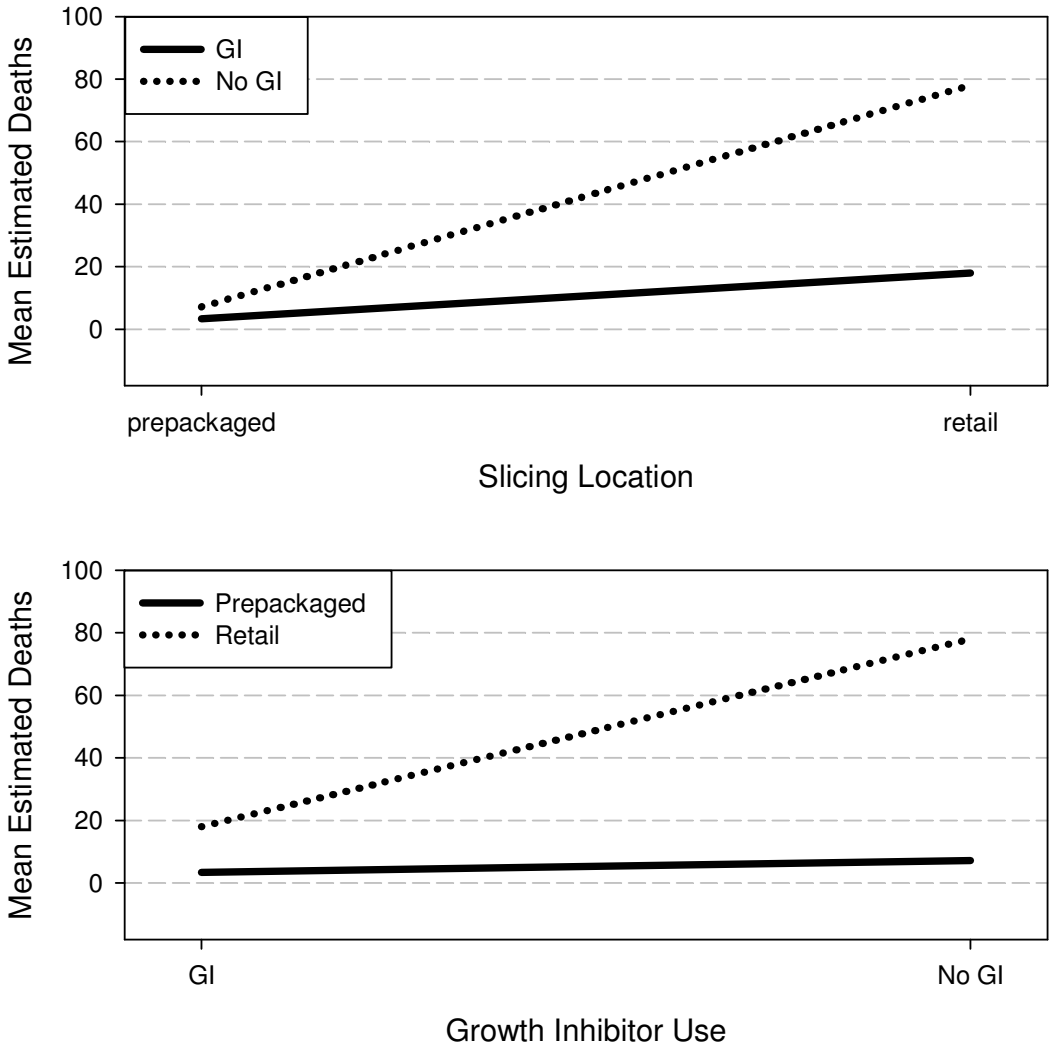


Figure 19. Interaction plots comparing the effect of growth inhibitor (GI) use and slicing location on the mean number of deaths from listeriosis.

3.3.1 Sensitivity Analysis.

Based on sensitivity analysis, an increase in the consumer storage time increased the total number of deaths and illnesses (Table 22). When the consumer storage time was halved, the number of deaths was decreased by nearly 25%. This finding shows that estimations made by this model are dependent on the accuracy of the consumer storage time assumption. More investigation into the typical consumer storage times for ready-to-eat meat and poultry products is necessary to enhance the accuracy of the model. Table 22 also includes the ratio of deaths resulting from retail-sliced versus prepackaged deli meats. The comparative risk ratio decreased as the consumer storage times for the retail-sliced meats decreased, however retail-sliced product is estimated to cause 1.7 times more deaths than prepackaged product even when stored for a quarter of the time.

Table 22. Estimated mean number of deaths and illnesses per annum by fraction of consumer storage time.

Storage Time Fraction	**25%**	**50%**	**75%**	**100%**
Deaths	70.7	105.5	127.1	139.3
Illnesses	397.8	589.9	708.0	774.7
Ratio of Deaths, Retail-sliced: Prepackaged	1.7	3.7	5.4	9.1

Maintaining the FDA-FSIS *Listeria* model's assumed consumer storage distribution, the EGR of *Listeria* on deli meat was then changed for both retail-sliced and prepackaged product based on a shelf life of 10, 14, and 21 days. Increasing the shelf life decreased the number of deaths (Table 23). The change in shelf life from 10 days to 14 days resulted in a 10% reduction in the mean number of deaths. A week long extension of shelf life from 14 days to 21 days resulted in a 5% reduction in the number of deaths. This suggests that the assumption of a 14-day shelf life may be adequate for predicting the number of deaths or illnesses due to listeriosis.

Table 23. Mean number of deaths and illnesses per annum by shelf life.

Shelf Life	10 day	14 day	21 day
Deaths	155.1	139.3	131.7
Illnesses	861.1	774.7	732.9
Ratio of Deaths, Retail-sliced: Prepackaged	8.1	9.1	7.9

The comparative risk ratios exhibited no definitive correlation with the change in shelf life; however, the EGR for product with growth inhibitor consistently decreased, while the EGR for product without growth inhibitor increased as the shelf life increased (Table 24). The differences in the comparative risk may be a result of the iterative process used to adjust the dose-response curve and may not necessarily indicate a true difference in the relative risk.

Table 24. EGR for product with and without growth inhibitor by shelf life.

Shelf Life	10 day	14 day	21 day
With GI	0.20	0.14	0.10
Without GI	0.30	0.31	0.32

3.4 Discussion and Conclusions

Results from this study suggest retail-sliced ready-to-eat meat and poultry products are over nine times more likely to cause listeriosis than prepackaged products. Transmission of *L. monocytogenes* in retail delis may be one factor contributing to the elevated risk of listeriosis from retail-sliced products compared to prepackaged products. Retail delis are most commonly out of compliance with the FDA Food Code for improper holding times and temperatures of product, poor personal hygiene of workers handling product, and a lack of adequate safeguards against contamination *(8)*. Vorst *et al.(30)* found that *L. monocytogenes* can spread from contaminated product to uncontaminated product by mechanical slicers during the slicing of turkey, bologna, and salami. Moreover, despite regular cleaning and disinfection, processing equipment may act as a vehicle for transfer of *L. monocytogenes* between processing plants *(19)*,

suggesting sanitation alone is not adequate for preventing *L. monocytogenes* contamination of retail-sliced ready-to-eat product.

Our results also suggest that consumption of retail-sliced meat and poultry products is more likely to result in listeriosis compared to consumption of prepackaged products, regardless of whether growth inhibitors are used. In addition, consuming retail-sliced products was found to be riskier than consuming prepackaged products, regardless of consumer age. Finally, if retail-sliced products are stored for a quarter of the time of prepackaged products, consumption of retail-sliced products is still nearly two times as likely to cause listeriosis than consumption of prepackaged products. These findings indicate that while growth inhibitors are an effective method for controlling *L. monocytogenes* levels in prepackaged deli meat products, further measures are necessary to reduce *L. monocytogenes* concentrations at the retail level.

There were limitations to this study. First, the AMI survey *(17)* used in this study did not distinguish between consumer storage times for retail-sliced and prepackaged products. Consumers may store retail-sliced products for shorter periods than prepackaged products, but additional studies are needed to support this conjecture. Therefore, our model assumed consumer storage times for retail-sliced and prepackaged products were identical. Second, in the study by Draughon *et al (6)*, a low number of samples tested positive for *L. monocytogenes* and these positive samples were reported as below the enumeration limit of 0.3 MPN/g. This resulted in the fitted distribution of the data being only approximate. An increase in the number of samples and a better method of reporting positive concentrations would improve the goodness of fit of the chosen distribution. Third, our model was developed using growth inhibitor data collected prior to the FSIS Interim Final Rule *(12)*. The use of growth inhibitors has since increased *(1)*, thereby causing the model to likely overestimate the absolute number of deaths and illness. However, it is important to note that this does not affect the relative risk ratio.

The above limitations notwithstanding, the results from our study provide compelling evidence that meat and poultry products sliced at retail delis are riskier for listeriosis than products sliced and packaged at federally-inspected plants. The elevated initial distribution of *L. monocytogenes* makes retail-sliced deli meats riskier than prepackaged deli meats regardless of growth inhibitor use. To better understand the reasons for this, additional studies are needed to address the extent of cross-contamination of *Listeria* at retail delis and to identify methods to

mitigate and control *L. monocytogenes* contamination in retail-sliced ready-to-eat meat and poultry products.

Chapter 4. Conclusions

The results of the dynamic in-plant model found that the minimum frequency of testing and sanitation of food contact surfaces, as presented in the FSIS proposed rule *(9)*, results in a small reduction in the levels of *L. monocytogenes* on deli meats at retail, but greater frequency of food contact surface testing and sanitation is estimated to lead to a proportionally lower risk of listeriosis. The use of growth inhibitors combined with a post-processing lethality step was estimated to save over 200 more lives than the FSIS proposed minimum sampling standard and 66 more lives than even sampling each lot of ready-to-eat product. Thus, the use of a combination of interventions (e.g., post processing lethality and the use of growth inhibitors) is more effective in mitigating potential contamination of RTE meat and poultry product with *L. monocytogenes* than sampling or any single intervention used alone. Subsequently, the use of a combination of interventions best reduces the risk of illness or death due to listeriosis. When relying on sampling alone to maintain food safety, a timely response to a positive food contact surface may help reduce the duration and severity of a contamination event due to the temporal clustering of food contact surface positives.

An analysis of the data collected from the NAFSS study *(6)* found that retail-sliced deli meat has both a higher prevalence and level of *L. monocytogenes* than prepackaged product. Cross contamination within the retail environment is suspected based on the clustering of positives by store and the putative statistical link between the positives and the time of day of sampling. There was no significant difference in prevalence of *L. monocytogenes* among the four geographically-dispersed FoodNet sites; so these findings seem applicable at a national level.

The comparative risk assessment results estimate retail-sliced RTE meat and poultry products are at a 9.1 times greater risk of listeriosis than prepackaged product and slicing location has a greater effect on the risk of listeriosis than the use of growth inhibitors. Prepackaged RTE meat and poultry product without growth inhibitor poses less risk of *L. monocytogenes* illnesses and deaths than retail-sliced RTE meat and poultry product with growth inhibitor. Consumer storage times also have a significant effect on the comparative risk ratio, however, even if retail-sliced deli meat products are stored for a quarter of the time prepackaged product is stored, retail-sliced deli meats are still nearly two times more likely to cause

listeriosis. To help mitigate *L. monocytogenes* contamination, regulatory action at the retail level is necessary.

References

1. Anonymous. 2007. Industry survey data collected with Form 10240-1 in accordance with 9 CFR 430. *In*, Production Information of Post-Lethality Exposed Ready-to-eat Products USDA/FSIS.

2. Bedie, G. K., J. Samelis, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2001. Antimicrobials in the Formulation To Control *Listeria monocytogenes* Postprocessing Contamination on Frankfurters Stored at 4° C in Vacuum Packages. *Journal of Food Protection*. 64:1949-1955.

3. Bureau, U. S. C. Date, 2007. Available at: http://www.census.gov. Accessed.

4. CDC. 2007. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food — 10 States, 2006. p. 336-339. *In*, Morbidity and Mortality Weekly Report, vol. 56. U.S. Government Printing Office.

5. Chen, Y., K. M. Jackson, F. P. Chea, and D. W. Schaffner. 2001. Quantification and Variability Analysis of Bacterial Cross-Contamination Rates in Common Food Service Tasks. *Journal of Food Protection*. 64:72-80.

6. Draughon, A. F. 2006. A collaborative analysis/risk assessment of Listeria monocytogenes in ready-to-eat processed meat and poultry collected in four FoodNet states. *In*, Symposium S-16: Contamination of Ready-to-Eat Foods: Transfer and Risk: Listeria monocytogenes and Other Microorganisms. International Association for Food Protection 93rd Annual Meeting, Calgary, Alberta.

7. FDA-FSIS. Date, 2003, Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Available at: http://www.foodsafety.gov/~dms/lmr2-toc.html. Accessed.

8. FDA. 2004. FDA Report on the Occurrence of Foodborne Illness Risk Factors in Selected Institutional Foodservice, Restaurant, and Retail Food Store Facility Types. *In* FDA National Retail Food Team.

9. FSIS. 2001. Performance Standards for the Production of Processed Meat and Poultry Products 66 Federal Registrar 39. p. 12590-12636. *In*.

10. FSIS. 2003. Interim Final Rule 9 CFR Part 430. *In*.

11. FSIS. 2003. Risk assessment for Listeria monocytogenes in deli meat. . *In*, Washington, D.C.

12. FSIS. 2008. Interim Final Rule 9 CFR Part 430. *In*.

13. Geornaras, I., P. N. Skandamis, K. E. Belk, J. A. Scanga, P. A. Kendall, G. C. Smith, and J. N. Sofos. 2006. Postprocess Control of *Listeria monocytogenes* on Commercial Frankfurters Formulated with and without Antimicrobials and Stored at 10° C. *Journal of Food Protection*. 69:53-61.

14. Gombas, D. E., Y. Chen, R. S. Clavero, and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in Ready-to-Eat Foods. *Journal of Food Protection*. 66:559-569.

15. Hintz, J. 2001. NCSS and Pass. Number Cruncher Statistical Systems *In*, Kaysville, UT.

16. Hynes, N. 2000. Draft: Multistate Outbreak - Findings of Epidemiological In-Plant Investigation. *In*, Waco, TX.

17. Institute, A. M. 2001. Consumer Handling of RTE Meats. *In*.

18. Lin, C.-M., K. Takeuchi, L. Zhang, C. B. Dohm, J. D. Meyer, P. A. Hall, and M. P. Doyle. 2006. Cross-Contamination between Processing Equipment and Deli Meats by *Listeria monocytogenes*. *Journal of Food Protection*. 69:71-79.

19. Lundén, J. M., T. J. Autio, and H. J. Korkeala. 2002. Transfer of Persistent *Listeria monocytogenes* Contamination between Food-Processing Plants Associated with a Dicing Machine. *Journal of Food Protection*. 65:1129-1133.

20. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-Related Illness and Death in the United States. *Emerging Infectious Diseases*. 5:607-625.

21. Midelet, G., and B. Carpentier. 2002. Transfer of Microorganisms, Including *Listeria monocytogenes*, from Various Materials to Beef. *Applied and Environmental Microbiology*. 68:4015-4024.

22. Montville, R., Y. Chen, and D. W. Schaffner. 2001. Glove barriers to bacterial cross-contamination between hands to food. *Journal of Food Protection*. 64:845-849.

23. Prevention, C. f. D. C. a. 2007. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food — 10 States, 2006. p. 336-339. *In*, Morbidity and Mortality Weekly Report, vol. 56. U.S. Government Printing Office.

24. Service, F. S. a. I. 1999. HACCP Implementation - Phase II for Small Plants. *In* USDA (ed.), Washington, D.C.

25. Team, R. D. C. 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing. *In*, Vienna, Austria.

26. Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the food-processing environment. *Journal of Food Protection*. 65:709-725.

27. Tsui, F. 2007. USDA Personal Communication. *In*.

28. Van Belle, G. 2002. Statistical rules of thumb. Wiley-Interscience, New York.

29. Voetsch, A. C., F. J. Angulo, T. F. Jones, M. R. Moore, C. Nadon, P. McCarthy, B. Shiferaw, M. B. Megginson, S. Hurd, B. J. Anderson, A. Cronquist, D. J. Vugia, C. Medus, S. Segler, L. M. Graves, R. M. Hoekstra, and P. M. Griffin. 2007. Reduction in the Incidence of Invasive Listeriosis in Foodborne Diseases Active Surveillance Network Sites, 1996–2003. p. 513-520. *In*, Clinical Infectious Diseases, vol. 44. Centers for Disease Control and Prevention, Atlanta, GA.

30. Vorst, K. L., E. C. D. Todd, and E. T. Ryser. 2006. Transfer of *Listeria monocytogenes* during Mechanical Slicing of Turkey Breast, Bologna, and Salami. *Journal of Food Protection*. 69:619-626.

31. Wallace, F. M., J. E. Call, A. C. S. Porto, G. J. Cocoma, T. E. S. P. Team, and J. B. Luchansky. 2003. Recovery Rate of *Listeria monocytogenes* from Commercially Prepared Frankfurters during Extended Refrigerated Storage. *Journal of Food Protection*. 66:584-591.

32. Wederquist, H. J., J. N. Sofos, and G. R. Schmidt. 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives. *Journal of Food Science*. 59:498-500, 516.

33. Wiedmann, D. M. 2002. Blinded industry data on the ratio of *Listeria* spp. to *Listeria monocytgenes*. *In* Cornell University.