EXECUTIVE SUMMARY

Listeria monocytogenes

In the Processing of Ready-to-Eat Beef Products
The foodborne pathogen, *Listeria monocytogenes* (*Lm*), is recorded as the causative agent in approximately 2,500 serious illnesses and 500 deaths in the U.S. every year. While the incidence of serious *Lm* infections is not high, the mortality rates are estimated at 20%. The infections generally occur only in the immunocompromised, including the elderly and very young children. Infections during pregnancy can result in miscarriage and stillbirth.

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

The annual incidence of listeriosis decreased by 44% between 1989 and 1993. Analysis of the incidence trend from 1996 to 2002 revealed a 38% decline. In 2002, an outbreak that resulted in 54 illnesses, 8 deaths, and 3 fetal deaths in 9 states was traced to consumption of contaminated turkey meat. No outbreaks occurred in 2003 or 2004.

*Lm* can grow in most ready-to-eat meat products at refrigeration temperatures. Contamination of cooked meat products most frequently occurs when a product or food contact surface is contaminated between the cooking step and packaging (e.g. during slicing or peeling operations).
USDA, Food Safety Inspection Service, (FSIS) Microbiological Testing Program yielded the following results for calendar year 2002:

<table>
<thead>
<tr>
<th>Product Category</th>
<th>% Prevalence for L. monocytogenes</th>
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</thead>
<tbody>
<tr>
<td>Sliced, diced and shredded</td>
<td>1.96%</td>
</tr>
<tr>
<td>Small mass chopped &amp; formed</td>
<td>1.05%</td>
</tr>
<tr>
<td>Large mass chopped &amp; formed</td>
<td>0.93%</td>
</tr>
<tr>
<td>Large mass whole muscle</td>
<td>0.49%</td>
</tr>
<tr>
<td>Salads and spreads</td>
<td>0.42%</td>
</tr>
</tbody>
</table>

In January 2001, USDA, FSIS issued the Draft Assessment of the Relative Risk to Public Health from Foodborne Listeria monocytogenes among Selected Categories of Ready-to-Eat Foods. FDA’s Center for Food Safety and Applied Nutrition (CFSAN) and USDA/FSIS wrote the assessment, in consultation with the Centers for Disease Control and Prevention. The draft risk assessment and the release of “Risk Management Action Plan” to address Lm were in response to the May 5, 2000, presidential directive to reduce Lm related disease by 50% by the year 2005.

USDA-FSIS has published an interim final rule to set forth requirements for food processors to control Listeria on food contact surfaces and eliminate Lm in RTE meat and poultry products. Consistent with the rule, efforts to control Listeria have already been made by food processors.
OBJECTIVES

The overall objectives in Phase I of the study were to:

• Identify critical entry points for *Lm* in RTE facilities.

• Re-establish *Lm* as an environmental contaminant by zones of the facility.

• Determine *Listeria* spp. presence in different zones of the facility.

• Evaluate and compare the ribotypes of the confirmed samples and identify source to enable control of the environment based on the results.

Findings led to the objectives of Phase II, which were:

• Based on the critical entry points for *Lm*, establish and implement plant specific corrective actions to improve the control of *Lm* in ready-to-eat products.

• Identify the incidence rates and ribotypes of *Lm* in the raw materials utilized in ready-to-eat products.

MATERIALS AND METHODS

Sample Collection (Phase I)

Environmental samples were collected from five commercial ready-to-eat (RTE) facilities located in diverse geographical regions. The test facilities were selected based on the variety of products produced to maximize product types (hot dogs, roast beef, sliced luncheon meats, pepperoni, tacos, taco meat, pastrami and pizza toppings).
Samples were collected on three separate sample dates from each facility at approximately thirty-day intervals between each sampling time. Each facility was divided into four zones based on the contamination risk to the RTE product. A total of 134 sample sites were selected prior to each facility visit. The selected sites were sampled at pre-operational, operational and end-of-shift for a total of 402 samples collected per plant visit (total of 6030).

The zones are identified at left:

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

The corresponding percentage of samples collected from each zone was:

- Zone 1 – 40%
- Zone 2 – 30%
- Zone 3 – 20%
- Zone 4 – 10%

Valuable information was gained in Phase I and positive changes were made between visits. Based on results and lessons learned, three of the five plants were selected for Phase II based on predominant areas of opportunity: (1) Sanitation, (2) Segregation of Raw and Cooked areas and (3) Traffic Patterns.

After the first sampling visit, the data were presented and corrective action plans were discussed with key management. Each establishment was given approximately forty-five days to implement the corrective actions prior to the second and third sampling visit, which consisted of collecting samples on two consecutive

**Sample Collection (Phase II)**
production dates. Additionally, on each of the sampling dates, samples were collected at each of three sampling times: (1) Pre-operational; (2) Operational; and (3) End-of-shift.

A total of 195 sample sites were selected prior to each facility visit. The selected sites were sampled at each of the three sample times for a total of 585 samples collected per sampling day. The zone identification and frequency of sampling in each zone was the same as in Phase I.

**Ribotyping**

When discussing the background or origin of microbes, the terms “serotypes” and “DNA fingerprinting” may be used. Serotyping and Ribotyping differ in the following manner. Serotyping is based on antigen-antibody reactions to cell wall and flagellar proteins. Accordingly, it would show a phenotypic likeness between microbes. Ribotyping is a raw form of DNA typing so it represents more of the genotypic material responsible for the different serotypes. It gives a more specific identification of microbes because you can have several ribotypes under the same serotype.

Six hundred *Lm* isolates were selected for ribotype identification in Phase I. *Lm* isolates were ribotyped from additional environmental and raw material samples in Phase II. The percentage of isolates selected by zone mirrored the sample site selection criteria as previously described.

Comparison and classification of the riboprint patterns were carried out using pattern-matching software included in the Riboprinter system. In order to obtain an identification, the similarity between the test isolate and the database isolate must be >0.85.
**SUMMARY OF RESULTS AND DISCUSSION**

*L. monocytogenes* was present in 13% and *Listeria* spp. in 27% of the 6030 environmental samples analyzed in Phase I. Overall, there was a reduction in incidence of *Lm* on visits 2 and 3, from 18% on visit 1 to 10% and 12% on visit 2 and 3, respectively (Fig 1). In general, regardless of the level of *Lspp* during Pre-operational sampling, the incidence of *Lm* and *Lspp* increased approximately 1.5 times in the subsequent sampling times (Op and End) (Fig 2).

As expected, the highest incidence levels during all three sampling times were from Zone 3 (Fig 3). Overall, pre-operational, Zone 1 samples had the lowest incidence rate for *Lm* and *Lspp*. However, over the sampling time, Zone 1, 2 and 4 appear to equilibrate to similar levels. Typically, facilities concentrate sampling only in Zone 1 (RTE areas). The data suggest that the pathogen is being tracked into Zone 1 from the surrounding areas of the facility (Fig 4). Accordingly, it is evident that facilities need to further concentrate sampling efforts and control *Listeria* in the adjoining areas of the facility in order to improve control in the RTE zones.

In general, based on the data, the industry needs to continue to focus on floors, drains and traffic patterns in order to maintain control of *Listeria* and reduce the potential for cross contamination.

The overall impact of this study on the three common test plants from Phase I to Phase II was an incidence reduction from 18% to 9% for *Lm* and a 27% to 17% for *Lspp*. (Fig 5). The percent reductions in each plant for *Lm* were 22%, 45%, and 74% for
facility A, B, and C, respectively (Fig 6). It is evident that corrective actions implemented as a result of this study positively impacted the incidence of *Listeria*.

Taken as a whole, all facilities improved between the sampling visits. Additionally, as expected the highest incidence levels are from Zone 3, which are the raw processing areas and coolers for raw material storage. In evaluating all plants combined, Zone 1 and Zone 2 are very similar in incidence rates for *Lm* and *Lspp*, which underscores the fact that the facilities need to continue to improve the control of contamination entering Zone 1 from the other zones (Fig 7).

As expected from Phase I, there is an increase in incidence of *Lm* and *Lspp* from the Pre-operational samples to the Operational and End of shift. The data suggest that the level of *Listeria* hits a plateau after the start of operations and remains constant throughout the remainder of the production day (Fig 8).

In addition to environmental samples, the boots of RTE operations and management personnel throughout the production shift were sampled to evaluate and compare the incidence of *Listeria*. The RTE employees had 5%, 5% and 35% for pre-operational, operational and end sampling, respectively. The management staff had incidence levels of 30%, 40% and 50% for the same time periods. Typically, management personnel are more mobile and are exposed to a multitude of environments (raw and RTE) during the day versus an employee that is assigned to one area. The pre-operational levels underscore the importance of an effective boot cleaning policy for all employees.
From the 321 raw meat samples collected, 25% and 41% were positive for *Lm* and *L. spp.*, respectively (Fig 9).

**Environmental**

Ribotyping is an automated DNA fingerprinting system that allows comparison of multiple isolates at the sub-species level for differentiation amongst the samples. For the purpose of this study, ribotype identification was determined to enhance the overall knowledge of type of *Lm* in the processing environment and raw materials.

There were 17 different ribotypes identified from the 600 selected isolates (Fig 10). *Lm* isolates ribotyped in Phase II showed a similar distribution of riboprints in Phase I. The predominant ribotypes at pre-operational sampling remained predominant and increased throughout the production day. In the compilation of all plants, Zone 1, 2 and 3 had similar numbers of ribotypes identified. Zone 4 had the lowest number of different ribotypes identified at each individual facility. The dominant ribotype for each facility was noted in all four zones.

The study also indicated that the ribotypes that persisted in the processing facility were significantly more prevalent among industrial isolates than among isolates associated with human illness.

**Raw Material**

Thirteen different ribotypes were identified from the 80 raw meat isolates evaluated, with DUP-1039 and DUP-1052 comprising 45% and 21% of the isolates (Fig 11). There were four ribotypes found in the environment that were not identified in any raw meat sample.
It is imperative for the industry to react and take appropriate corrective actions on all *Listeria* spp. positive findings. The first step in *L. monocytogenes* control is to get a true assessment of the environment by performing mapping studies. This allows the investigators to develop a plan for improvement.

Information gained from these studies provided and emphasized guidelines that can and should be followed by any processing facility to reduce the risk of product contamination from *Listeria monocytogenes*.

**ACTION POINTS TO REDUCE RISK OF *Listeria* CONTAMINATION.**

- **Aggressive drain program for the entire facility – not just Z1 & Z2.**
- **Captive boot policy – adequate cleaning of footwear.**
- **Properly functioning, effective barrier systems between critical areas of the facility are a must (i.e. door foamers, boot scrubbers, hand dips, etc.) with regular monitoring of sanitizer strength.**
- **Control and cleaning systems (to include hand scrubbing), need to be in place for any item that moves from area to area – including people, ladders, forklifts, palletjacks, carts, dollies, etc.**
- **Facility design and maintenance to facilitate proper cleaning is essential (i.e. spiral freezer, concrete curbing and peripheral areas around spirals, slicing equipment, equipment control panels, etc.).**
- **Use ribotyping as a tool to identify *Listeria* entry points and track movements throughout a processing facility.**

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