Control of *Listeria monocytogenes* along the food chain: from outbreak detection to interventions

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Take home messages

- *Listeria monocytogenes* and human listeriosis will likely continue to increase as an issue
  - Better outbreak detection through molecular subtyping
  - Growth of susceptible population
  - High risk foods will become more commons
- Listeriosis is a rare (but severe) human disease, but *Listeria monocytogenes* is common (“ubiquitous”).
  - High infectious dose
  - Presence of *L. monocytogenes* in environments has to be expected
- Key interventions and controls include
  - Prevent post kill step re-contamination of Ready-To-Eat foods
  - Prevent *L. monocytogenes* growth in Ready-To-Eat foods
  - Reduce exposure of susceptible subgroups
Outline

• *Listeria monocytogenes* – Introduction and overview
• Outbreak detection: trends and use of molecular methods
• Importance of environmental *L. monocytogenes* sources
• Environmental sampling programs and associated preventive strategies
• Other control strategies
Listeria monocytogenes – Intro

• Typically about 3 to 5 cases/million population
• Approx. 1,300 human cases/year and 255 deaths/year in the US
  – Human listeriosis can occur as epidemic and sporadic cases
• Causes septicemia, abortion and encephalitis in humans and more than 40 animal species
• Potentially long incubation period (7-60 days)
• Affects predominantly elderly and immunocompromised people, pregnant women and newborns
  – High infectious dose: at 1 x 10^{10} cfu/serving, the dose-response model predicts a median death rate of 1 in 667 servings for pregnancy associated/neonatal listeriosis
• Grows at refrigeration temperatures and is fairly resistant to may stress conditions (salt)
  – Killed by standard pasteurization type heat treatments
• Common in certain/many environments
**L. monocytogenes prevalence**

- Natural environments: 1.3% to 8% (NYS data)
- Urban environments: 7.3% (NYS data)
- Ruminant farms
  - Bovine farms with listeriosis cases: 24.3% (n=616)
  - Bovine farms without listeriosis cases: 20.1% (n=643)
  - Small ruminant farms with listeriosis: 32.9% (n=322)
  - Small ruminant farms without listeriosis: 5.9% (n=475)
- Listeria species are often found at around 30% prevalence
Cellular Pathogenesis of Listeriosis
Listeria: more than L. monocytogenes

• The genus Listeria includes:
  – Human and animal pathogens: L. monocytogenes and L. ivanovii
  – Non-pathogenic species: L. innocua, L. seeligeri, L. welshimeri, and the divergent L. grayi
  – 11 new non-pathogenic species described since 2010 (L. marthii, L. rocourtiae, L. weihenstephanensis, L. fleischmannii, L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis, L. booriae, L. newyorkensis)
    • Many of these new non-pathogenic species have been isolated from produce related sources
• Listeria spp. sometimes used as “indictor” or “index” organisms that reveal conditions where L. monocytogenes could reside
Animal listeriosis

- Caused by *Listeria monocytogenes* and in rare cases *Listeria ivanovii*
- *L. monocytogenes* has been linked to clinical disease in more than 40 animal species
  - Cattle, goats, and sheep are most commonly affected by listeriosis
- Clinical symptoms include meningitis & encephalitis ("circling disease"), abortions, neonatal septicemia, as well as in rare occasions mastitis and ocular infections
- Source: often improperly fermented silage
- Essentially no evidence of direct zoonotic transmission of *L. monocytogenes* from animals to humans
  - Except for a few anecdotes of veterinarians having *L. monocytogenes* skin infections after helping with birth of infected calves
Role of animal infections in human listeriosis

• Most foods that are sources of human listeriosis cases are contaminated post-processing
  – Infected animals or animal products not a direct source of *L. monocytogenes*

• Raw milk as well as dairy products made from raw milk as potential source
  – *Raw milk cheeses***

• Fruits and vegetables
  – Manure as a source of contamination
Listeriosis outbreak linked to hispanic style cheese (L.A., 1985)

- Jalisco brand Mexican-style cheese was implicated as the vehicle of infection
- A total of 142 cases involving 93 pregnant women or their offspring and 49 nonpregnant immunocompromised adults were documented in Los Angeles County, CA.
  - 48 deaths were recorded (mortality rate of 33.8%)
  - An additional 160 cases occurred in other parts of California,
- 62% of afflicted individuals were pregnant Hispanic women
- The mean incubation period in pregnant women was 31 days (range of 11-70 days)
- Implicated cheese was most likely manufactured from a combination of raw as well as pasteurized milk, serotype 4b outbreak strain was recovered from unopened packages of cheese
Human listeriosis outbreak - Canadian Maritime provinces, 1981

- 41 listeriosis cases, including 17 deaths
- Food preference survey to assess risk factors
  - coleslaw identified as vehicle
  - *L. monocytogenes* serotype in patient’s blood detected in coleslaw from patient’s refrigerator
- Coleslaw produced using cabbage that appears to have been fertilized with raw manure form a sheep farm with a history of listeriosis
- Indirect link established between invasive human listeriosis and pre-harvest food production system
Foods linked to human listeriosis cases and outbreaks

• Foods that (i) support *L. monocytogenes* growth and (ii) are stored for prolonged time at refrigerated temperatures (hence allowing growth)
  – Trends to extended shelf life products may increase listeriosis risk
  – Refrigeration temperature plays a huge role: very slow growth at temperatures of 4 C and below significantly reduces risk
• “Classical” foods linked to listeriosis cases and outbreaks: soft cheeses with high pH; Ready-To-Eat deli meats
<table>
<thead>
<tr>
<th>Relative Risk Ranking</th>
<th>Predicted Median Cases of Listeriosis for 23 Food Categories</th>
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<th>Predicted Median Cases of Listeriosis for 23 Food Categories</th>
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<tr>
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<td>Per Serving Basis ^a</td>
<td>Cases</td>
<td>Per Annum Basis ^b</td>
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<td>2</td>
<td>Frankfurters, not reheated</td>
<td>6.5x10^8</td>
<td>Pasteurized Fluid Milk</td>
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<td>3</td>
<td>Pâté and Meat Spreads</td>
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<td>High Fat and Other Dairy Products</td>
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<tr>
<td>4</td>
<td>Unpasteurized Fluid Milk</td>
<td>7.1x10^9</td>
<td>Frankfurters, not reheated</td>
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<td>5</td>
<td>Smoked Seafood</td>
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<td>Cooked Ready-to-Eat Crustaceans</td>
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<td>9</td>
<td>Pasteurized Fluid Milk</td>
<td>1.0x10^9</td>
<td>Smoked Seafood</td>
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</table>

L. monocytogenes in produce

• 1981: Outbreak in Canada linked to coleslaw
• 2009: Outbreak linked to sprouts?
• 2010: Outbreak linked to diced celery in Texas (10 cases)
  – FDA investigation of facility included >200 environmental and 19 product samples; outbreak strains was detected in environmental and product samples
• 2011: Outbreak linked to cantaloupe: 146 illnesses, 30 deaths, and 1 miscarriage (28 states)
• 2014/15: Caramel apples: 35 cases, 7 deaths
• Recalls due to L. monocytogenes contamination
  – Bagged salads, spinach and lettuce; fresh shelled peas; alfalfa/soybean sprouts; peaches
Outline

• *Listeria monocytogenes* – Introduction and overview
• **Outbreak detection: trends and use of molecular methods**
  • Importance of environmental *L. monocytogenes* sources
  • Environmental sampling programs and associated preventive strategies
• Other control strategies
Strain differentiation (subtyping/fingerprinting)

• Tools which allow sensitive differentiation of bacterial subtypes
  – Detection of contamination sources
• Strain differentiation methods commonly applied include serotyping, ribotyping, Pulsed Field Gel Electrophoresis (PFGE)
• These methods are used to detect foodborne disease outbreak and identify pathogen sources throughout the food chain
Examples of different PFGE patterns
Case study – human listeriosis outbreak
Human listeriosis cases in NYS: 1/97-10/98
### Subtyping results – part I

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Subtyping results – part II

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Epidemic curve for 1/97 - 2/99 in NYS
### Similarity Search Results

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| FSL-M1-188 | DUP-1844 | 0.98 |
| FSL-C1-124 | DUP-1844 | 0.98 |
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| FSL-M1-272 | DUP-1844 | 0.98 |
| FSL-C1-144 | DUP-1844 | 0.98 |
| FSL-J1-213 | DUP-1844 | 0.98 |
| FSL-M1-268 | DUP-1844 | 0.98 |
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Conclusions

• 101 human cases and 21 deaths in 22 US states linked to infection by the same sub-type of *Listeria monocytogenes*

• Outbreak traced back to a single specific plant in Michigan
  – Plant had an appropriate HACCP plan
  – *L. monocytogenes* source was post-CCP contamination from plant environment
Possibilities for international traceback – a hypothetical example

Food isolate, deposited into PulseNet

Human case

Human case
Food Safety News

CDC/FDA Partnership Targets Whole Genome Sequencing of Listeria Monocytogenes

By Brian Sanders | November 27, 2013

In a prior APHLTech blog post (NGS in Action: FDA’s Genome TRAKR Network), Victor Waddell of the Arizona State Public Health Laboratory described the newly formed network of laboratories formed by the U.S. Food and Drug Administration (FDA). Known collectively as Genome TRAKR, the member laboratories perform whole genome sequencing (WGS) on bacterial foodborne pathogens isolated primarily from food and environmental sources.

On Sept. 1, 2013, the Centers for Disease Control and Prevention (CDC) began a partnership with the FDA Genome TRAKR network to utilize the network to conduct WGS of all Listeria monocytogenes collected from reported human illness cases in the United States. This effort leverages public health resources to evaluate and
Multistate Outbreak of Listeriosis Linked to Roos Foods Dairy Products

- Feb 2014
- A total of eight persons infected with the outbreak strain of Listeria monocytogenes were reported from two states: California (1) and Maryland (7)
  - 7 ill persons were hospitalized. One death was reported in California. Five of the illnesses (2 mother-newborn pairs and a newborn) were related to pregnancy.
- Whole-genome sequences of the Listeria strains isolated from Roos Foods cheese products were available after the recall and were found to be highly related to sequences of the Listeria strains isolated from the patients
Listeria Outbreaks and Incidence, 1983-2014

No. outbreaks

<table>
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<tr>
<th>Era</th>
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<th>Early PulseNet</th>
<th>Listeria Initiative</th>
<th>WGS</th>
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<tbody>
<tr>
<td>Outbreaks per year</td>
<td>0.3</td>
<td>2.3</td>
<td>2.9</td>
<td>8</td>
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<tr>
<td>Median cases per outbreak</td>
<td>69</td>
<td>11</td>
<td>5.5</td>
<td>4.5</td>
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</table>

Data are preliminary and subject to change
Recall -- Firm Press Release

FDA posts press releases and other notices of recalls and market withdrawals from the firms involved as a service to consumers, the media, and other interested parties. FDA does not endorse either the product or the company.

Blue Bell Creameries Voluntarily Expands Recall to Include All of its Products Due to Possible Health Risk

Contact:
Consumer:
1-866-608-3940

Media:
Joe Robertson
979-830-9830
media@bluebell.com

FOR IMMEDIATE RELEASE – April 20, 2015 – BRENHAM, TX – Blue Bell Ice Cream of Brenham, Texas, is voluntarily recalling all of its products currently on the market made at all of its facilities including ice cream, frozen yogurt, sherbet and frozen snacks because they have the potential to be contaminated with Listeria monocytogenes, an organism which can cause serious and sometimes fatal infections in young children, frail or elderly people, and others with weakened immune systems. Although healthy individuals may suffer only short-term symptoms such as high fever, severe headaches, stiffness, nausea, abdominal pain and diarrhea, Listeria infection can cause miscarriages and stillbirths among pregnant women.
At a Glance:
Case Count: 10
States: 4
Deaths: 3
Hospitalizations: 10
Recall: Yes
Case study: *L. monocytogenes* outbreak in a goat farm

- Notified of several listeriosis cases from a large meat goat farm (200 does) during kidding of older does in January, 2002
- 70 kids were affected by encephalitic form of listeriosis
- *L. monocytogenes* found in tissue of necropsied animals
- Baleage (primary feed source) pH range 5.5 - 7.6.
- Second group scheduled to kid in April, 2002
Case study: *L. monocytogenes* outbreak in a goat farm

- *L. monocytogenes* positive samples
  - Fecal: 4/10
  - Soil: 6/10
  - Feedstuff: 8/12
  - Water: 5/10
  - Milk: 0/10
  - Compost 2/8
  - Run-off 2/2
*L. monocytogenes* ribotype patterns from goat case farm clinical isolates
### Selected *L. monocytogenes* Ribotypes

<table>
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<tr>
<th>Ribotype</th>
<th>Location/Description</th>
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<td>DUP-1045</td>
<td>duodenum 2606</td>
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<tr>
<td>DUP-1045</td>
<td>lymph node 2606</td>
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<tr>
<td>DUP-1045</td>
<td>mother (196) of affected kid (225)</td>
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<tr>
<td>DUP-1045</td>
<td>mother (161) of affected kid (liver biopsy)</td>
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<tr>
<td>DUP-1042B</td>
<td>mother (8) of healthy kid(s)</td>
</tr>
<tr>
<td>DUP-1038B</td>
<td>mother (9) of healthy kid(s)</td>
</tr>
<tr>
<td>DUP-1045</td>
<td>water bucket in pen of sick kid/roe</td>
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<tr>
<td>DUP-1045</td>
<td>water trough in small healthy group pen</td>
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<td>DUP-1045</td>
<td>automatic waterer in barn</td>
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<td>DUP-1045</td>
<td>water bucket in pen of sick kid/roe</td>
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<tr>
<td>DUP-1045</td>
<td>water bucket in pen of sick kid/roe</td>
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<td>1st cut purchased baleage core sample</td>
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<td>bedding inside coverall</td>
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<tr>
<td>DUP-1045</td>
<td>bedding in drop pen</td>
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</table>
Outline

• *Listeria monocytogenes* – Introduction and overview
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L. monocytogenes ecology and contamination patterns in seafood processing plants

- Environmental Listeria contamination as significant problem in the food industry
- Controlling environmental L. monocytogenes contamination in food plants is key to better control (“Seek and destroy”)
DNA fingerprinting can identify persistence in plants

<table>
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<tr>
<th>Sample</th>
<th>Ribotype</th>
<th>Sample Source</th>
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<td>1039C</td>
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<td>20-35-6</td>
<td>1039C</td>
<td>Floor drain, hallway to finished area</td>
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<td>20-22-1</td>
<td>1039C</td>
<td>Troll Red King Salmon, in brine, head area</td>
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<tr>
<td>20-23-1</td>
<td>1039C</td>
<td>Troll Red King Salmon, in brine, belly area</td>
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<td>20-28-1</td>
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# House bugs & pet *Listeria*

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<td></td>
<td>1 app</td>
<td>1 app</td>
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</tr>
</tbody>
</table>

**Raw In Process Areas:**

- Floor drain, raw sodium (26)
- Sodium reserve floor drain

**Sodium Reserve Areas:**

- Sodium reserve, drums (65-FV1)
- Sodium reserve, drums (65-FV2)
- Sodium reserve, 5 drums
- Sodium reserve, neat flour
- Sodium reserve, neat flour, neat cleaning
- Sodium reserve, pallet, neat cleaning

**Raw Sodium Reserve, pallet, neat cleaning:**

- Raw sodium reserve, pallet, neat cleaning

**Finished Product Areas:**

- Trench drain, processing (1 app)
- Trench drain, smoke room
- Trench drain, trench drain, in next

**Cart wheels, neat trolley 1 app:**

- Cart wheels, neat trolley

- Floor, under conveyer belt 1 app

- Punch Room, floor plates 1 app

- Punch Room, floor plates 2 app

- Punch Room, floor plates, reg. Clean

- Punch Room, floor plates, reg. Clean

- Punch room, 1200 rpm (200), weekend

- Punch room, 1200 rpm (200), weekend

- De-dryer valves cover, processing

- Platform under Oslo #1 till

- Sliding door handles, titanium 1 app

**Food Contact Surfaces:**

- Glass #1, lunchroom 1 app

- Sliding machine 1 app

- Oslo #2, luncher 1 app

- 20/30 conveyor belt 1 app

**Finished Product Sample:**

- 1 app

---

42
<table>
<thead>
<tr>
<th>Raw Product Samples</th>
<th>1-2 ppm</th>
<th>3-10 ppm</th>
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<tbody>
<tr>
<td>Raw In Process Areas</td>
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<tr>
<td>Floor drain, raw salmon</td>
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<tr>
<td>Salmon receiving floor drain</td>
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<td>L spp</td>
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<td>L spp</td>
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<td>Raw salmon room, Drum (SB-FD2)</td>
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<td>Raw salmon room, 5 floor tanks</td>
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<td>L spp</td>
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<td>Raw salmon room, autofect post cleaning</td>
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<td>L spp</td>
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<tr>
<td>Raw salmon room, plate filler</td>
<td>-</td>
<td>L spp</td>
<td>L spp</td>
</tr>
<tr>
<td>Raw salmon room, pallet post cleaning</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raw salmon room, pallet post cleaning</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EB: Agrim, employee in raw area</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Cleaning raw material packaging</td>
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<td>L spp</td>
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<thead>
<tr>
<th>Finished Product Areas</th>
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</thead>
<tbody>
<tr>
<td>E1: Trench drain, processing</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E2: Trench drain, smoke room</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Smoke room trench drain, in use</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>E4: Cart wheels, fish box transfer</td>
<td>-</td>
<td>L spp</td>
<td>L spp</td>
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<tr>
<td>E5: Floor, under conveyor belt</td>
<td>-</td>
<td>L spp</td>
<td>L spp</td>
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<tr>
<td>Finish Room, floor mats #1</td>
<td>-</td>
<td>L spp</td>
<td>L spp</td>
</tr>
<tr>
<td>Finish room, floor mats #2</td>
<td>-</td>
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<td>L spp</td>
</tr>
<tr>
<td>Finish room, floor mats, reg. Clean</td>
<td>L spp</td>
<td>L spp</td>
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</tr>
<tr>
<td>Finish room, floor mats, reg. Clean</td>
<td>L spp</td>
<td>L spp</td>
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<tr>
<td>Finish room, 12:00 pm (Mon, weekend)</td>
<td>-</td>
<td>L spp</td>
<td>L spp</td>
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<tr>
<td>Finish room, 12:00 pm (Mon, weekend)</td>
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<td>L spp</td>
</tr>
<tr>
<td>Handipump valve cover, process unit</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E9: Platform under Geha #2 #1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E10: Tailing door handle, chimney</td>
<td>-</td>
<td>L spp</td>
<td>L spp</td>
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<tr>
<td>Food Contact Surfaces</td>
<td></td>
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<tr>
<td>E7: Gloved hands, fish prep</td>
<td>-</td>
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<tr>
<td>E11: Geha #5 silver</td>
<td>L spp</td>
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<tr>
<td>E12: 30/30 vac bag</td>
<td>-</td>
<td>L spp</td>
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</table>

<table>
<thead>
<tr>
<th>Finished Product Sample</th>
<th>0 ppm</th>
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</thead>
</table>
L. monocytogenes persisted in rubber floor mats despite sanitation

Listeria can be protected from sanitizer in “micro-cracks”, but can be squeezed out by pressure if people stand on mats
Growth niches

Locations harboring the organism after the routine sanitation process for that area has been completed.

Examples
- Hollow roller on conveyor transporting food product
  Hollow rollers not disassembled, cleaned and sanitized or heat treated in a manner to eliminate any contaminating organisms can become growth niches.
An Outbreak of *Listeria Monocytogenes* Serotype 3a Infections from Butter in Finland

*The Journal of Infectious Diseases* 2000;181:1838–41

The outbreak strain was first isolated in samples of butter from the implicated dairy in 1997, which led to processing-line cleaning and increased monitoring of the products and environment. Despite intensified sampling, the dairy did not detect *Listeria* before February 1999. However, the process seems to have been contaminated for a longer period, because *L. monocytogenes* was detected in samples from several batches manufactured between September 1998 and February 1999. Long-

eration. The outbreak strain was isolated from the butter-producing equipment and the dairy environment. We could not confirm any error in operation. The source of *L. monocytogenes* may have been the screw conveyor in the butter wagon, which
Persistence in processing equipment

The possibility of the transfer of persistent *Listeria monocytogenes* contamination from one plant to another with a dicing machine was evaluated, ... A dicing machine that diced cooked meat products was transferred from plant A to plant B and then to plant C. After the transfer of the dicing machine, *L. monocytogenes* PFGE type I, originally found in plant A, was soon also found in plants B and C. This *L. monocytogenes* PFGE type I caused persistent contamination of the dicing lines in plants B and C. All persistent *L. monocytogenes* PFGE type I isolates were found in an area with high hygienic standards, with the dicing machine being the first point of contamination. These observations show that the dicing machine sustained the contamination and suggest that the dicing machine transferred the persistent *L. monocytogenes* PFGE type from one plant to another.

Lunden et al., 2012
2000 US outbreak - Environmental persistence of *L. monocytogenes*?

- 1988: one human listeriosis case linked to hot dogs produced by plant X
- 2000: 29 human listeriosis cases linked to sliced turkey meats from plant X
From the Centers for Disease Control and Prevention
Leads From the Morbidity and Mortality Weekly Report
Atlanta, Ga.
Multistate Outbreak of *Salmonella* Serotype Agona Infections Linked to Toasted Oats Cereal—United States, April-May, 1998

Information as of May 13, 2008 (FINAL Update)
Click Here for Advice to Consumers

CDC is collaborating with public health officials in multiple states across the United States and with the U.S. Food and Drug Administration (FDA) to investigate a multi-state outbreak of *Salmonella* Agona infections. An investigation that includes interviews of persons with *Salmonella* Agona infections and comparison of the DNA fingerprints suggests that cereal from Malt-O-Meal unsweetened Puffed Rice Cereals and unsweetened Puffed Wheat Cereals is likely related to these illnesses.
Summary – environmental pathogen sources and persistence

- Persistent environmental contamination with *L. monocytogenes* has been reported in almost all types of food processing plants, including RTE seafood plants (> 10 years), dairy plants; RTE meat plants (>12 years), etc.
- A number of listeriosis outbreaks have been linked to persistent *L. monocytogenes* contamination in source plants
- Industry has adapted the “Seek and Destroy” strategy to address this issue
- Similar issues with *Salmonella* and *Cronobacter*
CDC: Listeria Probably Contaminated WI Cheese During Production Process
BY NEWS DESK | APRIL 8, 2014

A Listeria outbreak in the Midwest linked to one death and a miscarriage likely was caused by contamination during the cheese-making process, according to a new report from the U.S. Centers for Disease Control and Prevention.

“Inspection of the cheese-making facility revealed that substantial sanitation deficiencies during the cheese-making process itself, after the milk was pasteurized, likely led to contamination,” the agency’s April 4 Morbidity and Mortality Weekly report stated.
Outline

- *Listeria monocytogenes* – Introduction and overview
- Outbreak detection: trends and use of molecular methods
- Importance of environmental *L. monocytogenes* sources
- Environmental sampling programs and associated preventive strategies
- Other control strategies
Listeria monocytogenes interventions

- Prevent post kill step re-contamination of products (at plants as well as at retail)
  - Sanitary equipment design
  - Environmental testing
  - Appropriately designed and implemented SSOPs (sanitation standard operating procedures)
  - Post-Lethality Treatment of Product

- Prevent growth:
  - Assure appropriate refrigeration: temperatures of 4°C and below throughout storage can significantly decrease exposure and human cases
  - Reformulate Ready-To-Eat foods to prevent growth

- Reduce exposure of susceptible subgroups
  - Education and outreach
  - Targets pregnant women, elderly and immunocompromised and those caring for them
Behavior of *Listeria monocytogenes* at 7 °C in commercial turkey breast with or without antimicrobials, after simulated contamination for manufacturing, retail and consumer settings

Alexandra Lianou*, Ifigenia Geornaras*, Patricia A. Kendall*, John A. Scanga*, John N. Sofos*~*~

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Optimization of combinations of bactericidal and bacteriostatic treatments to control *Listeria monocytogenes* on cold-smoked salmon


---

Quantitative Risk Assessment for *Listeria monocytogenes* in Selected Categories of Deli Meats: Impact of Lactate and Diacetate on Listерiosis Cases and Deaths


---
Listeria monocytogenes interventions

• Prevent post kill step re-contamination of products (at plants as well as at retail)
  – Sanitary equipment design
  – Environmental testing
  – Appropriately designed and implemented SSOPs (sanitation standard operating procedures)
  – Post-Lethality Treatment of Product
• Prevent growth:
  – Assure appropriate refrigeration: temperatures of 4 C and below throughout storage can significantly decrease exposure and human cases
  – Reformulate Ready-To-Eat foods to prevent growth
• Reduce exposure of susceptible subgroups
  – Education and outreach
  – Targets pregnant women, elderly and immunocompromised and those caring for them
Goals of a microbial environmental testing program

• Identify problem areas harboring pathogen sources ("niches") and locate contamination sources
  – Need to set up a system that encourages collection of samples that yield positive results
• Confirm effectiveness of problem-solving procedures
• Secondary goal may be to characterize transmission pathways
• It is essential to consider regulatory environment
  – Considerable differences by country and within country how different agencies view environmental testing
  – Some agencies may view food contact surface positives with L. monocytogenes as evidence of finished product contamination
Seek and Destroy

• Systematic approach to finding sites of persistent growth ("niches") in food processing plants
  • Environmental sampling with follow up on every positive sample
• Goal is to either eradicate or mitigate effects of niches
• Seek and Destroy can be applied to specific equipment (e.g., new equipment qualification) or the facility as a whole
Designing environmental sampling plans

• Effective environmental sampling plans can prevent food contamination before it occurs
• Sampling plans need to be developed individually for each plant
  – Layout, production schedules, facility design
• For many products *Listeria* and *Salmonella* as key targets
  – Environmental sampling for other pathogens and spoilage organisms may also be relevant
• Trend is towards regulatory agencies recommending environmental sampling
  – Regulators often perform sampling if there are no data supporting that sampling is done by the facility
Where to test?

• Food contact surfaces
  – Food contact surface positives may have to be followed up with finished product testing
• Non-food contact surfaces
  – Sites in coolers (floors, walls, cooler coils, condensate collectors etc.)
  – Tubs, conveyances, underneath tables
  – Floors, floor mats, walls, & drains in production areas
Where to test – the zone concept

• Plant is divided into different zones; zones are defined based on relative potential for finished product contamination a site or area represents; sampling and corrections triggered by positive samples differ by zones.
  – Zone 1: Finished product contact surfaces
  – Zone 2: Non-food contact surfaces in finished product area
  – Zone 3: Product contact surfaces in raw product handling areas
  – Zone 4: Areas remote from finished product handling (e.g., non-product contact surfaces in the raw product handling areas)

• Some plans have 3 not 4 zones
Zone 1
Product Contact Surfaces
(Slicers, peelers, fillers, hoppers, screens, conveyor belts, air blowers, employee hands, knives, racks, work tables)

Zone 2
Non-Product (Near) Contact Surfaces
(Exterior, under, & framework of equipment: refrigeration units, equipment housing; switches)

Zone 3
Other Areas within Finished Product (RTE) Room
(Air return covers, phones; hand trucks, forklifts, drains, wheels)

Zone 4
Area Outside of RTE Room
(Locker rooms, cafeteria, hallways, loading dock, maintenance areas)
Where to test

• Niches:
  – Hallow rollers, table legs, etc.; floor wall junctures; floor cracks; difficult to clean areas; seals on doors, etc.
  – Sampling of niches more likely to identify source

• Transfer points:
  – Hands, door handles
  – Sampling of transfer points requires follow up to identify source

• Some areas could be both
  – Key boards
Challenges with environmental sampling
Where to sample if you hear

“If sampling reveals the presence of Listeria species, it is important that the processor immediately shuts down the plant and implements an aggressive sanitation protocol and resampling until Listeria is not found.” (KSU professor in IFT ePerspectives)

“Our company goal for 2016 is zero Listeria environmental positives” (Anonymous)

“FDA will collect 100s of environmental samples in your plant if your records show a single Listeria positive” (industry rumors after an FDA visit and record review, followed by FDA swab-a-thon)
When to test?

- **Pre-op**
  - Less likely to yield positive samples
  - More easy to interpret, will identify sanitation weaknesses

- **Mid-op**
  - More likely to yield positive
  - Will provide information on spread of target pathogen during processing
  - Sample site positive may not be the site where the pathogen survives
    - Positive sites typically will require pre-op follow-up sampling to identify pathogen source/niche
How to collect samples

• Sterile sampling techniques (sponges with gloves or handles)
• Typically use sponge for sampling
  – rarely use swabs, only for very difficult to reach areas
How often to test?

• Can range from daily/multiple times a day to weekly or maybe even monthly (in very small operations)
• Sites are typically pre-determined, but may be randomly rotated so that not all sites are sampled every times
  – For example, only 15 of 30 predetermined sites may be sampled every time
• Sampling frequency and sample numbers should be determined through a risk-based approach
Innovation Center for US Dairy recommendations

• **Minimum:** PEM samples are collected at least weekly and include samples at eye level, below and above. A minimum of 30 swabs are taken per 50,000 sq. ft. per week: Raw:7, RTE/HH: 20, Zone 4: 3

• **Best of class:** PEM samples are collected at least weekly and include samples at eye level, below and above. Greater than 55 swabs are taken per 50,000 sq. ft. per week: Raw:14, RTE/HH 35, Zone 4: 6. As facility ages, swabbing increases to reflect increased risks.

Test methods

• **Traditional methods:**
  – Often time consuming
  – With traditional methods *Listeria* spp. testing is faster than *L. monocytogenes* testing

• Detection of surface molecules and other antigens
  – Antibody-based methods (e.g., ELISA)
  – Recombinant phage protein

• **Nucleic acid amplification methods**
  – Polymerase chain reaction (PCR)
  – Other nucleic acid amplification methods
What to do with testing results

• Review testing results every time results are reported
  – This should include review of at least last 4-8 sampling results to identify trends (e.g., site that has positives with intervening negatives)
  – Take corrections on each positive sample and document action
• Organize testing results in one location (folder, three-ring binder or ideally electronically)
  – Include documentation of corrections in same location
• Conduct regular (quarterly, yearly; depends on testing frequency & volume) review of testing results
  – Tabulate and evaluate long-term trends
Guidelines for follow-up and corrections

• Corrections based on positive samples need to be plant specific and may differ by zone
• Trend towards increased frequency of pathogen detection needs to be investigated to determine reason and action needs to be taken to reduce frequency
• Additional samples should be taken from environmental area that showed positive results ("vector swabbing")
• Positive samples should be followed up with additional investigations and root cause analyses as well as intensified cleaning and sanitation
• Corrective actions may furthermore include:
  – Cleaning and sanitation procedures and SSOPs may need to be changed
  – Maintenance may be needed and preventive maintenance program may need to be improved
  – Equipment may have to be modified and replaced
  – Problem areas may have to be shut down temporarily
• Consider if a test and hold program is needed
Correction Plan: Flow Chart for Zone 3 & 4 Environmental Testing in Dairy Plant

- Pre-operational sites in pilot plant area swabbed each month for *Listeria* spp.

  - Positive
    - Evaluate site for possible contamination host(s). Make notes of possible host(s). Note modifications made, if any. Focused cleaning & sanitizing at positive site. Resample pre-operational after cleaning.
    - Negative
      - Drop additional site from monthly testing. Make report of findings. Audit area as necessary.
    - Positive
      - Add Notes to final report of investigation and any action taken.

- 2 consecutive monthly Negative samples

  - Positive
    - Create an isolation area at positive site. Cease use of area. Consult with chemical company for recommendations. Follow further cleaning instructions. **Add additional site from contamination query to consecutive monthly testing**
    - Negative
      - Perform extra cleaning and conc. sanitizing. Retest after confirmation with plant manager that proper clean-up has been achieved. Identify and record five (5) vector sites either as starburst or traffic pattern (depending on site parameters). Sample additional sites pre-operational.
    - Positive
      - Add Notes to final report of investigation and any action taken.
Need to have specific and separate written records for corrections

Corrective Action:

**Corrective Action Record for "Name of Plant"**

**Plant A**

Date of Environmental Sampling/Swabbing: ___________ 5/15/2013

Site Found Positive: ___________ Circle one: *Listeria monocytogenes* or *Listeria* species

Date action taken: ___________ 5/23/2013

Detailed description of action taken on positive site:

Through cleaning with an acid cleaner (vs. our old chlorine bleach) was performed

__________

__________

__________

Mark which applies:

- Perform immediate out of cycle testing
- Swab again during next scheduled testing

Follow-up Environmental testing Results (circle one): *Negative* or *Repeat Positive*

ADA 6/13/2013
Validation of environmental sampling plans - *Listeria* example

- Performed to assure that routine sampling correctly monitors for pathogens
- Sample size calculations performed to assure enough samples are collected to make sure prevalence detected during routine sampling does not severely underestimate true prevalence
- Outside experts perform sampling
## Prevalence data – routine sampling and validation

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<thead>
<tr>
<th>Plant ID</th>
<th>Prevalence (from routine)</th>
<th>Goal for validation</th>
<th>Prevalence (from validation)</th>
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<tbody>
<tr>
<td>A</td>
<td>5.12% (34/664)</td>
<td>&lt;10.24%</td>
<td>1.33% (2/150)</td>
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<tr>
<td>E</td>
<td>11.97% (88/735)</td>
<td>&lt;23.95%</td>
<td>10% (6/60)</td>
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<tr>
<td>F</td>
<td>&lt;0.3% (0/334)</td>
<td>&lt;10%</td>
<td>6% (3/50)</td>
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<tr>
<td>G</td>
<td>8.33% (19/228)</td>
<td>&lt;16.67%</td>
<td>2.35% (2/85)</td>
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<tr>
<td>H</td>
<td>22.64% (24/106)</td>
<td>&lt;45.28%</td>
<td>8% (2/50)</td>
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<tr>
<td>J</td>
<td>0.94% (1/106)</td>
<td>&lt;10%</td>
<td>14% (7/50)</td>
</tr>
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Take home messages

• *Listeria monocytogenes* and human listeriosis will likely continue to increase as an issue
  – Better outbreak detection through molecular subtyping
  – Growth of susceptible population
  – High risk foods will become more commons
• Listeriosis is a rare (but severe) human disease, but *Listeria monocytogenes* is common (“ubiquitous”).
  – High infectious dose
  – Presence of *L. monocytogenes* in environments has to be expected
• Key interventions and controls include
  – Prevent post kill step re-contamination of Ready-To-Eat foods
  – Prevent *L. monocytogenes* growth in Ready-To-Eat foods
  – Reduce exposure of susceptible subgroups
Action items

• Improve foodborne disease surveillance and use molecular tools
• Encourage industry to develop and implement environmental sampling plans that find *Listeria* and take corrective actions
  – Do not punish companies for occasional *Listeria* positives as long as appropriate corrective action has been taken
• Encourage reformulation of RTE foods to prevent *Listeria* growth
  – Approve appropriate antimicrobials
• Pursue consumer education