Results of Data Analysis for the *Listeria monocytogenes* RLm Risk-based Sampling Program, Calendar Year 2008

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ACRONYMS AND ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
DAIG	Data Analysis and Integration Group
EIAO	Enforcement, Investigations, and Analysis Officer
FSA	Food Safety Assessment
FSIS	Food Safety and Inspection Service
GMP	Good manufacturing practice
HACCP	Hazard Analysis and Critical Control Point
IVT	Intensified Verification Testing
Lm	*Listeria monocytogenes*
LSFS	Laboratory Sample Flow System
NOIE	Notices of Intended Enforcement
NR	Noncompliance Record
OFO	Office of Field Operations
OSEL	Outbreaks Section of the Eastern Laboratory Microbiology Branch
PBIS	Performance-based Inspection System
PFGE	Pulsed field gel electrophoresis
PHV	Public Health Veterinarian
RLm	Routine *Listeria monocytogenes* Risk-based Sampling Program
RTE	Ready-to-eat
USDA	United States Department of Agriculture

EXECUTIVE SUMMARY

The Food Safety and Inspection Service's (FSIS's) *Listeria monocytogenes* Risk-based RLm sampling program is designed to detect *L. monocytogenes* (*Lm*) contamination from three types of samples: Post-lethality environmentally exposed ready-to-eat (RTE) meat and poultry products, RTE food contact surfaces and noncontact environmental sources. The Agency analyzed results of *Lm* testing of meat and poultry product, food contact surface, and environmental (nonfood contact) samples collected under the RLm sampling program for calendar year 2008. These analyses, which included 6,006 samples from 204 establishments, focused on the following:

- incidence and categorization of positive *Lm* samples from sampled establishments;
- types, sources, and pulsed field gel electrophoresis (PFGE) subtyping of *Lm* isolates from the positive samples;
- descriptive summaries with respect to
  - *Lm* control alternatives employed by the establishment,
  - establishment Hazard Analysis and Critical Control Point (HACCP) size,
  - establishment production volume,
  - FSIS District,
  - geographic location of the establishment, and
  - season or month of sample collection; and
- trends in percentage of positive results from April 2006 (program inception) through 2008.

For calendar year 2008, 0.5% (5/959) of product samples, 0.6% (19/3,322) of contact surface samples, and 2% (35/1725) of environmental samples tested positive for *L. monocytogenes*. This included five product samples from four separate establishments. About 1 in 25 establishments had *Lm*-positive contact surface samples, and about 1 in 6 establishments had *Lm*-positive environmental samples. The five *Lm*-positive products were three chicken products, one beef product, and one pork product. Containers, blades, tables, and trays were the chief types of positive contact surface samples. Drains, wheels, floors, floor mats, and squeegees were the most common sources of positive environmental samples; 20% of the tested floor mat samples were positive for *Lm*. PFGE subtyping results yielded 31 distinct patterns among 59 isolates tested. Matching PFGE subtypes were obtained among multiple isolates from each of six establishments.

Results of analysis based on *Lm* control alternatives showed that the majority of positive samples were obtained from establishments employing *Lm* control Alternative 2b (antimicrobial treatment/high-risk) and Alternative 3 (sanitation only/highest risk). Most of the positive RLm samples were from establishments that produced deli meats, hot dogs, and cooked products. Positive product and contact surface samples were isolated mainly from establishments that produced between 10,000 and 10,000,000 pounds of product per year. (This encompasses about 85% of all establishments, but less than 15% of total production volumes). Positive environmental samples were obtained at all times of the year, whereas most of the positive contact surface samples were obtained mainly between May and September.

Analysis of multi-year (2006-2008) data for the RLm program revealed statistically significant increases for RLMPROD (RLm product) and RLMCONT (RLm food contact surface) samples over time. There also was a downward trend in the percentage of establishments with at least one *Lm*-positive sample. The net effect of these results is that between 2006 and 2008, more *Lm*-positive samples were being isolated from a stable or decreasing percentage of *Lm*-positive establishments.

1. INTRODUCTION

FSIS conducts regulatory microbiological testing of ready-to-eat (RTE) meat and poultry products for three microorganisms: *Listeria monocytogenes* (*Lm*), *Salmonella,* and *Escherichia coli* O157:H7. FSIS began risk-based testing of RTE products for *L. monocytogenes* in 2004. One of the first of these risk-based sampling and testing programs was ALLRTE (random verification sampling of all RTE meat and poultry products). All establishments were considered at equal risk under the ALLRTE sampling program.

FSIS regulations also mandated the reporting of various production factors by establishments producing RTE meat and poultry products that were exposed to the environment after a lethality treatment. This served as the basis for sampling programs based on the risk characteristics of the producing establishment. RTE001, a sampling and testing program for RTE meat and poultry products based on establishment risk factors, was initiated in January 2005. An *Lm* risk-based sampling project named the Routine *Lm* Risk-based (RLm) Sampling Program was then initiated in April of 2006. While RTE001 involves sampling and testing of the RTE meat and poultry products themselves, the RLm program includes sampling and testing of products, product contact surfaces, and environmental surfaces. This makes the RLm program a *proactive* sampling project; that is, RLm provides a means of identifying establishments with a higher risk of *Lm* contamination in the food processing environment before product contamination can actually be demonstrated. In addition, a Food Safety Assessment (FSA) is conducted at the establishment in conjunction with RLm sampling and testing. Unlike the ALLRTE and RTE001 programs in which samples are also tested for *Salmonella* and *E. coli* O157:H7, in the risk-based RLm program, samples are collected and tested for *L. monocytogenes* only.

The RLm testing program consists of the following three concurrent sampling projects:

1. RLMPROD—the routine risk-based testing of intact RTE food product samples throughout the selected production shift;
2. RLMCONT—the routine risk-based testing of surfaces that have direct contact with RTE product(s) in the RTE production area (e.g., conveyor belts, storage racks, slicer blades, loaders, table tops); and
3. RLMENVR—the routine risk-based testing of environmental (nonfood contact) surfaces in the RTE production areas (e.g., floors, drains, walls, floor mats).

Samples collected under the RLm program are limited to establishments subject to 9 CFR Part 430 (i.e., establishments in which RTE products are exposed to the post-lethality environment (see http://www.access.gpo.gov/nara/cfr/waisidx_08/9cfr430_08.html)).

In accordance with FSIS Directive 10,240.5, Verification Procedures for Enforcement, Investigations and Analysis Officers (EIAOs) for the *Listeria monocytogenes* (*Lm*) Regulation and Routine Risk-Based *Listeria monocytogenes* (RLm) Sampling Program, FSIS Enforcement, Investigations, and Analysis Officers (EIAOs) and Public Health Veterinarians (PHVs) trained in EIAO methodologies are responsible for conducting RLm sampling and assessing whether the establishment's food safety system complies with 9 CFR Part 430 (see http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/10240.5Rev2.pdf).

The selection of establishments for food contact and environmental swab sampling and number of samples for testing was previously based on an FSIS risk-based sampling algorithm. The risk-ranking algorithm was maintained using data from various Agency resources, including information from FSIS Form 10,240-1 (in particular, food product category and sanitation alternative for controlling *Listeria*) and the establishment's sample history. Once the risk ranking had been established, additional scheduling

criteria were employed to select establishments for testing. However, starting in July 2008, the top 95% volume establishments were sampled. Scheduling criteria for the RLm program for 2008 were posted on the FSIS Web site and are included here as Appendix A. The risk ranking is updated monthly. A scheduling memo is sent to districts to inform them of the establishments selected for RLm sample collection activity.

FSIS previously tabulated, analyzed, evaluated, and reported on *Lm* data collected under the RLm program since its inception in April 2006 through calendar year 2007 (see http://www.fsis.usda.gov/PDF/Results_Data_Analysis_Lm.pdf). Accordingly, the objectives of this report were to (1) obtain, tabulate, and review sampling results for calendar year 2008; (2) evaluate the data with respect to various parameters; and (3) identify possible trends in the data based on annual results for 2006 through 2008.

1.1 Background

The RLm program was conceived as a means of routinely collecting and testing three types of samples (RTE meat and poultry product, food contact surface, and environmental) in post-lethality production areas where *L. monocytogenes* may be present. The impetus for this sampling and testing effort was the need to determine if, or how well, establishments were controlling *Lm* contamination in post-lethality exposed RTE products based on regulation 9 CFR 430. FSAs are conducted in conjunction with sample testing to evaluate the food safety practices of establishments producing post-lethality exposed RTE products, particularly those establishments considered to be high risk.

This examination of high-risk establishments on a routine basis was conceptually similar to that of Intensified Verification Testing (IVT). IVT involves the collection and testing of food contact surface and non-food contact environmental samples (in addition to product) in response to RTE meat and poultry product samples that initially test positive for *L. monocytogenes* in the ALLRTE and RTE001 sampling programs. What sets the RLm program apart from IVT (and indeed, from other FSIS sampling programs for foodborne pathogens) is that the routine testing of samples from food contact surfaces and processing environments is not done in response to positive product samples. Rather, the purpose of such testing is to proactively detect the presence of *L. monocytogenes* in establishments, even in the absence of actual product contamination, and to take corrective actions accordingly. (With respect to positive samples from the RLm program, one way of ensuring the effectiveness of any corrective actions is IVT itself, employed as a follow-up when a sample tests positive for *L. monocytogenes*.)

In conducting the RLm program, FSIS anticipated it would be able to assess the compliance of establishments with regulation 9 CFR 430 regarding the control of *L. monocytogenes* in post-lethality exposed RTE products and help ensure that RTE products are safe for consumption at the end of the production process. With the RLm program in place, FSIS has the ability to verify and evaluate *Lm* control alternatives and sanitation practices at individual establishments that would not be possible with normal day-to-day inspection. The RLm program was also designed, in part, to increase confidence in the effectiveness of a given establishment's control measures and interventions (alternatives).

RLm testing involves collecting multiple samples as a unit—3 product samples, 10 product contact samples, and 5 environmental (non-product contact) samples—during a production shift.[1] (It should be noted that it is up to the EIAO to select products based on which ones are considered to be the highest risk, as well as selecting appropriate contact and environmental surfaces for collection and testing). This contrasts markedly with RTE001 and ALLRTE, in which a single RTE product sample is collected at a given point in time. Moreover, because RLm sampling at each establishment is done in conjunction with an FSA, an in-depth evaluation of food safety practices is possible. The product, contact surface, and environmental sample data collected from the establishments can help identify possible risk factors that could be associated with positive results. For example, testing of food contact and environmental samples may permit the identification of establishments where there is evidence of control issues, such as harborage (sites of *Lm* survival or persistence) or poor sanitation practices.

Because the RLm program was intended to be a routine sampling program to complement the FSA process, FSIS has the expectation that establishments selected for sampling should be in compliance with all regulatory standards because those establishments are selected for sampling on the basis of risk rather than for any particular cause. Accordingly, FSIS evaluates establishments producing RTE products, first and foremost, to ensure the safety of these products and, thus, to protect the public from foodborne *Listeria* infections. If a given establishment has positive results from the RLm sampling, FSIS takes enforcement actions as necessary to address product contamination and adulteration. Furthermore, the positive results serve as the impetus for focusing inspection efforts and intensifying inspection resources in that establishment. Such results may indicate poor HACCP design, execution, or both. In addition to determining the vulnerabilities and the adequacy of the establishment's food safety practices with respect to *L. monocytogenes*, FSIS develops and implements policies to improve the effectiveness of the establishment's *Listeria* contamination control practices.

[1] Initially, the numbers of 18-sample units used at each establishment were based on the number of production lines. Currently, the number of sampling units is based on establishment Hazard Analysis and Critical Control Point (HACCP) size, with three, two, and one sample units used at establishments classified as HACCP sizes Large, Small, and Very Small, respectively. This system provides for consistency with respect to the logistics of sample collection and testing. It should also be noted that the ratio of 10:5 for contact and environmental samples is because positive contact samples have defined regulatory consequences, which is not the case for positive environmental samples.

2. DATA COLLECTION DESIGN AND IMPLEMENTATION

Data routinely generated from the 2008 RLm program were used for all analyses. FSIS tabulated the routine risk-based sample information for *L. monocytogenes* in RTE meat products that were collected on FSIS sampling forms. The data consisted of product, contact surface, and environmental test results for samples that were collected and tested for *L. monocytogenes*. Information regarding establishment parameters such as control alternative and production volumes, was obtained from establishment-provided data contained in FSIS Form 10,240-1. These data were extracted from the FSIS Data Warehouse (M2K database) via Laboratory Sample Flow System (LSFS). Supplementary data were obtained using the Performance-based Inspection System (PBIS) reader.

The PFGE pattern data related to isolate subtyping of *Lm*-positive samples were provided by the Outbreaks Section of the Eastern Laboratory Microbiology Branch (OSEL) of FSIS. Actual PFGE pattern designations and pattern matching were done by the Centers for Disease Control and Prevention (CDC).

3. DATA ANALYSIS PROCEDURES

FSIS calculated the numbers of positive samples and percentage of positive samples for product, contact surface, and environmental samples for calendar year (CY) 2008. The data analyzed were based on sample collection dates (January 1, 2008 through December 31, 2008)[1]. The analyses which included 6,006 samples from 204 establishments in 2008, focused on the following:

- the incidence and categorization of positive *Lm* samples from sampled establishments;
- types, sources, and PFGE subtyping of *Lm* isolates from the positive samples;
- descriptive summaries with respect to the following:
  - *Lm* control alternatives employed by the establishment,
  - establishment HACCP size,
  - establishment RTE production volumes,
  - FSIS District,
  - geographic location of the establishment,
  - season or month of sample collection; and
- trends in percentages of positive results from 2006-2008 (all based on sample collection date).

All data analyses were performed through data handling and evaluation techniques using Microsoft Office Excel.

[1] FSIS routinely posts summarized annual data for their microbiological testing programs for ready-to-eat (RTE) meat and poultry products (http://www.fsis.usda.gov/Science/Micro_Testing_RTE/index.asp#results08). Prior to calendar year 2008, results that were posted were based on sample analysis completion date. Beginning in January 2008, results are being posted by sample collection date to include all samples collected within the calendar year. This aligns FSIS' activities in this area with those of other Federal partners. As it happens, the 2008 RLm results by collection date are directly comparable with the RLm results from 2006 and 2007 because those results are the same regardless of whether collection date or completion date is used (i.e., in 2006 and 2007, all samples collected had their analyses completed within the same calendar year).

4. RESULTS AND DISCUSSION

Data collection under the RLm sampling program began in April 2006. The results for the periods April through December 2006 and calendar year 2007 are found in an online report at http://www.fsis.usda.gov/PDF/Results_Data_Analysis_Lm.pdf. Sampling data collected under RLm in calendar year 2008 included product, contact surface, and environmental sampling results. Some analyses, notably for types of samples and for data trends over time (2006 through 2008), required the evaluation of combined multi-year data. Beginning in 2008, summarized RLMPROD and other RLm data are being reported by FSIS based on the date of sample collection in order to better align FSIS data reporting activities with that of other Federal partners. Thus, all data analyzed for this report, including any data that involved multiple years (2006-2008) are based exclusively on sample collection dates in order to make them directly comparable on an annualized basis. However, no changes needed to be made in the above-cited 2006-2007 report, because, in this instance, the 2006 and 2007 results are identical regardless of whether collection date or analysis completion date is utilized for reporting (i.e., in 2006 and 2007, all samples collected had their analyses completed within the same calendar year).

4.1 RLm Testing Results for Calendar Year 2008

Table 4.1.1 and Figures 4.1.1 and 4.1.2 show the results of testing 6,006 samples from 204 establishments in the RLm sampling program for calendar year 2008 (based on sample collection date). This encompasses the following:

- 959 RTE food product samples,
- 3,322 contact surface samples, and
- 1,725 environmental surface samples.

Overall, 1% of the samples (59 of 6,006 samples) were positive for *L. monocytogenes*. Five (0.5%) of the product samples were positive for *L. monocytogenes*, while positive results were obtained for 19 (0.6%) contact surface and 35 (2%) environmental samples.

Table 4.1.1. Detection of *L. monocytogenes* in Product, Contact Surface, and Environmental Samples, Calendar Year 2008

Sample Type	Total Collected	Positive Samples	
		No.	%
Product	959	5	0.52
Contact surface	3,322	19	0.57
Environmental	1,725	35	2.03
Total	**6,006**	**59**	**0.98**

Figure 4.1.1. Samples Collected and Tested for *L. monocytogenes*, Calendar Year 2008

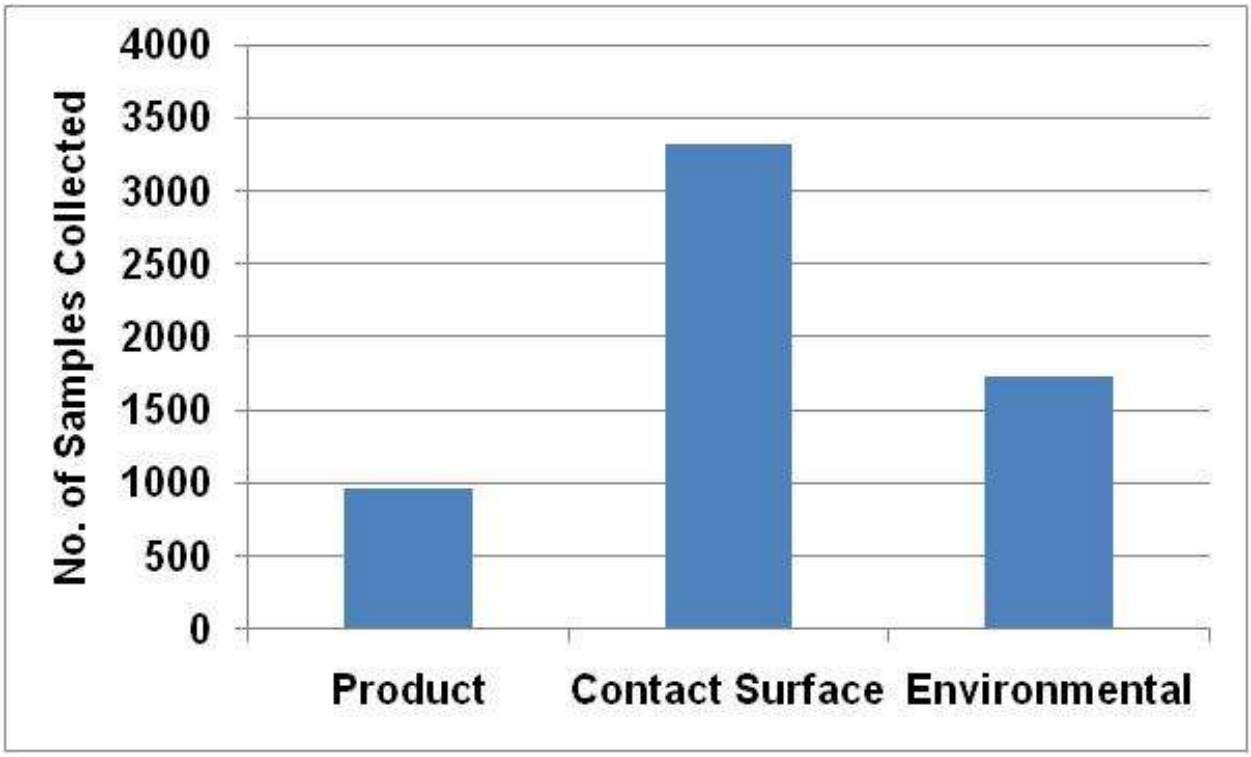
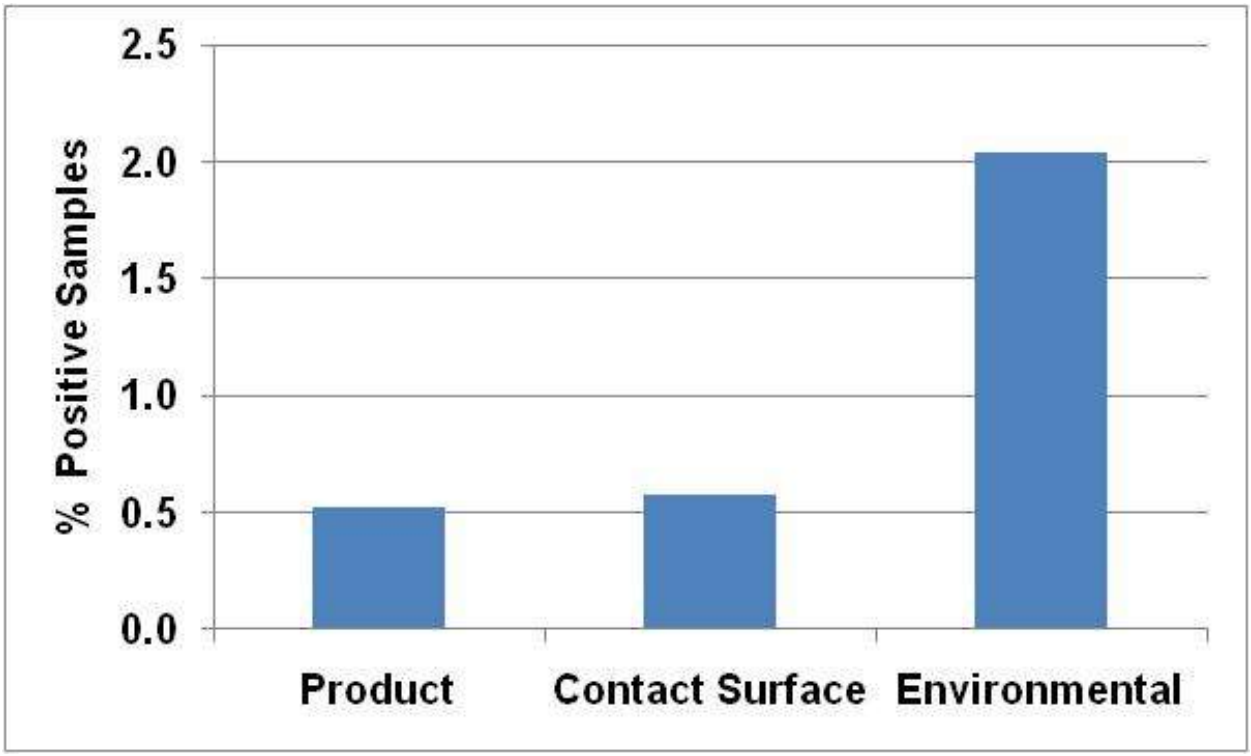


Figure 4.1.2. Percentage of *Lm*-Positive Samples, Calendar Year 2008



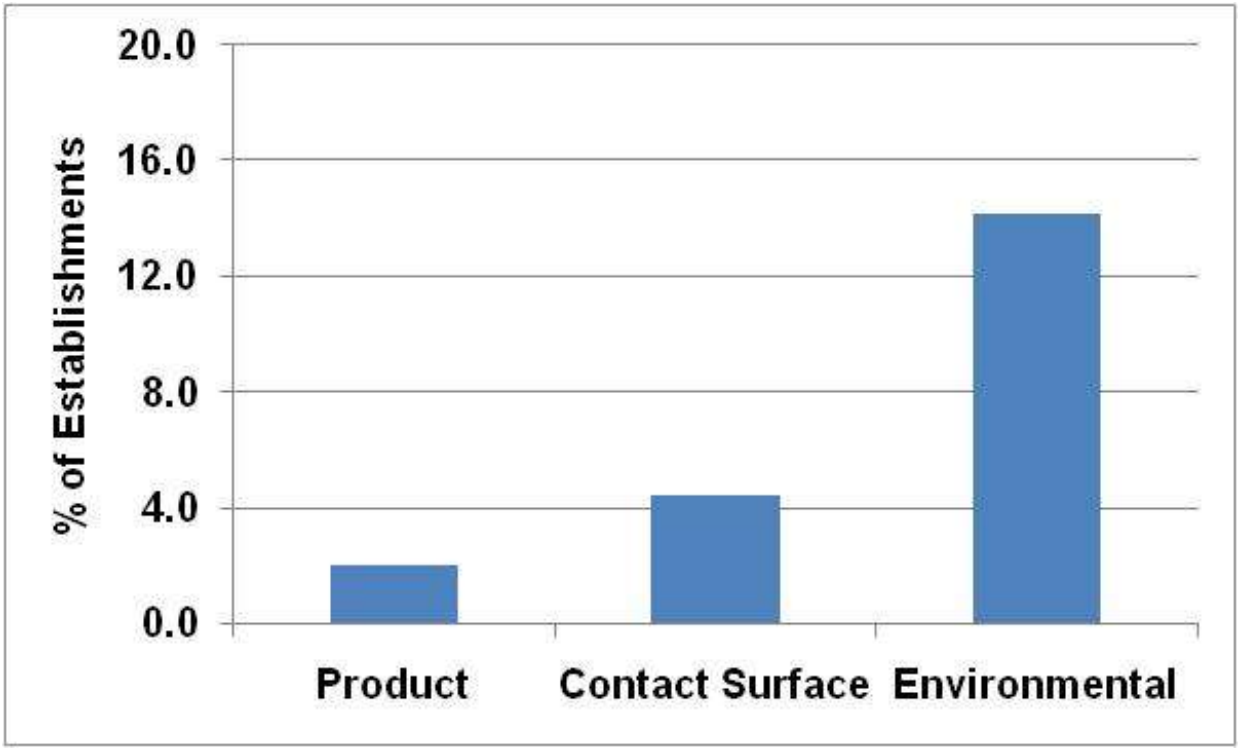
Of the 204 establishments tested in 2008, 1 of every 6 establishments (33, or 16.3%) had at least one *Lm*-positive sample (product and/or contact and/or environmental). The results of sorting the establishment data by type of testing program are shown in Table 4.1.2 and Figure 4.1.3. Most of the establishments with *Lm*-positive samples had positive results for environmental sampling (29, or about 14%), while 9 (4.4%) had positive results for contact surface, and 4 (2%) had positive results for product sampling.

Table 4.1.2. Number and Percentage of Establishments with at Least One *Lm*-Positive Sample, Calendar Year 2008

Sample Type	Establishments	
	No.	**%**
Product	4	2.0
Contact surface	9	4.4
Environmental	29	14.1
Combined (204 establishments sampled)	**33***	**16.1**

*Total includes establishments with more than one positive sample type. See Section 4.2.

Figure 4.1.3. Percentage of Establishments with *Lm*-Positive Samples, Calendar Year 2008



4.2 Classification of *Lm*-Positive Samples, within Establishments, Calendar Year 2008

Lm-positive samples from the establishments were further characterized based on which establishments had samples that were positive in all three sample sites or in six other possible combinations of sample sites. The results of this categorization are shown in Table 4.2.1. A total of 33 establishments (or about 16% of the 204 establishments for which samples were collected and tested) had *Lm*-positive samples:

- Most of the positive establishments (24 of 33, or about 72% of all establishments with at least 1 positive sample) were positive only for environmental samples.
- Another three establishments (9.1%) were positive only for contact surface samples.
- Two establishments (6.1%) were positive for both contact surface and environmental samples.
- Of the four establishments with positive product samples, one also was positive for contact surface samples, while the other three yielded positive results in all three sample sites.

These results suggest that in establishments with *Lm*-positive product samples, one may also encounter positive contact and/or environmental samples. Still, over 80% of these establishments had positive samples in only one category (largely environmental) within the RLm sampling program.

Table 4.2.1. Classification of *Lm*-Positive Samples from Establishments with at Least One Positive Sample, Calendar Year 2008

Time Period	Environmental Only	Contact Surface Only	Environmental and Contact Surface	Product and Contact Surface	All Types	Total Establishments
CY 2008	24 (72.1%)	3 (9.1%)	2 (6.1%)	1 (3.0%)	3 (9.1%)	33

Note: Values in parentheses are percent positive relative to all establishments with at least one positive sample.

4.3 Types and Sources of *Lm*-Positive Samples, Calendar Year 2008

A total of 59 *Lm*-positive samples were obtained under the RLm sampling program in 2008. Of these, 5 were from product, 19 were from contact surfaces, and 35 were from environmental surfaces. Tables 4.3.1 through 4.3.5 show a breakdown of the types and sources of *Lm*-positive samples by category. These results are as follows:

- There were four different product types represented among the five meat and poultry products that were positive in calendar year 2008 (Table 4.3.1).
- Of the 19 positive contact surface samples, there were 4 positive container (or bin, tub, or bag) samples, 3 positive blade samples, 3 positive table samples, and 2 positive tray samples. One positive was found in 7 other contact surface sample types (Table 4.3.2).
  - The rates of *Lm*-positive samples relative to the numbers of containers, blades, tables, and trays sampled ranged from 0.8 to 3% (Table 4.3.3).
- Of the 35 positive environmental surface samples (Table 4.3.4), the highest number of positives were found in drains (11, or about 31% of all environmental positives), followed by floors (4), wheels (4), and floor mats (3).
  - The rates of *Lm*-positive samples relative to the approximately 440 drain, wheel, and floor samples ranged from 3.6 to 4.6% (Table 4.3.5).
  - The rate of *Lm*-positive floor mats relative to the 15 floor mats sampled was 20%.

Table 4.3.1. Types of *Lm*-Positive Product Samples, Calendar Year 2008

Product Type	Positives
Beef, Large Mass, Whole Muscle (Brisket)	1
Chicken, Small Mass, Whole Muscle (Chicken Teriyaki)	1
Pork, Sliced, Diced/Shredded (Pork Product)	1
Chicken, Sausage Type Product, Peeled (Chicken Franks)	2
Total	**5**

Table 4.3.2. Types of *Lm*-Positive Contact Surface Samples, Calendar Year 2008

Contact Surface Type	Positive Samples	
	No.	%
Container/Bag/Tub/Bin	5	26.3
Blade (2 slicer; 1 shredder)	3	15.8
Table	3	15.8
Tray	2	10.5
Packaging Film	1	5.3
Gloves	1	5.3
Grinder	1	5.3
Hands	1	5.3
Loader	1	5.3
Shredder Entry Chute	1	5.3
Total	**19**	**100.0**

Table 4.3.3. *Lm*-Positive Rate for the Main Types of Contact Surface Samples, Calendar Year 2008

Contact Surface Type	Total Collected[a]	Positive Samples	
		No.	%
Container/Bag/Tub/Bin	135	4	3.0
Blade	175	3	1.7
Table	390	3	0.8
Tray	85	2	2.4

[a]Approximate numbers from sample description listings.

Table 4.3.4. Types of *Lm*-Positive Environmental Samples, Calendar Year 2008

Environmental Surface Type	Positive Samples	
	No.	% of All Positives
Drain	11	31.4
Floor	4	11.4
Wheel(s)	4	11.4
Floor Mat	3	8.6
Squeegee	2	5.7
Dolly	1	2.9
Foil Wrap	1	2.9
Fork Lift	1	2.9
Freezer Curb (under drain)	1	2.9
Gloves	1	2.9
Hose	1	2.9
Other ("Sauger Green with Duct Tape")	1	2.9
Pallet	1	2.9
Slicer	1	2.9
Trash Can Bottom	1	2.9
Wall	1	2.9
Total	**35**	**100.0**

Table 4.3.5. *Lm*-Positive Rate for the Main Types of Environmental Samples, Calendar Year 2008

Environmental Surface Type	Total Collected[a]	Positive Samples	
		No.	%
Drain	240	11	4.6
Floor	90	4	4.4
Wheel(s)	110	4	3.6
Floor Mat	15	3	20.0
Squeegee	20	2	10.0

[a]Approximate numbers from sample description listings (actual number for mat).

While absolute numbers of RLMENVR samples from floor mats are relatively low, the percent positive rate for floor mats was 20% in calendar year 2008. Retrospective data analysis of percent positive rates for floor mats in RLMENVR also exceeded 20% in 2006-2007. A similar situation exists for INTENV samples (environmental samples collected as part of Intensified Verification Testing). These results raise concerns that floor mats in RTE production establishments may be an important environmental source of *L. monocytogenes* contamination.

Table 4.3.6. *Lm*-Positive Rate for RLMENVR and INTENV Floor Mat Samples, Calendar Years 2006-2007 and 2008

Sampling Program	Total Collected[a]	Positive Samples	
		No.	%
RLMENVR (2006-2007)	12	3	25
RLMENVR (2008)	15	3	20
INTENV (2006-2007)	11	4	36.4
INTENV (2008)	8	2	25

[a]Actual numbers from sample description listings.

4.4 Isolate PFGE Pattern Typing Results

PFGE analysis was performed on all 59 isolates derived from the positive RLm samples. The results from RLm sampling and PFGE analysis of isolates can be used to determine cross-contamination between products and/or contact surfaces and or the production environment. Furthermore, in conjunction with PFGE analysis of isolates from Intensified Verification Testing (IVT), RLm sampling and PFGE analysis can help to identify both possible cross-contamination and harborage (persistence of specific PFGE pattern types over time) within establishments. As shown in Table 4.4.1, a total of 31 different PFGE pattern types were observed among the 59 isolates.[1] Ten of these patterns were isolated multiple times, with 1 pattern type isolated 13 times, 1 pattern type isolated 6 times, 1 pattern type isolated 5 times and 7 pattern types isolated 2 times (Table 4.4.1).

[1] PFGE pattern types are designated by CDC and are part of the PulseNet database, which was accessed for purposes of this analysis. Actual pattern types are not shown.

Table 4.4.1. Patterns and Occurrence of *Lm* PFGE Subtypes Isolated in the RLm Program, Calendar Year 2008

PFGE Pattern Type	Occurrence of Pattern Type			No. of Total Isolates	% of Total Isolates	Rank in FSIS List of Top 10 PFGE Patterns[a]
	RLMPROD	RLMCONT	RLMENVR			
1	2	8	3	13	22.0	1
2	1	4	1	6	10.2	—
3	2	2	1	5	8.5	—
4			2	2	3.4	10
5			2	2	3.4	—
6			2	2	3.4	2
7			2	2	3.4	—
8		1	1	2	3.4	—
9			2	2	3.4	5
10			2	2	3.4	3
11		1		1	1.7	—
12		1		1	1.7	—
13		1		1	1.7	—
14		1		1	1.7	—
15			1	1	1.7	4
All other pattern types (single occurrences)			16	16	27.1	—
Total	**5**	**19**	**35**	**59**	**100**	

[a] The FSIS list of top 10 PFGE patterns encountered is maintained by the Microbiology Division of the Office of Public Health Science (OPHS).

It should also be noted that FSIS (specifically, the Microbiology Division of OPHS) keeps a list of the 10 top PFGE patterns encountered. Of the 31 PFGE patterns encountered, 6 patterns from 22 isolates (representing about 37% of all isolates) were on the current top 10 pattern list. The number one pattern from the FSIS list was also the number one pattern from the RLm sampling program (Table 4.4.1). The fact that the other patterns were not on the current FSIS pattern list does not exclude the possibility that any of these isolates could be involved in *Listeria* infections in humans.

4.5 Cross-contamination and Harborage

Several of the establishments had multiple isolations of the same PFGE pattern type from positive samples. In five of six instances, the same PFGE pattern was observed for multiple product and/or contact surface and/or environmental isolates obtained within the same establishment. This indicated possible cross-contamination between products, product contact surfaces, and environmental locations (Table 4.5.1). This included the following:

- Two establishments with matching product, contact surface, and environmental isolates;
- two establishments with matching product and contact surface isolates; and
- one establishment with matching contact surface and environmental isolates.

In the sixth establishment (#5 in Table 4.5.1), two different environmental isolates had the same PFGE pattern, which could also be considered to be evidence of cross-contamination. In all, these results demonstrate the utility of collecting multiple product, contact surface or environmental samples for the purpose of detecting isolates with a common PFGE pattern from different sources within a single establishment.

Evidence of harborage over time could not be determined from the RLm results alone because establishments with positive results were not sampled multiple times under this program. FSIS systematically reviews PFGE data across all *Lm* sample sites to determine whether harborage or cross-contamination may have occurred within particular establishments and may use the information as a basis to take further actions. A comparison of PFGE pattern types from RLm and IVT isolates obtained at different times from the same establishments in 2006 and 2007 revealed harborage in 2 of 4 (50%) of those establishments. This information is being used for the purpose of developing regulations that would require IVT in establishments with positive RLMPROD and/or positive RLMCONT samples. (At this time, positive RLMENVR samples do not have regulatory impact). The combined RLm, IVT, and PFGE results should yield important information regarding both harborage and cross-contamination in establishments with contaminated products and/or contact surfaces.

Table 4.5.1. Incidence of Multiple Isolations of the Same PFGE Subtype within the Same Establishment, Calendar Year 2008

Establishment	PFGE Pattern Type Within Establishment	Occurrence of Pattern Type			Total
		RLMPROD	RLMCONT	RLMENVR	
1	1	1	2		3
2	1	1	6	1	8
3	2	1	4		5
4	3	2	2	1	5
5	5			2	2
6	8		1	1	2

Note: Establishment is a numerical ranking, not an identifier. Pattern types are the same as in Table 4.4.1.

4.6 Results Based on Alternatives Used to Control *L. monocytogenes*

For the RLm sampling program, establishments use one or more of four possible contamination control procedures, or control alternatives, for eliminating or inhibiting the growth of *L. monocytogenes* in the particular RTE products produced by each establishment. The four alternative categories are the following:

- **Alternative 1**, the lowest-risk category, involves using both a post-lethality treatment (which could be a physical treatment or an antimicrobial agent) "that reduces or eliminates microorganisms on the product AND an antimicrobial agent or process that suppresses or limits the growth of *L. monocytogenes*" (FSIS Directive 10,240.4, Revision 1, 3/15/2006).

- **Alternatives 2a** (or Alternative 2 choice 1) and **2b** (or Alternative 2 choice 2), the next higher-risk categories, provide the option of either a post-lethality treatment the kills or inhibit microorganisms (2a) or an antimicrobial agent or process that specifically inhibits *L. monocytogenes* (2b).
- **Alternative 3**, the highest-risk category, requires the "use of sanitation procedures only" (FSIS Directive 10,240.4).

Accordingly, one would expect the potential of encountering *L. monocytogenes* in product, contact surface, and environmental samples to be the least in Alternative 1 establishments and the greatest in Alternative 3 establishments.

The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* with respect to the four major RTE *Lm* control Alternatives (1, 2a, 2b, and 3) for calendar year 2008 are shown in Table 4.6.1 and Figures 4.6.1 and 4.6.2. All five *Lm*-positive product samples encountered in 2007 were from Alternative 2 or Alternative 3 establishments. The same was true for all *Lm*-positive contact surface samples. About 11% of environmental samples that were positive for *Lm* were from individual establishments that were using both Alternatives 1 and 3, while about 5% of the sample positives were from individual establishments that employed both Alternatives 1 and 2. Although the risk associated with employing Alternative 1 as a contamination control measure is low, it is uncertain whether these establishments were employing Alternative 1 or Alternatives 2 or 3 at the time of (or in the location of) sample collection. The results may represent a demonstration of the effectiveness of post-lethality treatments, as none of the *Lm*-positive product or contact surface samples were from establishments that employed Alternative 1 (or Alternative 2a alone) for pathogen control.

4.7 Results by Establishment HACCP Size

The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes*, with respect to each establishment's HACCP size of large, small, or very small for calendar year 2008, are shown in Table 4.7.1 and Figures 4.7.1 and 4.7.2.[2] In 2008, all five positive product samples were from small establishments, while all contact samples were from either small or very small establishments. Positive *Lm* environmental samples occurred in establishments across all three HACCP sizes, with the percentages of 2.5%, 2.1%, and 0.9% for large, small, and very small establishments, respectively.

4.8 Results by Production Volume

Results were analyzed as a function of the annual production volumes of RTE food products. This information, when available, is provided by the producing establishments on FSIS Form 10,240-1. The observations made are, thus, dependent on the accuracy of the production volumes supplied. The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* with respect to the production volumes for calendar year 2008 are shown in Table 4.8.1 and Figure 4.8.1. The results indicated that *Lm*-positive product and contact surface samples were most commonly found in establishments with production volumes in the range of 10,000-10,000,000 pounds per year. Positive environmental samples were present in establishments at all but the lowest production volumes (1,000 pounds per year or less). The data on a percentage basis suggest that *Lm*-positive product and contact surface samples may occur in establishments that produce in a specific range of volumes. Positive product and contact surface samples were absent at both the lower and upper ends of the production volume ranges**.** The decreased or absent levels of positive environmental samples in those establishments at the lower end of the production volume range (<=10,000 pounds per year) is also of interest. Further

[2] Large establishments have 500 or more employees, Small establishments have 10 or more employees, but fewer than 500, and Very Small establishments have fewer than 10 employees or less than $2.5 million in annual sales.

comparisons of results as a function of production volumes are warranted, provided that such volumes can be estimated as accurately as possible.

4.9 Results by Food Product Category

Positive samples were analyzed as a function of the nine food product categories found on FSIS Form 10,240-1.[2] The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* with respect to the nine above-named food product categories for calendar year 2008 are shown in Figure 4.9.1. The results indicated that *Lm*-positive samples were often found in establishments that produced multiple products, mainly deli meat and/or cooked products and/or hot dog products. However, the highest percentages of *Lm*-positive samples were found a) in establishments that produced deli/hotdog/cooked/dried products and b) in establishments that produced deli/cooked/salad products (see Figure 4.9.1).

4.10 Results by Geographic Region

To explore possible geographic influences on the detection of *L. monocytogenes*, individual states were classified into the following geographic regions[3]:

- **Northeast**: Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, and Washington, DC
- **North Central**: Illinois, Indiana, Iowa, Michigan, Minnesota, Ohio, and Wisconsin
- **Southeast**: Alabama, Florida, Georgia, Kentucky, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, West Virginia, Puerto Rico, and the U.S. Virgin Islands
- **Southwest**: Arkansas, Kansas, Louisiana, Missouri, Nebraska, New Mexico, Oklahoma, and Texas
- **West**: Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, North Dakota, Oregon, South Dakota, Utah, Washington, Wyoming, American Samoa, Guam, and the Northern Marianas Islands

The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* within these five broad geographic regions for calendar year 2008 are shown in Table 4.10.1 and Figure 4.10.1. In 2008, four of the five *Lm*-positive product samples were from establishments located in the Western region of the country (the fifth was from the North Central region). *Lm*-positive contact surface samples were fairly evenly distributed across the five regions (range, 0.8-1.4%). The *Lm* percent positive rates for environmental samples ranged from 1.6-4.4% across four of the five geographic regions; there were no positive environmental samples from the Southwest region in 2008.

[2] The nine 10,240-1 categories are deli products sliced at the producing establishment, deli products to be sliced after distribution, hot dog products, fully cooked products, fermented products, dried products, salt-cured products, frozen products, and salad/spread/pâté products.

[3] This classification is taken from the following FSIS manuscript: Naugle et al., Journal of Food Protection 69:2607-2614, 2006.

4.11 Results by Season and Month

To explore possible seasonal influences on the detection of *L. monocytogenes*, positive product, contact surface, and environmental results were categorized based either on season (quarter) or month of the year. The percentages of product, contact surface, and environmental samples positive for *L. monocytogenes* by season and by month for calendar year 2008 are shown in Figures 4.11.1 and 4.11.2, respectively. All of the *Lm*-positive product samples and most of the positive contact surface samples were obtained in the spring and summer (notably, between May and September). In contrast, *Lm*-positive environmental samples were isolated across all seasons or months of the year.

Table 4.6.1. Detection of *L. monocytogenes* by Control Alternative, Calendar Year 2008

Alternative	No. of Establishments	Product Samples			Contact Surface Samples			Environmental Samples			Total Samples		
		Amount	Positive Samples		Amount	Positive Samples		Amount	Positive Samples		Amount	Positive Samples	
			No.	%		No.	%		No.	%		No.	%
1	5	21	0	0.0	70	0	0.0	35	0	0.0	126	0	0.0
3	87	328	1	0.3	1167	7	0.6	625	12	1.9	2120	20	0.9
2A and 2B	18	90	0	0.0	339	0	0.0	169	4	2.4	598	4	0.7
2B	41	238	2	0.8	801	2	0.2	406	10	2.5	1445	14	1.0
Mixed 1/2/3	5	27	0	0.0	90	0	0.0	45	0	0.0	162	0	0.0
Mixed 1/2	7	38	0	0.0	128	0	0.0	65	3	4.6	231	3	1.3
Mixed 1/3	1	6	0	0.0	16	0	0.0	9	1	11.1	31	1	3.2
Mixed 2/3	40	211	2	0.9	711	10	1.4	371	5	1.3	1293	17	1.3
Total	**204**	**959**	**5**	**0.5**	**3322**	**19**	**0.6**	**1725**	**35**	**2.0**	**6006**	**59**	**1.0**

Figure 4.6.1. Percentage of *Lm*-Positive Product and Contact Surface Samples by Control Alternative, Calendar Year 2008

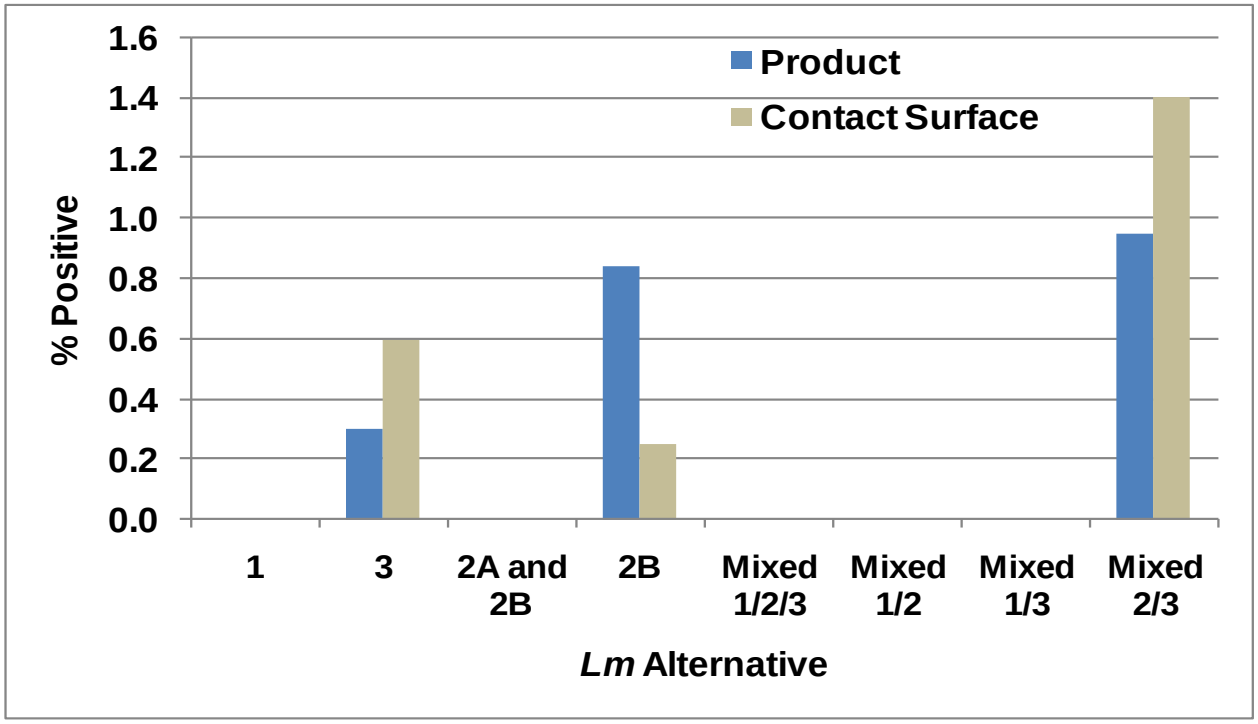


Figure 4.6.2. Percentage of *Lm*-Positive Environmental Samples by Control Alternative, Calendar Year 2008

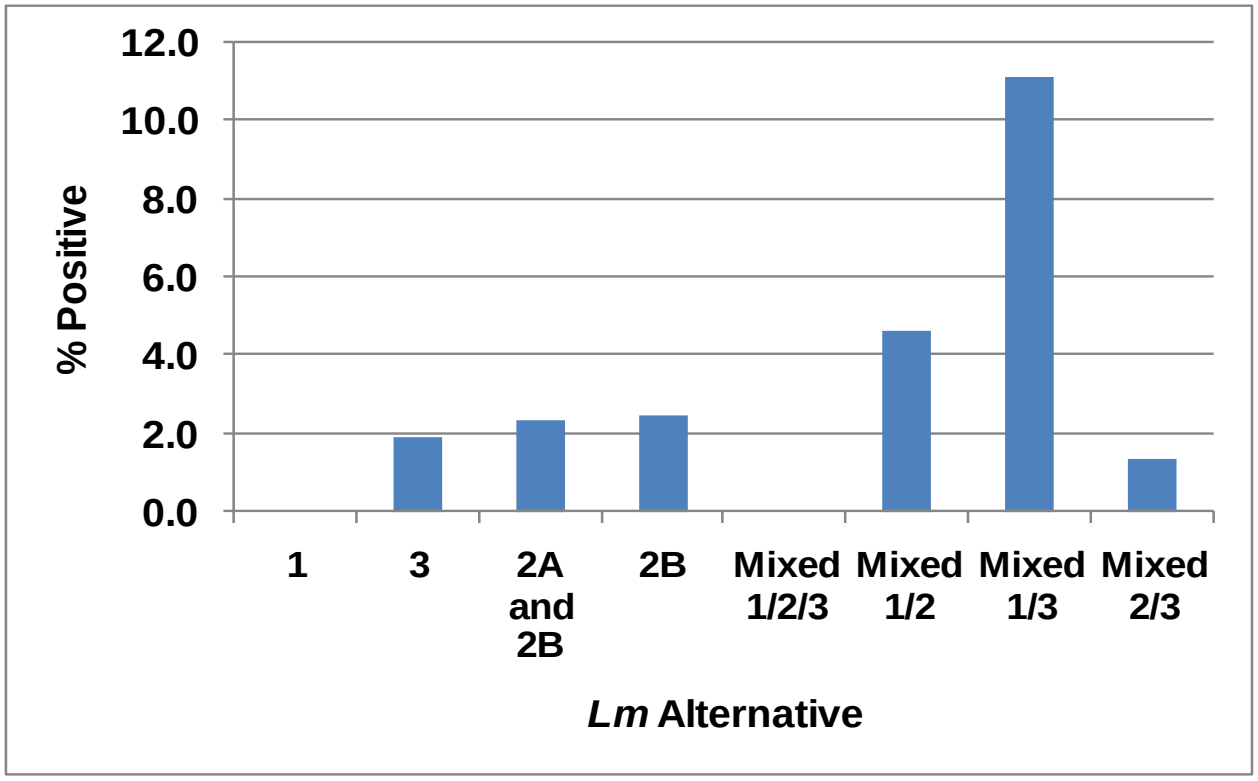


Table 4.7.1. Detection of *L. monocytogenes* by Establishment Size, Calendar Year 2008

Establishment HACCP Size	Product Samples	Positive Samples		Contact Surface Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
Large	214	0	0.0	737	0	0.0	367	9	2.5	1,318	9	0.7
Small	621	5	0.8	2,157	17	0.8	1125	24	2.1	3,903	46	1.2
Very Small	124	0	0.0	428	2	0.5	233	2	0.9	785	4	0.5
Total	**959**	**5**	**0.5**	**3,322**	**19**	**0.6**	**1725**	**35**	**2.0**	**6,006**	**59**	**1.0**

Table 4.8.1. Detection of *L. monocytogenes* as a Function of Establishment Annual Production Volumes, Calendar Year 2008

Establishment Annual Production Volume Range, Pounds*	Product Samples	Positive Samples		Contact Surface Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
~1000	6	0	0.0	20	0	0.0	10	0	0.0	36	0	0.0
~10000	56	0	0.0	186	1	0.5	92	1	1.1	334	2	0.6
~100000	156	1	0.6	556	5	0.9	290	5	1.7	1,002	11	1.1
~1000000	265	2	0.8	923	11	1.2	498	14	2.8	1,686	27	1.6
~10000000	401	2	0.5	1,378	2	0.1	706	11	1.6	2,485	15	0.6
~100000000	75	0	0.0	259	0	0.0	129	4	3.1	463	4	0.9

*Approximate, based on information collected on FSIS 10,240-1 forms.

Figure 4.7.1. Percentage of *Lm*-Positive Product and Contact Surface Samples by Establishment HACCP Size, Calendar Year 2008

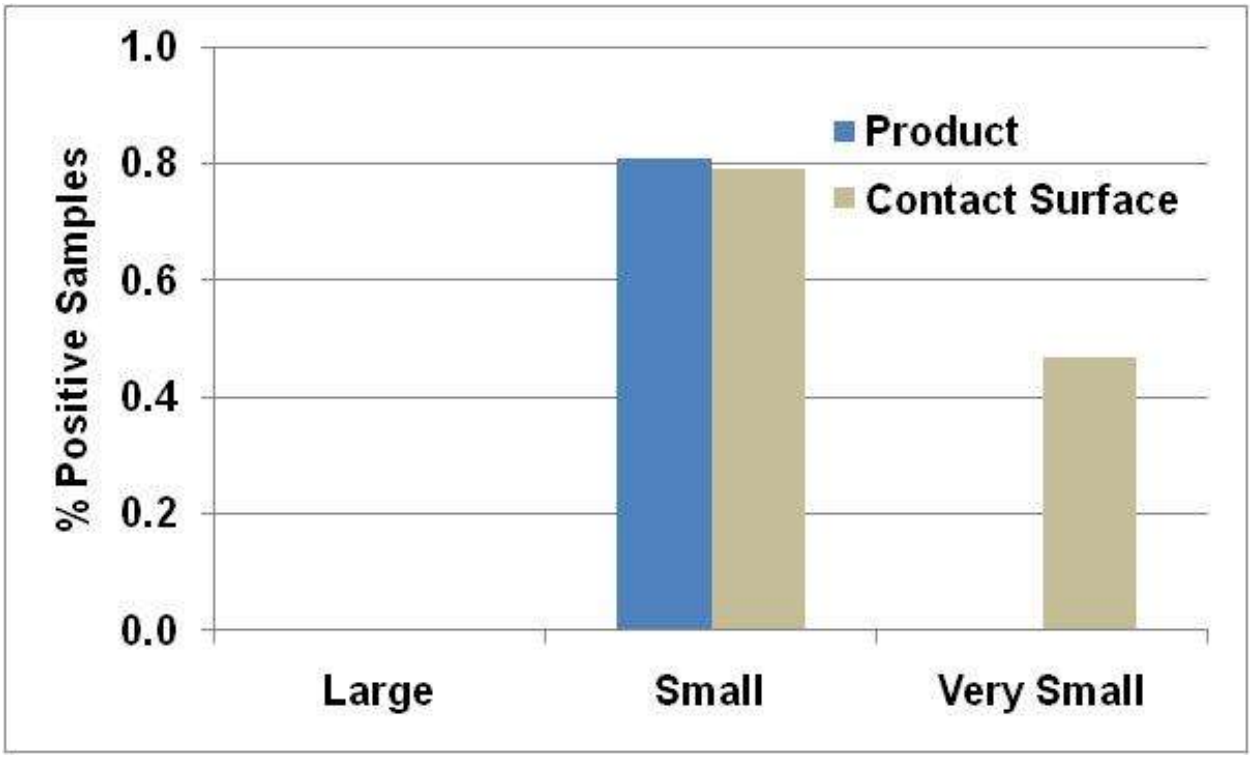


Figure 4.7.2. Percentage of *Lm*-Positive Environmental Samples by Establishment HACCP Size, Calendar Year 2008

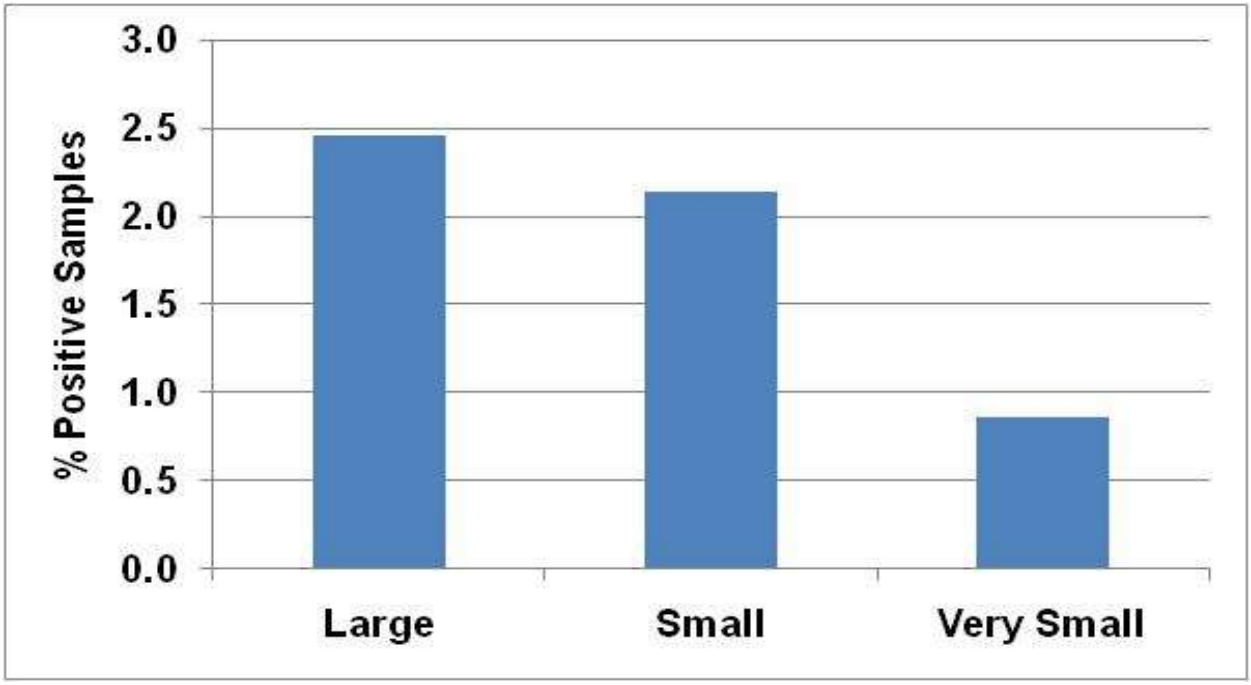


Figure 4.8.1. Percentage of *Lm*-Positive Samples as a Function of Establishment Annual Production Volumes, Calendar Year 2008

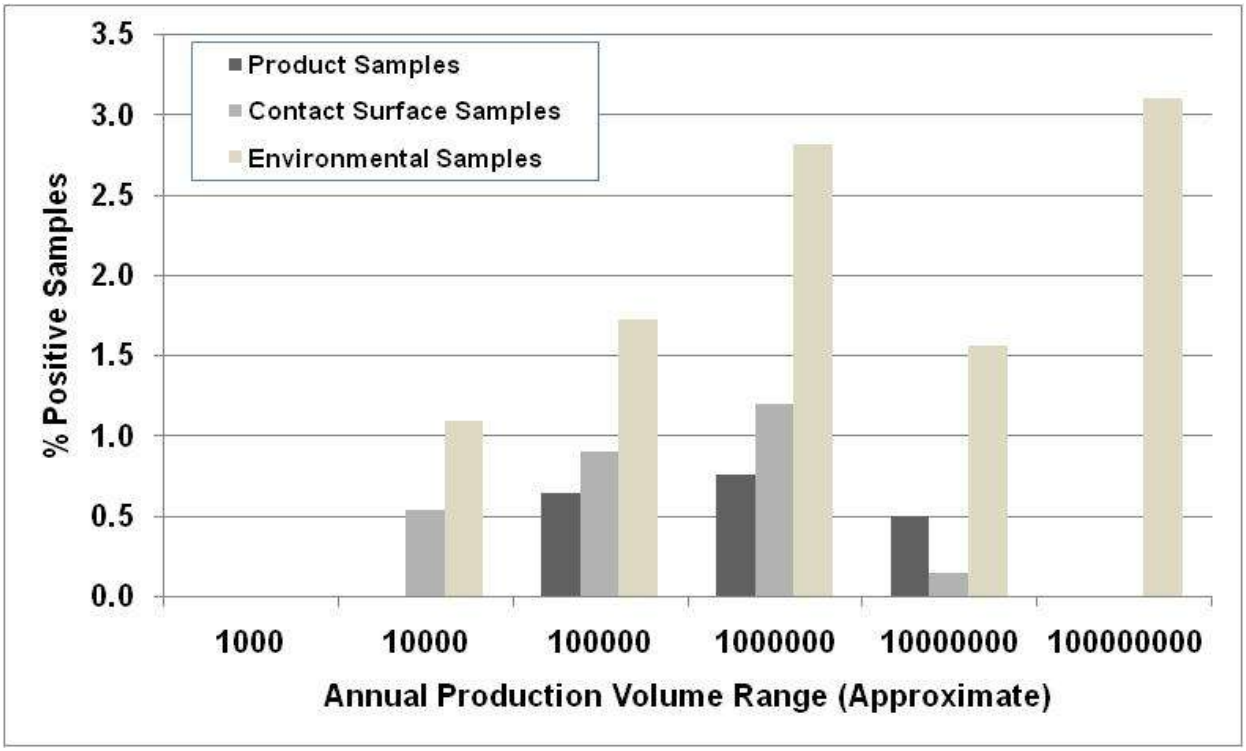


Figure 4.9.1. Percentage of *Lm*-Positive Samples by Establishment Food Production Category, Calendar Year 2008

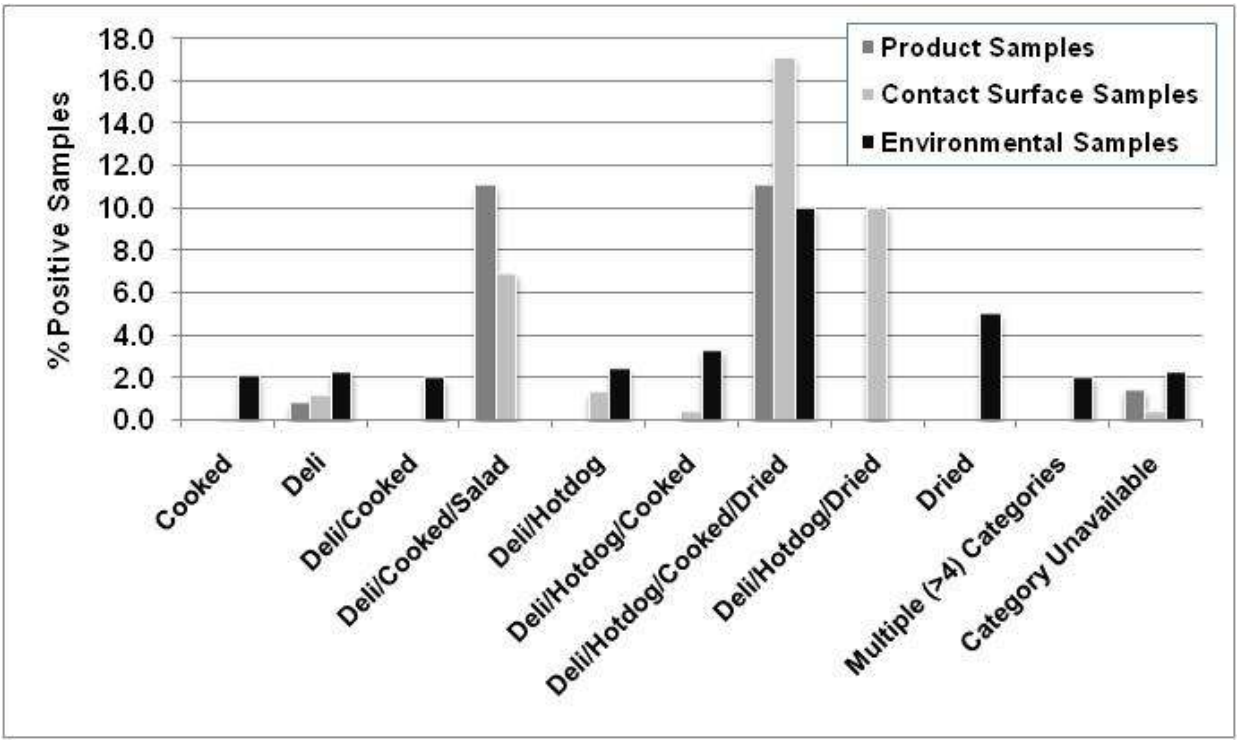


Table 4.10.1. Detection of *L. monocytogenes* by Geographic Region, Calendar Year 2007

Region	Product Samples	Positive Samples		Contact Samples	Positive Samples		Environmental Samples	Positive Samples		Total Samples	Positive Samples	
		No.	%		No.	%		No.	%		No.	%
North Central	229	1	0.4	778	6	0.8	392	10	2.6	1399	17	1.2
Northeast	153	0	0.0	534	5	0.9	295	13	4.4	982	18	1.8
Southeast	156	0	0.0	598	5	0.8	311	5	1.6	1065	10	0.9
Southwest	247	0	0.0	829	11	1.3	411	0	0.0	1487	11	0.7
West	174	4	2.3	583	8	1.4	316	7	2.2	1073	19	1.8
Total	**959**	**5**	**0.5**	**3322**	**35**	**1.1**	**1725**	**35**	**2.0**	**6006**	**75**	**1.2**

Figure 4.10.1. Percentage of *Lm*-Positive Product, Contact Surface and Environmental Samples by Geographic Region, Calendar Year 2008

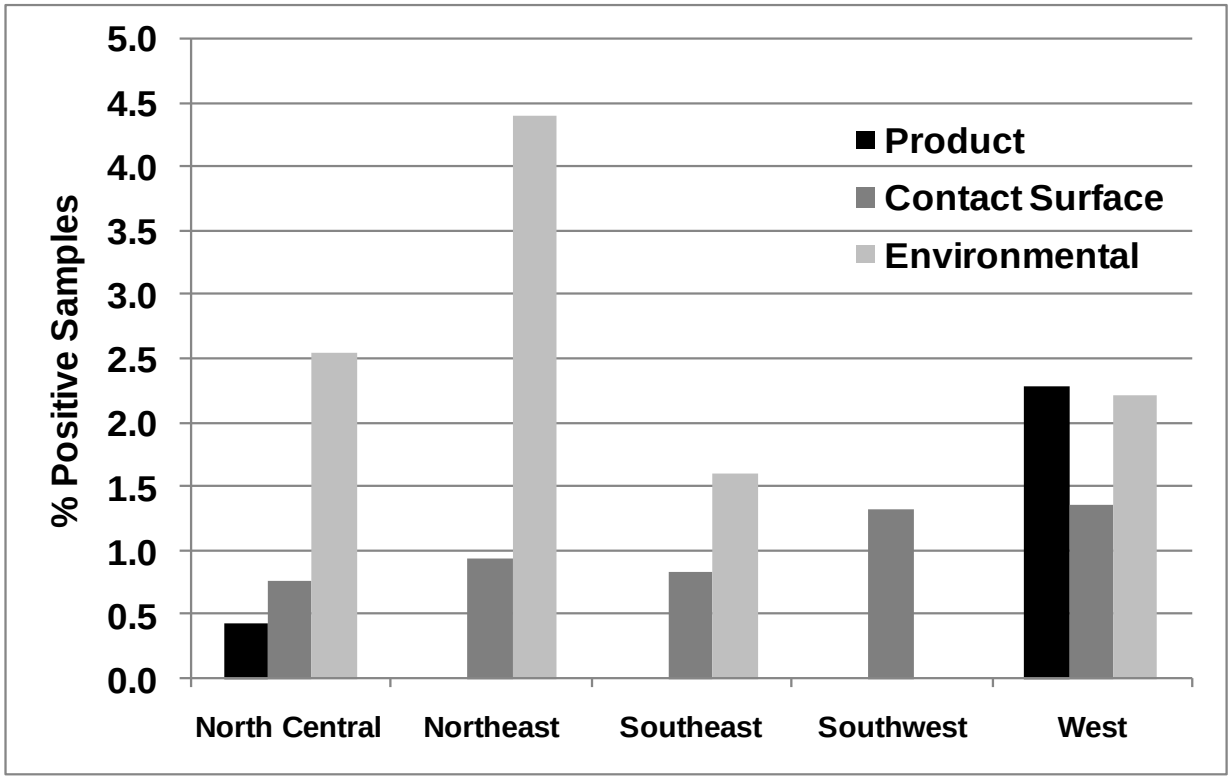


Figure 4.11.1. Percentage of Lm-Positive Product, Contact Surface and Environmental Samples by Season, Calendar Year 2008

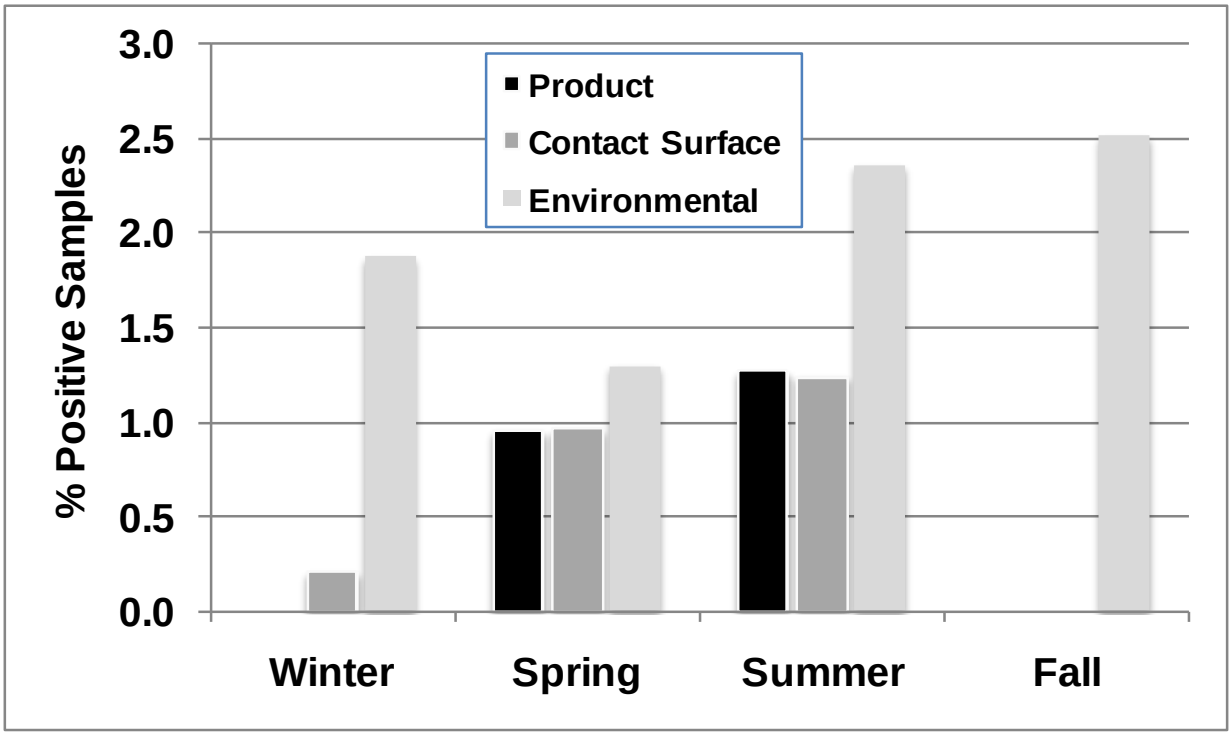
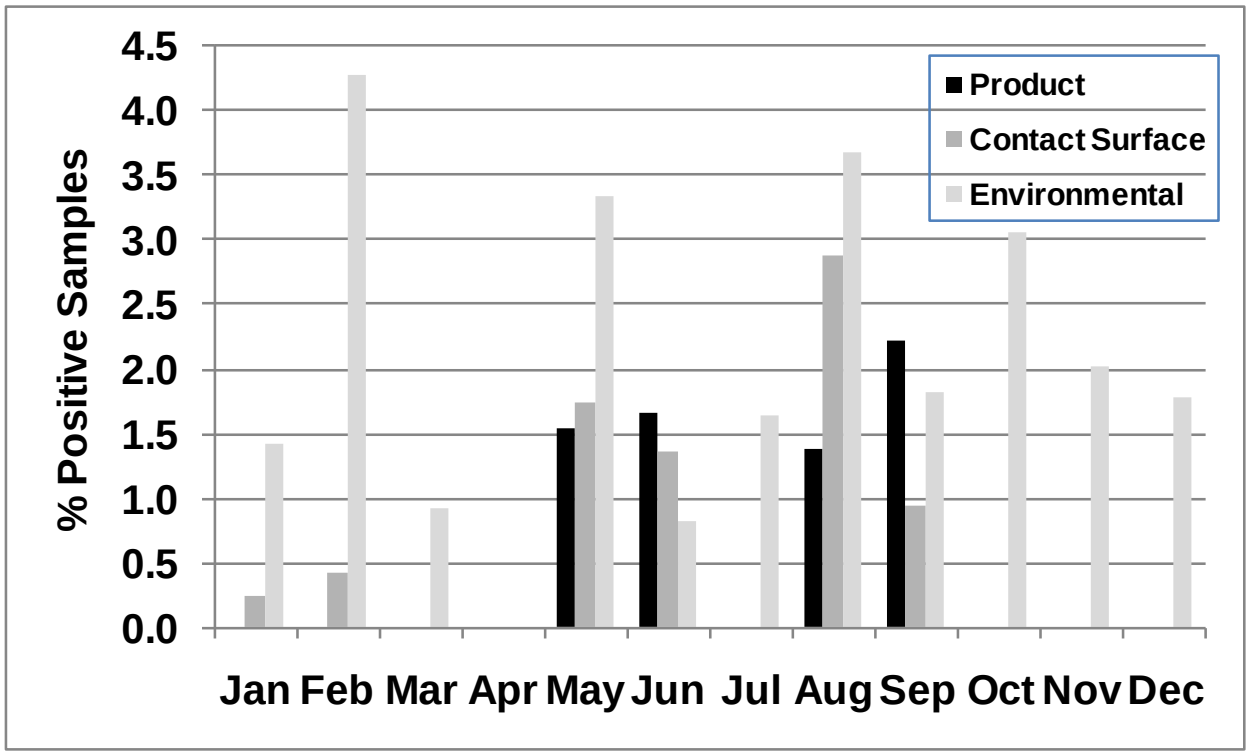


Figure 4.11.2. Percentage of *Lm*-Positive Samples by Month, Calendar Year 2008



4.12 Trend Analysis of Combined RLm Data: April 2006 through December 2008

Because the 2006 data begin in April, numbers of samples and *Lm*-positive results for 2006, 2007, and 2008 cannot be compared directly on a year-to-year basis. However, all 3 years' worth of data can be compared based on percentages of *Lm*-positive samples in each category analyzed. Accordingly, Figures 4.12.1 through 4.12.3 show comparative results for the rates of *Lm*-positive total, product, contact surface, and environmental samples for each year. Based on these results, the *Lm*-positive rates did the following:

- increased for product samples (0.2% and 0.5% in 2007 and 2008, respectively, versus 0.0% in 2006),
- increased for contact surface samples (0.4% and 0.6% in 2007 and 2008, respectively, versus 0.2% in 2006), and
- were virtually unchanged for environmental samples (1.8-2% over the 3-year sampling period).

Logistic regression analysis was used to evaluate the upward trends in the percentages of positive results observed for the product and contact surface samples (SAS PROC LOGISTIC procedures). This revealed statistically significant increases for product and contact surface samples with time ($P = 0.08$ and $P = 0.05$, respectively). If the product and contact surface data are pooled, the increase over time yields a P value of 0.01. The model assumes that samples were collected with equal probability. In actuality, the sampling was such that this approximation might not be valid. Nevertheless, the increased incidence for the food contact surface areas and product over the last 3 years points to the existence of potential problems that are not being addressed. No statistically significant changes were observed for the environmental samples over time ($P = 0.6$).

Trends in the percentages of establishments with at least one positive sample in any of the three sample sites for 2006 through 2008 are shown in Figure 4.12.4. In 2006 and 2007, about one of every five establishments had at least one positive sample; while in 2008, the same was true for about one of every six establishments. Figures 4.12.4 and 4.12.6 show comparative results for the rates of establishments with at least one positive product, contact surface, and environmental sample. Based on these results, the percentage of establishments with at least one positive sample

- increased for product samples (1.6% and 2% in 2007 and 2008, respectively, versus 0% in 2006),
- was similar for contact surface samples (about 4-5% over the 3-year sampling period), and
- was similar for environmental samples (about 14-17% over the 3-year sampling period).

There appeared to be a downward trend in the percentage of establishments with *Lm*-positive samples. However, logistic regression analysis showed that none of the establishment trends were statistically significant ($P>0.15$ for environmental, $P>0.3$ for product, and $P>0.5$ for contact samples).

If one examines the data for both samples and establishments, there is an interesting dichotomy: While in general the percentages of positive samples increased from 2006 through 2008, the percentages of establishments with positive samples trended down slightly during that same period. This means that in 2008, there were more positive samples within a smaller percentage of establishments than there were in either 2006 or 2007. However, it must be reiterated that such changes over time may not be comparable because of differences in establishment prioritization for the RLm sampling program.

Figure 4.12.1. Percentage of Total Samples Positive for *L. monocytogenes*, April-December 2006 and Calendar Years 2007 and 2008

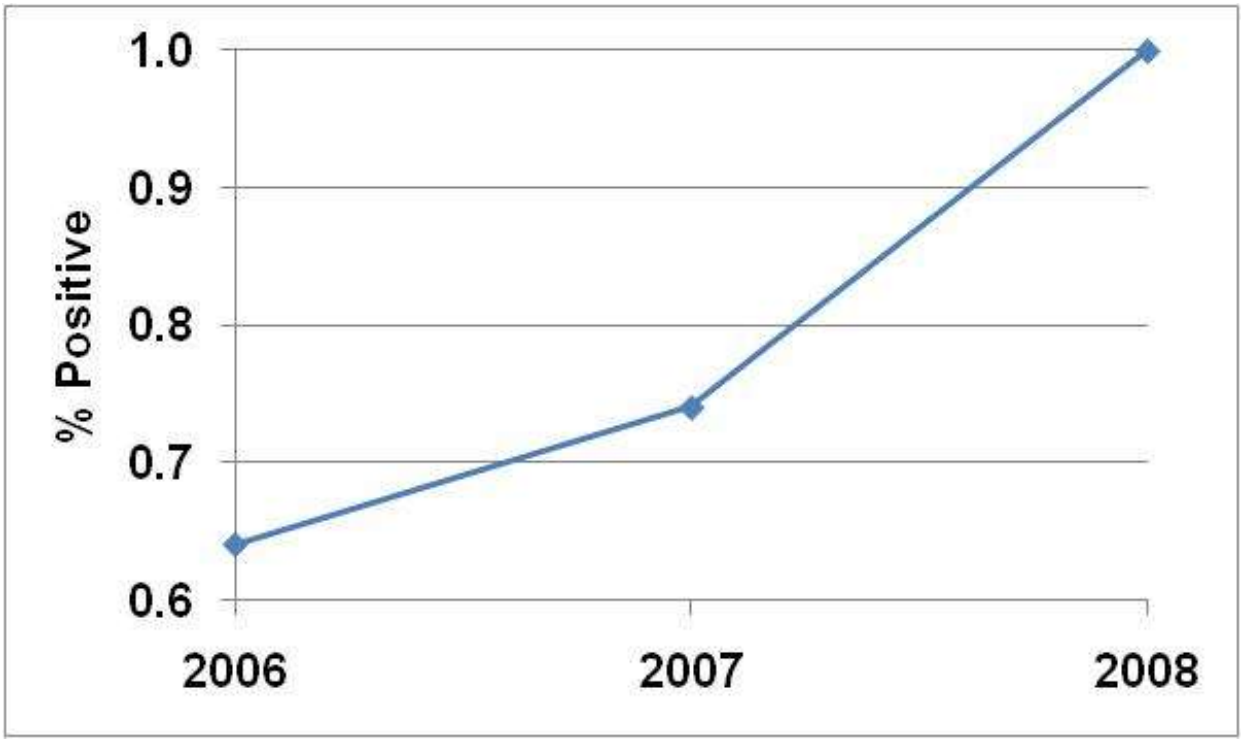


Figure 4.12.2. Percentage of Product and Contact Surface Samples Positive for *L. monocytogenes*, April-December 2006 and Calendar Years 2007 and 2008

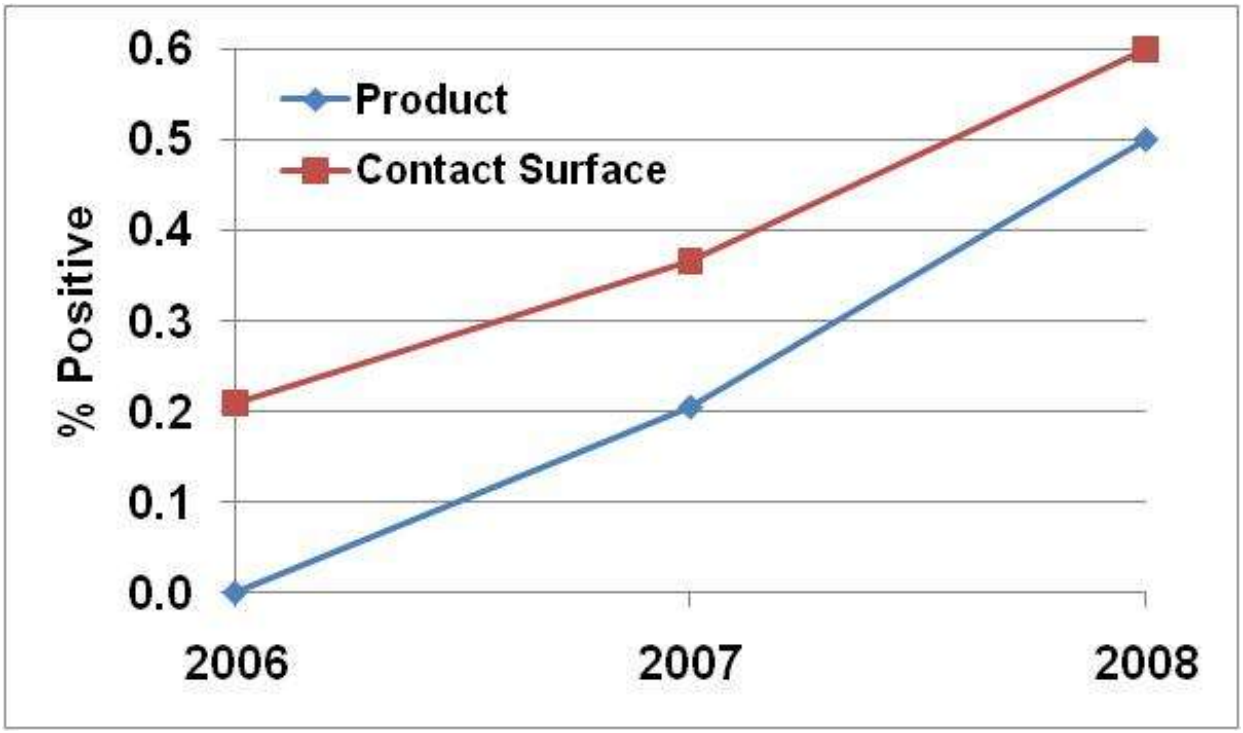


Figure 4.12.3. Percentage of Environmental Samples Positive for *L. monocytogenes*, April-December 2006 and Calendar Years 2007 and 2008

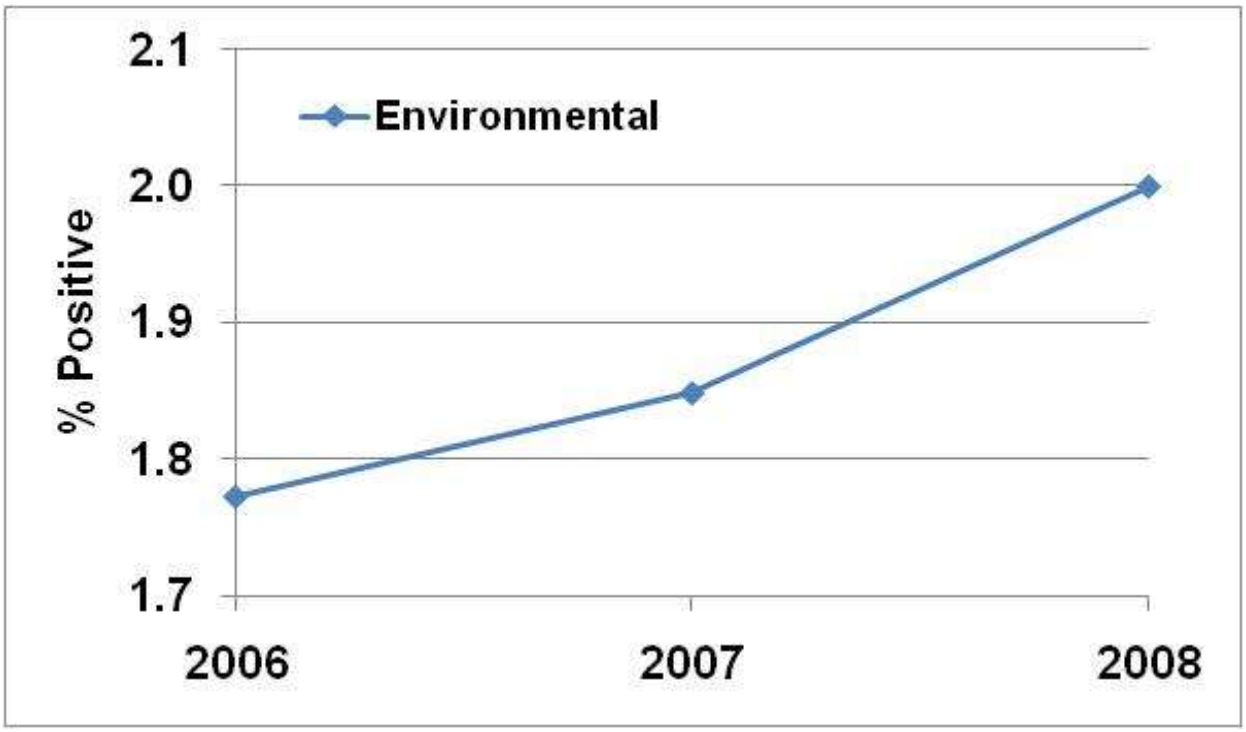


Figure 4.12.4. Percentage of Establishments with at Least One *Lm*-Positive Sample, April-December 2006 and Calendar Years 2007 and 2008

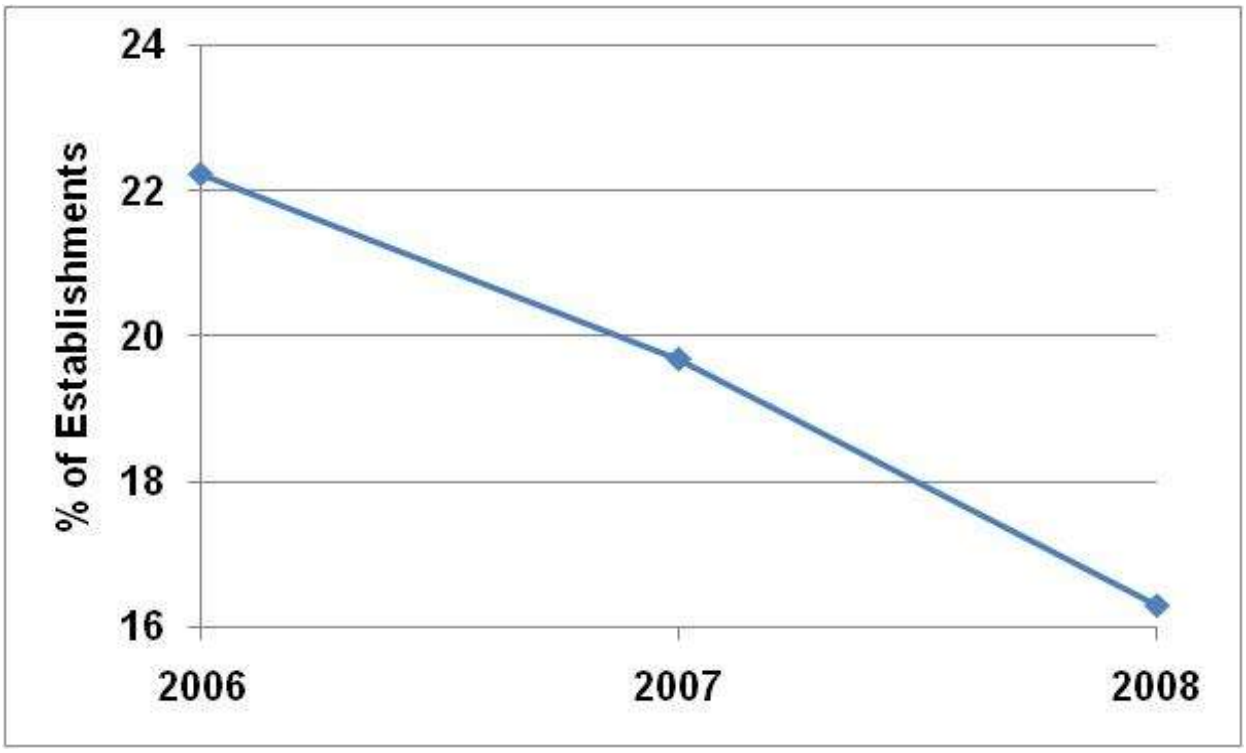


Figure 4.12.5. Percentage of Establishments with *Lm*-Positive Product and Contact Surface Samples, April-December 2006 and Calendar Years 2007 and 2008

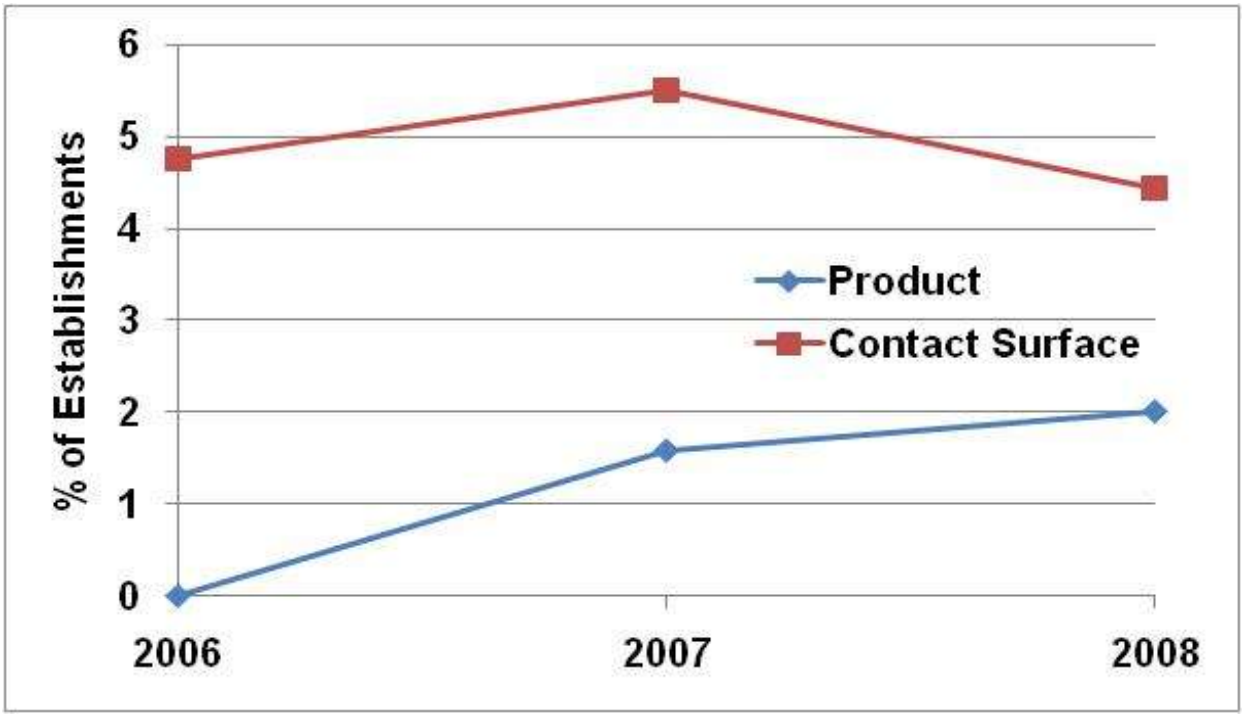
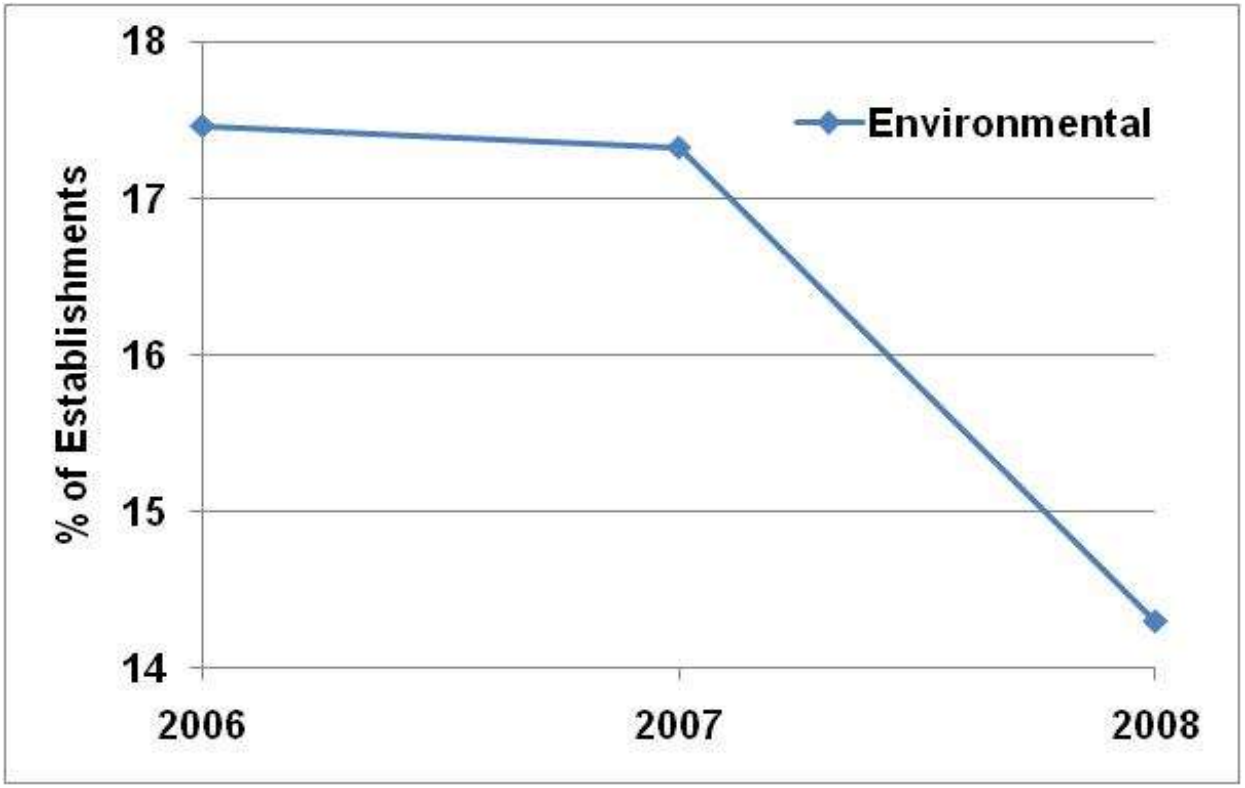


Figure 4.12.6. Percentage of Establishments with *Lm*-Positive Environmental Samples, April-December 2006 and Calendar Years 2007 and 2008



5. SUMMARY AND CONCLUSIONS

FSIS analyzed data with respect to the detection of *L. monocytogenes* in product, contact surface, and environmental samples collected under the RLm sampling program for calendar year 2008. Overall, 6,006 samples from 204 establishments were tested in 2008. Percentages of *Lm*-positive product, contact surface, and environmental samples were 0.5%, 0.6%, and 2%, respectively.

Results based on percentages of establishments with *Lm*-positive results. Four establishments, or about 2% of the 204 establishments in which samples were collected, had positive product samples, while about 1 in 25 (4.4%) had positive contact surface samples. About one in seven establishments (14.1%) had environmental samples that were positive for *L. monocytogenes*. A key aspect of the RLm program is in the identification of establishments that have *L. monocytogenes* somewhere in the environment and, thus, have a potential for product contamination. The data indicate that collecting multiple contact surface and environmental samples during a particular production shift increases the likelihood of finding *L monocytogenes* in an establishment. Thus, the RLm program serves as a proactive sampling project in identifying establishments in which the risk of product contamination may be elevated before contamination is actually identified in the actual products. The use of 1 to 3 sampling units per establishment (to collect 3 product, 10 contact, and 5 environmental samples per unit, respectively) contributes to the detection of positive contact surface and environmental samples.

Results based on type of *Lm*-positive sample. The five positive product samples were composed of three poultry products (two chicken franks, one chicken teriyaki), one beef product (brisket), and one type of shredded or sliced pork product. Contact surface samples that were positive for *L. monocytogenes* included containers, blades, tables, and trays, which accounted for 12 of the 19 positive samples of this type. Of the 35 *Lm*-positive environmental samples, drains, wheels, floors, floor mats and squeegees were isolated on more than one occasion. Of particular interest is that although only 3 floor mat samples were positive, this was out of a total of 15 floor mat samples, or 20%. As similar results were obtained in 2006 and 2007, floor mats may prove to be an important source of *Lm* contamination. In contrast, while there were 11 positive drain samples, this was from 240 collected drain samples, or less than 5% of all drain samples. It should be noted that the detection of *L. monocytogenes* in the environment is not, in and of itself, an indicator that a control problem exists. However, under FSIS Directive 10,240.5, *Lm*-positive environmental samples may be considered evidence that an establishment's products are produced under unsanitary conditions.

Results based on PFGE pattern. The collection and testing of multiple product, contact surface, and environmental samples from a given establishment has the potential to demonstrate how strains of *L. monocytogenes* could move from the environment to product contact surfaces and eventually contaminate a given product. Determining the PFGE pattern for each positive isolate aids in analyzing the likelihood of such occurrences. Finding the same PFGE pattern types (or subtypes) in isolates from different locations would provide evidence of cross-contamination. An analysis of the PFGE subtyping data for 2008 revealed multiple instances of cross-contamination between isolates from *Lm*-positive food product, contact surface, and/or environmental samples. Thus, transfer of *L. monocytogenes* between the environment and contact surfaces and/or products was a more common finding in 2008 than in 2006 or 2007. Four instances involved cross-contamination between contact surfaces and products, one instance was cross-contamination between environmental sources and contact surfaces, and there was one instance of multiple environmental samples having the same PFGE pattern. The RLm PFGE data for recovered isolates, if done in conjunction with an analysis of IVT "for cause" PFGE data, could permit the evaluation of harborage (presence of specific PFGE pattern types over time within a given establishment) in addition to cross-contamination. IVT results from establishments with positive RLm samples have, in fact, yielded instances of matching PFGE patterns for isolates from multiple sources (product, contact surface, and/or environmental) within a given establishment. This information is a factor in the

development of regulations that would mandate IVT in response to *Lm*-positive product and/or contact surface samples.

Results based on *Lm* control alternative. Results based on *Lm* control alternatives employed by the establishments showed that most of the positive samples were obtained from *Lm* control Alternative 2b and 3. These data appear to reinforce the concept that Alternative 3 (sanitation only/highest risk) and Alternative 2b (antimicrobial treatment/higher-risk) establishments are more at risk than Alternative 1 (and possibly even category 2a) establishments with respect to detecting *Lm*-positive samples. This observation was more applicable to positive product and contact samples than to positive environmental samples, which were found in establishments that were classified as using mixed Alternatives 1 and 2 and, in particular, mixed Alternatives 1 and 3. The analysis of data related to *Lm* control alternatives has benefited from improvements in information entered on FSIS 10,240-1 forms.

Results based on size, product category, production volumes, District, geographic region, month, and season. Results based on establishment HACCP sizes indicated that in 2008, most positive product and contact surface samples came from small or very small establishments. However, environmental samples were in establishments in all HACCP categories. Results based on food product categories showed that most of the *Lm*-positive samples were from establishments that produced deli meats, hot dogs, and cooked products. Results based on establishment production volumes (which, as with control alternative data, were from FSIS 10,240-1 forms) indicated that most positive product and contact surface samples were from establishments producing between 10,000 and 10,000,000 pounds of product per year. Presentation of results by FSIS District and geographic region appears to be mainly descriptive in nature, but may be more meaningful on a comparative basis. Analysis of results by month and season indicated that *Lm*-positive environmental samples were obtained at all times of the year (as was the case in 2006 and 2007). In contrast, positive product and contact surface samples were obtained at specific times of the year, mainly between the months of May and September.

Results based on year-to-year trends. Trends in the percentages of *Lm*-positive results from 2006 to 2008 permitted an expanded view of trends. In particular, the percentages of *Lm*-positive product and contact surface samples trended higher between 2006 and 2008, and these increases were statistically significant. Trends for environmental samples were essentially flat. The percentages of establishments with at least one *Lm*-positive sample trended down slightly between 2006 and 2008, though there were upward trends for establishments with positive product and contact surface samples. However, the net effect for the RLm program between 2006 and 2008 was that while the percentage of *Lm*-positive establishments appeared to be decreasing over time, the percentages of positive samples within those establishments were increasing. Still, these results should be interpreted with caution because even 3 years' worth of RLm data may not be sufficient for an objective evaluation of the effectiveness of the RLm sampling program. Changes observed between 2006 and 2008 may reflect true trends or may reflect normal variations in sampling results. Furthermore, the data represent results from only about 400 of over 2,000 establishments that are subject to regulation 9 CFR 430. Because of the nature of scheduling of establishments within the RLm sampling program (i.e., which establishments are sampled when), these year-to-year results are not truly representative of all establishments sampled in the RLm program. It is expected that additional data over multiple years will provide for an expanded evaluation of the data trends. In fact, effective August 2009, FSIS is committed to performing RLms (in conjunction with routine FSAs) in each of the 2000-plus 9 CFR 430 establishments over a 4-year period. This represents a significant expansion of the RLm sampling program.

Next steps. The following is a list of actions that are being implemented subsequent to the completion of this report and its presentation to, and acceptance by, the appropriate FSIS offices and Management Council:

- An ongoing examination of the data from this report with respect to applying them to preventing foodborne outbreaks of *L. monocytogenes*. (This includes modifications of existing compliance guidelines and other regulatory practices that help protect public health.)
- Initiation of RLm sampling in all (approximately 2100) 9 CFR 430 establishments over a four-year period, which commenced in August 2009.
- Reporting of RLm test results on a quarterly, in addition to an annual, basis.
- IVT for those establishments in which positive RLMPROD and/or RLMCONT samples are obtained.
- Publication of these data analysis in a peer-reviewed scientific journal.
- Re-initiation of hands-on training for RLm/IVT sample collection.
- Analysis of 2009 RLm results.

Appendix A: FSIS Scheduling Criteria for Routine *Lm* Risk-based (RLm) Sampling Program

Before the month when samples are to be collected, FSIS uses a statistical algorithm to generate a risk ranking of establishments producing post-lethality exposed RTE meat and poultry products. The following criteria are then used to identify establishments from the risk ranking to be tested for *Listeria monocytogenes* in food product, contact surface, and environmental samples under the Routine *Lm* Risk-based (RLm) Sampling Program:

1. Once RLm sampling has been conducted in an establishment, that establishment will not be eligible for scheduling again for a 24-month period.
2. If there is a current-month positive result from any FSIS *Lm* sampling project, the Agency conducts FSAs and IVT at the establishment:
    a. If positive results are found during the IVT, the RLm will not be scheduled until 6 months after the IVT and FSA and any accompanying regulatory actions are complete.
    b. If the IVT test results were negative, RLm sampling would revert back to the 24-month sampling cycle.
3. RLm sampling at an establishment will also not be scheduled for 6 months after closeout of an *Lm*-related NOIE, suspension, or other enforcement action.
4. Previously, FSIS did not schedule RLm testing in more than one establishment operated by the same corporation in the same month. This restriction will not apply in FY 2008.
5. Collecting RLm samples will no longer take precedence over the other RTE sampling programs (i.e., ALLRTE and RTE001). If FSIS *Lm* sampling projects are scheduled at the same establishment over the same time period, all samples will be collected as scheduled.