Cornell University
College of Agriculture and Life Sciences

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

Martin Wiedmann
Department of Food Science
Cornell University, Ithaca, NY
E-mail: mw16@cornell.edu
607-254-2838


New York
78° W
76° W
72° W
80° W
CANADA
ONTARIO
Lake Ontario
Lake Erie
Massena
Malone
Ogdensburg
Canton
Plattsburgh
Lake Champlain
Saranac Lake
VERMONT
Tupper Lake
Mt. Marcy
5,344 ft
(1,629 m)
Adirondack Mts.
Watertown
Black
44° N
NEW HAMPSHIRE
Connecticut
Merrimack
Oswego
Fulton
Rome
Utica
Glens Falls
Saratoga Spgs.
Amsterdam
Schenectady
Troy
Albany
Mohawk
Syracuse
Auburn
Irondequoit
Rochester
Erie Canal
Niagara Falls
Lockport
Batavia
Buffalo
Lackawanna
Canandaigua
Geneseo
Geneva
Penn Yan
Finger Lakes
Cortland
Norwich
Oneonta
Ithaca
Genesee
Dunkirk
Fredonia
Salamanca
Jamestown
Olean
Hornell
Elmira
Binghamton
Catskill Mts.
Hudson
Kingston
Monticello
Poughkeepsie
Newburgh
Middletown
Port Jervis
MASSACHUSETTS
CONNECTICUT
RHODE ISLAND
42° N
New City
White Plains
Yonkers
New Rochelle
Hempstead
New York
Long Island Sd.
Long Island
Riverhead
ATLANTIC OCEAN
NEW JERSEY
PENNSYLVANIA
Allegheny
Susquehanna
Delaware
0 50 Miles
0 50 Kilometers
© MAPQUEST.COM

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

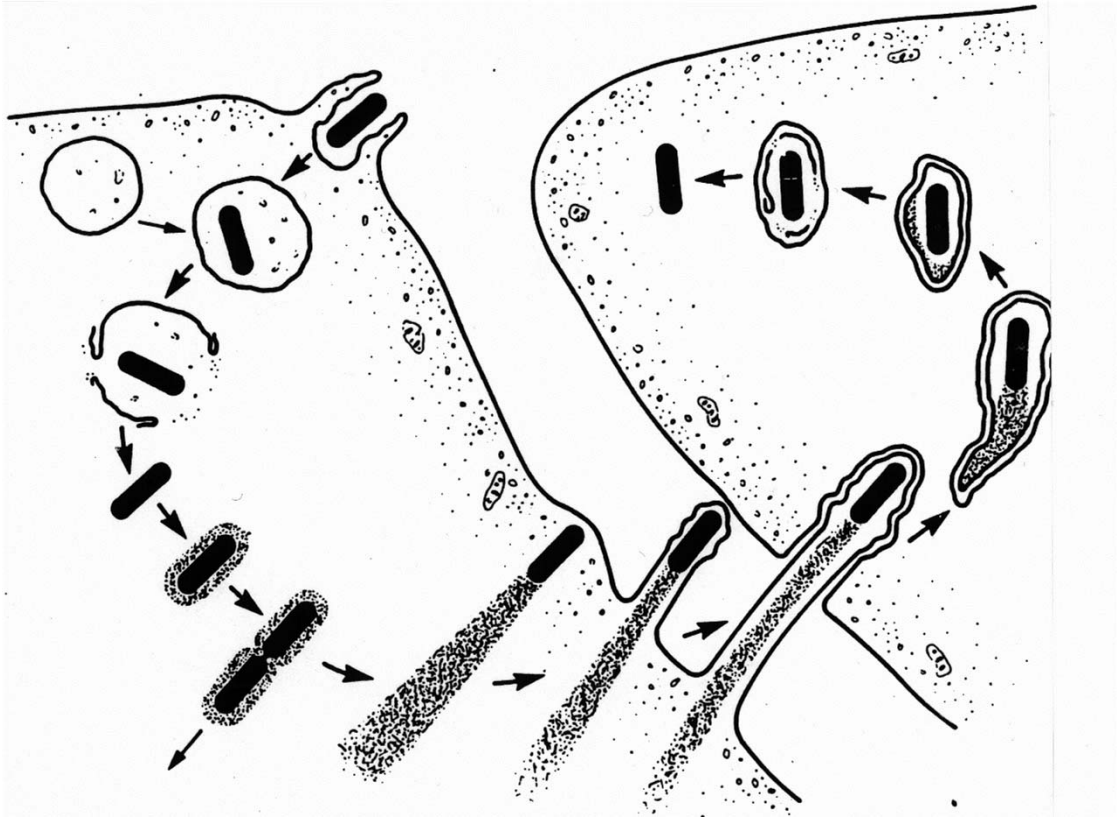
Listeria monocytogenes – Intro

- 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% prevalance

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

Summary Table 1. Relative Risk Ranking and Predicted Median Cases of Listeriosis for the Total United States Population on a per Serving and per Annum Basis

Relative Risk Ranking	Predicted Median Cases of Listeriosis for 23 Food Categories					
	Per Serving Basis[a]			Per Annum Basis[b]		
		Food	Cases		Food	Cases
1	High Risk	Deli Meats	7.7×10^{-8}	Very High	Deli Meats	1598.7
2	High Risk	Frankfurters, not reheated	6.5×10^{-8}	High Risk	Pasteurized Fluid Milk	90.8
3	High Risk	Pâté and Meat Spreads	3.2×10^{-8}	High Risk	High Fat and Other Dairy Products	56.4
4	High Risk	Unpasteurized Fluid Milk	7.1×10^{-9}	High Risk	Frankfurters, not reheated	30.5
5	High Risk	Smoked Seafood	6.2×10^{-9}	Moderate Risk	Soft Unripened Cheese	7.7
6	High Risk	Cooked Ready-to-Eat Crustaceans	5.1×10^{-9}	Moderate Risk	Pâté and Meat Spreads	3.8
7	Moderate Risk	High Fat and Other Dairy Products	2.7×10^{-9}	Moderate Risk	Unpasteurized Fluid Milk	3.1
8	Moderate Risk	Soft Unripened Cheese	1.8×10^{-9}	Moderate Risk	Cooked Ready-to-Eat Crustaceans	2.8
9	Moderate Risk	Pasteurized Fluid Milk	1.0×10^{-9}	Moderate Risk	Smoked Seafood	1.3

FDA-FSIS Risk Assessment for *L. monocytogenes* in Ready-to-Eat Foods (2003)

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

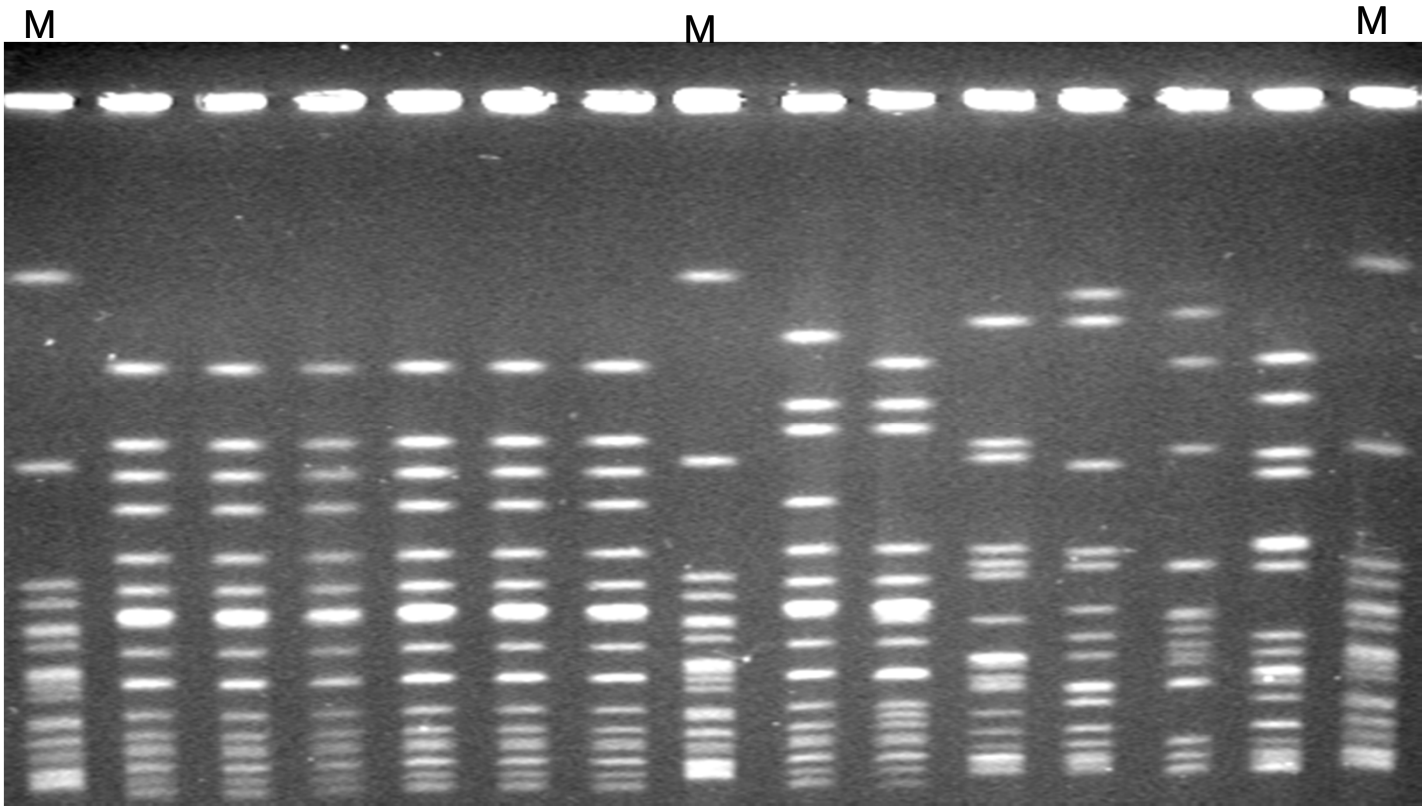
Outline

- *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

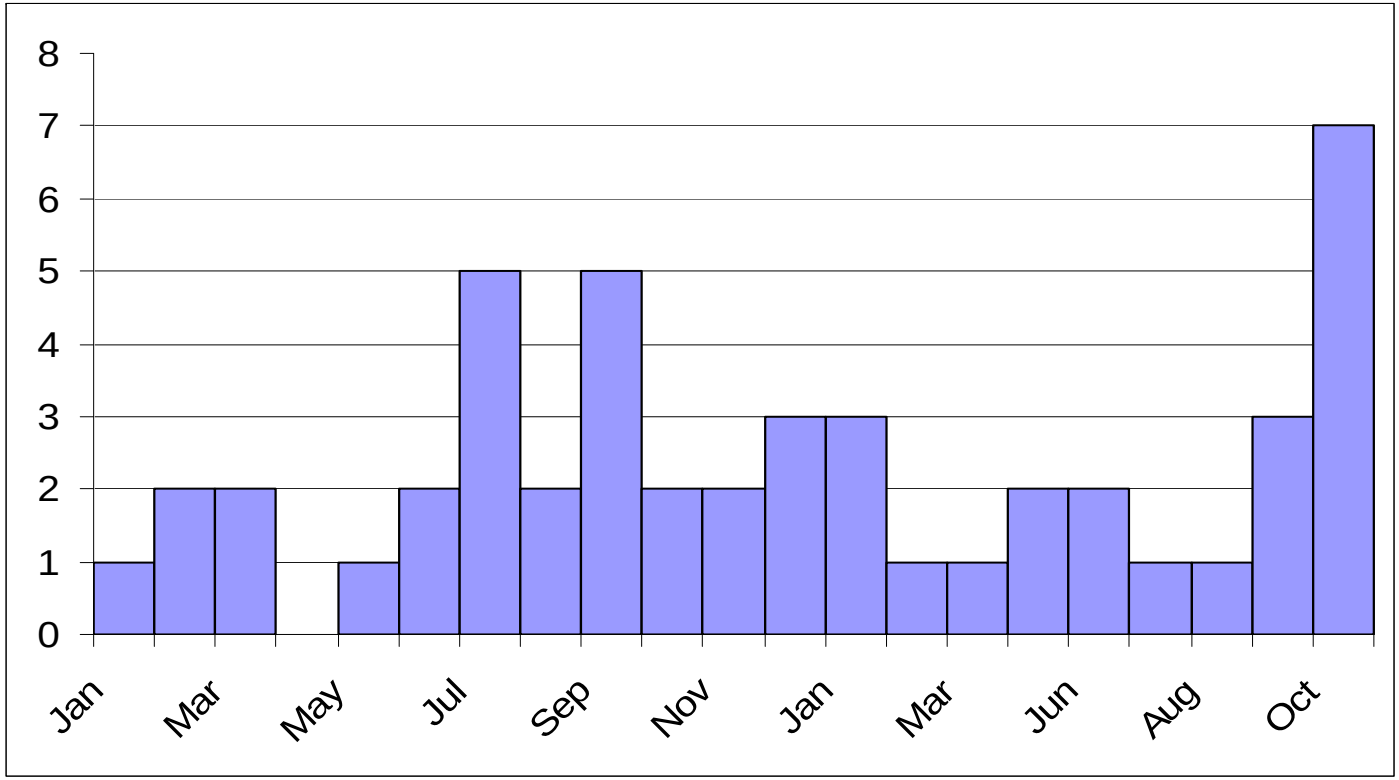
Examples of different PFGE patterns



Cornell University
College of Agriculture and Life Sciences

Case study – human listeriosis outbreak

Human listeriosis cases in NYS: 1/97-10/98

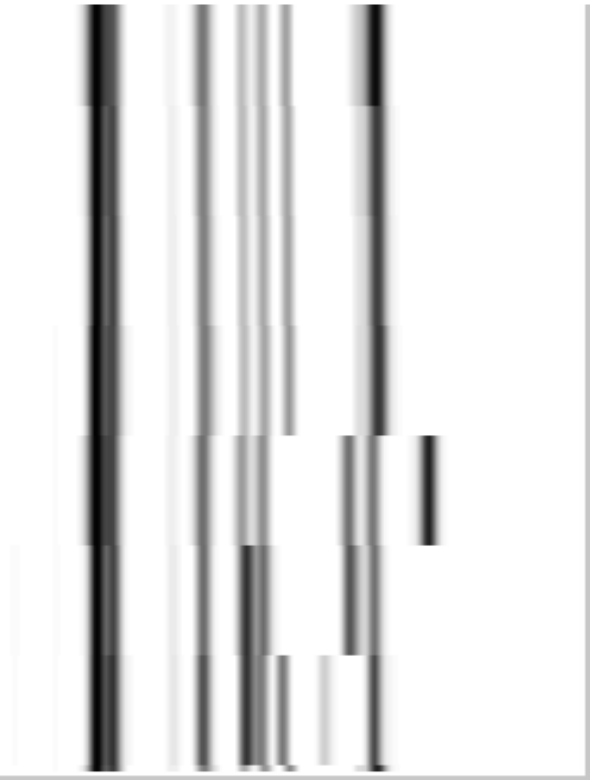


Subtyping results – part I

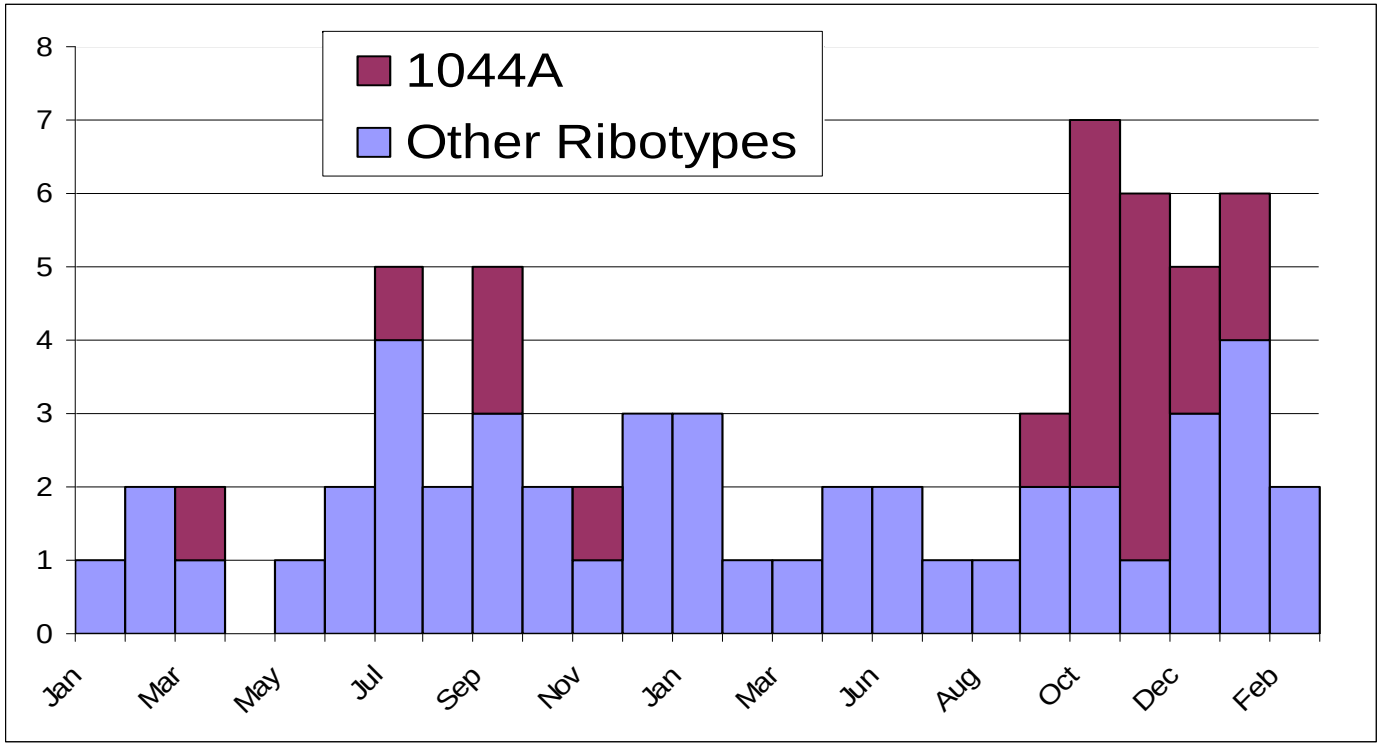
B98-2192	DUP-1039
B98-3297	DUP-1045
B98-3556	DUP-1042
B98-3853	DUP-1052
B98-4054	DUP-1044
B98-3412	DUP-1044
B98-4051	DUP-1044
B98-4193	DUP-1044

Subtyping results – part II

B98-4254	DUP-1044
B98-4295	DUP-1044
B98-4338	DUP-1044
B98-4450	DUP-1044
B98-4289	DUP-1052
B98-4340	DUP-1062
B98-4374	DUP-1056



Epidemic curve for 1/97 - 2/99 in NYS



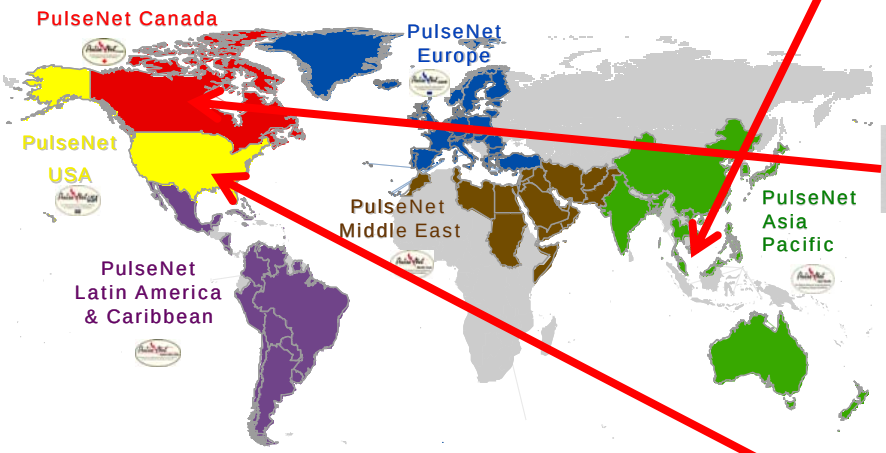
Similarity Search Results

FSL-N1-179	DUP-1044	0.98
FSL-N1-188	DUP-1044	0.98
FSL-C1-124	DUP-1044	0.98
FSL-C1-129	DUP-1044	0.98
FSL-N1-272	DUP-1044	0.98
FSL-C1-144	DUP-1044	0.98
FSL-J1-213	DUP-1044	0.98
FSL-N1-268	DUP-1044	0.98
FSL-N1-270	DUP-1044	0.98
FSL-J1-193	DUP-1044	0.98
FSL-C1-109	DUP-1044	0.98
FSL-N1-187	DUP-1044	0.98
FSL-N1-245	DUP-1044	0.98
FSL-C1-151	DUP-1044	0.98
FSL-C1-108	DUP-1044	0.98
FSL-C1-112	DUP-1044	0.98
FSL-C1-145	DUP-1044	0.98
FSL-N1-271	DUP-1044	0.98
FSL-J1-202	DUP-1044	0.98
FSL-N1-242	DUP-1044	0.99
FSL-N1-287	DUP-1044	0.99
FSL-C1-149	DUP-1044	0.99
FSL-C1-139	DUP-1044	0.99
FSL-N1-276	DUP-1044	0.99
FSL-N1-243	DUP-1044	0.99
FSL-C1-103	DUP-1044	0.99
FSL-N1-275	DUP-1044	0.99
FSL-N1-190	DUP-1044	0.99
FSL-N1-201	DUP-1044	0.99
FSL-C1-123	DUP-1044	0.99
FSL-N1-269	DUP-1044	0.99
FSL-M2-002	DUP-1044	0.99
FSL-C1-105	DUP-1044	0.99
FSL-M2-003	DUP-1044	0.99
FSL-C1-121	DUP-1044	0.99
FSL-N1-287	DUP-1044	1.00

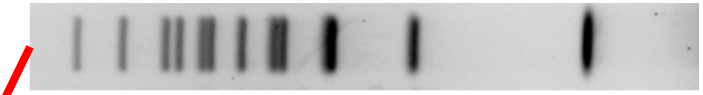
Conclusions

- 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

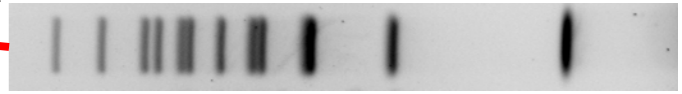
Possibilities for international traceback – a hypothetical example



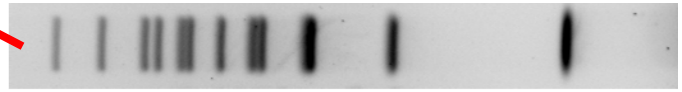
Food isolate, deposited into PulseNet



Human case



Human case



Cornell University
College of Agriculture and Life Sciences

Food Safety News
Breaking news for everyone's consumption

CDC/FDA Partnership Targets Whole Genome Sequencing of Listeria Monocytogenes

By Brian Sauders | November 27, 2013

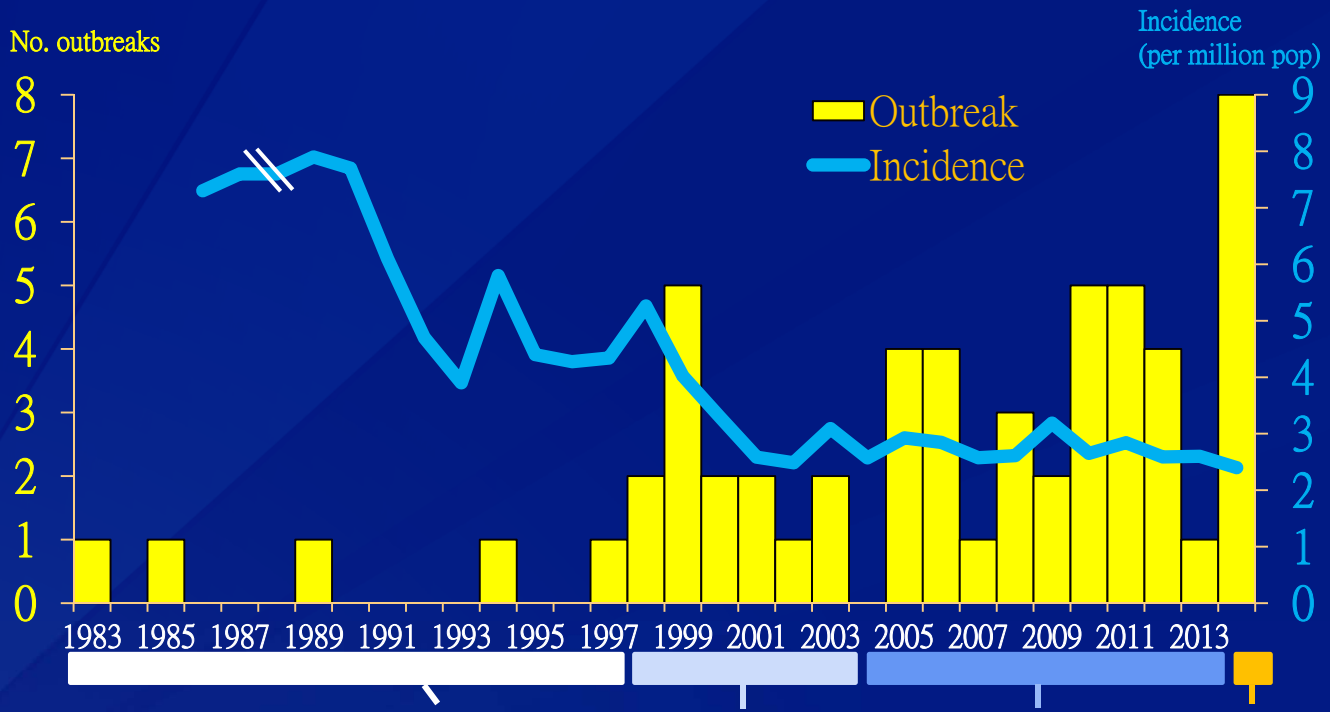
In a prior APHLTech blog post (NGS in Action: FDA's Genome TRAKR Network), Victor Waddell of the Arizona State Public Health Laboratory described the newly formed network of laboratories formed by the U.S. Food and Drug Administration (FDA). Known collectively as Genome TRAKR, the member laboratories 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



Era	Pre-PulseNet	Early PulseNet	*Listeria* Initiative	WGS
Outbreaks per year	0.3	2.3	2.9	8
Median cases per outbreak	69	11	5.5	4.5

Data are preliminary and subject to change

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

A to Z Index | Follow FDA | En Español

Search FDA

Home | Food | Drugs | Medical Devices | Radiation-Emitting Products | Vaccines, Blood & Biologics | Animal & Veterinary | Cosmetics | Tobacco Products

Safety

Home > Safety > Recalls, Market Withdrawals, & Safety Alerts

Recall -- Firm Press Release

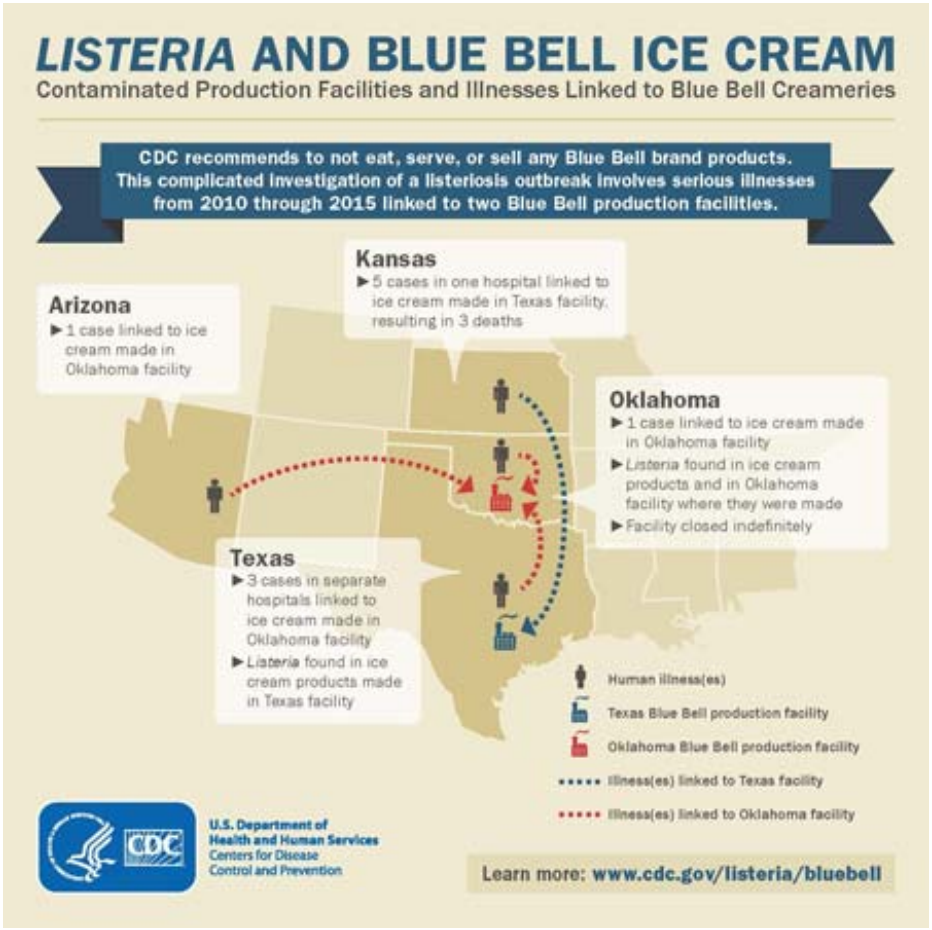
FDA posts press releases and other notices of recalls and market withdrawals from the firms involved as a service to consumers, the media, and other interested parties. FDA does not endorse either the product or the company.

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

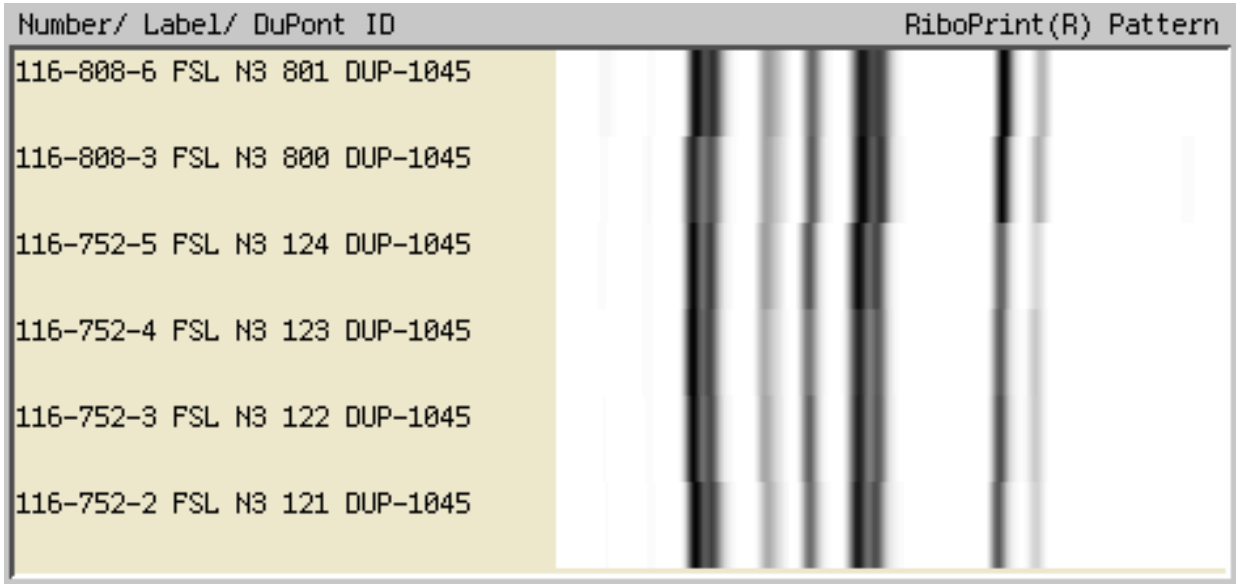
Case study: *L. monocytogenes* outbreak in a goat farm

- 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

DUP-1045	duodenum 2606
DUP-1045	lymph node 2606
DUP-1045	mother (196) of affected kid (225)
DUP-1045	mother (161) of affected kid (liver biopsy)
DUP-1042B	mother (8) of healthy kid(s)
DUP-1038B	mother (9) of healthy kid(s)
DUP-1045	water bucket in pen of sick kid/doe
DUP-1045	water trough in small healthy group pen
DUP-1045	automatic waterer in barn
DUP-1045	water bucket in pen of sick kid/doe
DUP-1045	water bucket in pen of sick kid/doe
DUP-1045	1st cut purchased baleage core sample
DUP-1045	1st cut purchased baleage core sample
DUP-1045	owner's baleage core sample
DUP-1045	owner's baleage core sample
DUP-1045	owner's baleage core sample
DUP-1045	2nd cut purchased baleage core sample
DUP-1045	entrance to drop pen
DUP-1045	bedding inside coverall
DUP-1045	bedding in drop pen

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

Cornell University
College of Agriculture and Life Sciences

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

	Sample Pattern		Ribotype	Sample Source	RiboPrint®
VISIT 1	1015-3	*	1039C	(E) Floor drain, raw materials area	
	2D-35-6	*	1039C	(E) Floor drain, hallway to finished area	
VISIT 2	2D-22-1	*	1039C	(IP) Troll Red King Salmon, in brine, head area	
	2D-23-1	*	1039C	(IP) Troll Red King Salmon, in brine, belly area	
	2D-27-1	*	1039C	(IP) Brine, Troll Red King Salmon	
	2D-28-1	*	1039C	(IP) Faroe Island Salmon, in brine, head area	
	2D-34-1	*	1039C	(F) Smoked Sable	
	2D-42-1	*	1039C	(F) Cold-Smoked Norwegian Salmon	
	2D-30-1		1044A	(E) Floor drain, brining cold room 1	
	2D-10-1		1044A	(R) Raw Troll Red King Salmon, head area	
	2D-31-2		1044A	(IP) Brine, Faroe Island Salmon	
	2D-11-1		1045	(R) Raw Troll Red King Salmon, belly area	
	2D-29-3		1045	(IP) Faroe Island Salmon, in brine, head area	
	2D-24-1		1053	(IP) Norwegian Salmon, in brine	
	2D-16-1		1062	(E) Floor drain #1, raw materials preparation	
VISIT 3	3D-10-3	*	1039C	(E) Floor drain #1, raw materials preparation	
	3D-11-13	*	1039C	(E) Floor drain, brining cold room 1	
	3D-13-4	*	1039C	(E) Floor drain #2, raw materials preparation	
	3D-14-1	*	1039C	(E) Floor drain #2, raw materials receiving	
	3D-6-21	*	1039C	(E) Floor drain, finished product area	
	3D-8-26	*	1039C	(E) Floor drain, hallway to finished area	
	3D-36-2	*	1039C	(IP) Brine, Troll Red King Salmon	
	3D-50-1	*	1039C	(F) Smoked Sable	
	3D-38-1		1044A	(IP) Sable, in brine	
	3D-42-3		1044A	(IP) Brine, Faroe Island Salmon	
	3D-37-1		1062	(IP) Brine, Norwegian Salmon	

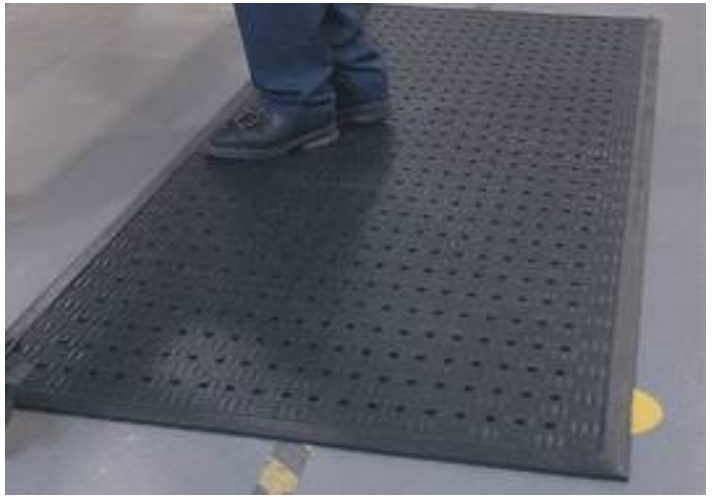
House bugs & pet *Listeria*

Samples	Plant B n=129	Plant C n=173	Plant D n=229	P-value
Ribotype		% Prevalence		
1039C	0.0	0.0	10.0	0.0000
1042B	0.8	1.2	0.4	0.8221
1042C	6.2	0.6	0.4	0.0003
1044A	0.0	2.3	3.1	0.1494
1045	5.4	0.0	0.9	0.0006
1046B	0.0	2.3	0.0	0.0144
1053	0.0	0.6	1.7	0.2686
1062	0.8	0.6	2.6	0.1822

Plant A2	2/28/01	3/26/01	4/24/01	5/22/01	6/19/01	7/17/01	8/14/01	9/18/01	10/9/01	11/6/01	12/12/01	1/29/02	2/25/02
Raw Product Samples	1062D	1060A	-	-	-	-	-	-	-	L.spp	-	L.spp	
	1 of 6	1 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	3 of 6	6 of 6	1 of 6	
Raw/In-Process Areas													
E3: Floor drain, raw salmon ro	1053A	-	-	-	-	-	-	-	-	L.spp	L.spp	L.spp	
Salmon receiving floor drain				-	-	L.spp	1053A	-	-	L.spp	-	-	
Raw salmon room, Drain (SB-FD1)													
Raw salmon room, Drain (SB-FD2)													
Raw salmon room, 3 floor mats													
RawSalmon room, mats- post cleaning													
Raw salmon room, platic pallet													
Raw Salmon room, pallet, post cleaning													
Raw salmon room, pallet jacket handle													
E8: Apron, employee in raw are	1062D	-	-	1053A	-	1053A	1025A	-	1053A	-	1053A	-	
Incoming raw material packaging												-	
Finished Product Areas													
E1: Trench Drain, processing r	L.spp	-	-	116-693	L.spp	L.spp	L.spp	-	L.spp	L.spp	-	L.spp	
E2: Trench Drain, smoke room	-	-	-	-	-	-	-	-	-	-	-	-	
Smoke room trench drain, in use													
E4: Cart wheels, for box transp	L.spp	-	-	-	-	-	-	L.spp	-	L.spp	-	-	
E5: Floor, under conveyor belt	L.spp	-	-	-	-	L.spp	L.spp	L.spp	L.spp	L.spp	L.spp	-	
Finish Room, floor mats #1													L.spp
Finish room, floor mats #2													L.spp
Finish room, floor mats, reg. Clean													
Finish room, floor mats, reg. Clean													L.spp
Finish room, 1200 ppm Quat, weekend													L.spp
Finish room, 1200 ppm Quat, weekend													-
Bootdip valve cover, processin	-	-	L.spp										
E6: Platform under Geba #1 sli	-	-	-	-	-	-	L.spp	L.spp	L.spp	-	-	-	
E9: Sliding door handle, skinnir	L.spp	-	-	-	-	-	-	1053A	L.spp	-	-	-	
Food Contact Surfaces													
E7: Gloved hands, finish prod.	-	-	-	-	-	-	-	-	-	-	-	-	
E10: Skinning machine	L.spp	-	L.spp	-	-	-	L.spp	L.spp	L.spp	-	L.spp	-	
E11: Geba #5 slicer	L.spp	-	-	-	-	-	-	-	-	-	L.spp	-	
E12: 20/20 vac belt	-	-	-	L.spp	-	-	-	-	-	-	-	-	
	6 of 6	6 of 6	6of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	
Finished Product Sample	-	-	-	-	-	-	-	-	-	-	-	-	

Plant A2	2/28/01	3/26/01	4/24/01	5/22/01	6/19/01	7/17/01	8/14/01	9/18/01	10/9/01	11/6/01	12/12/01	1/29/02	2/25/02	3/5/02	4/2/02	4/16/02	5/14/02	6/10/02	7/1/02	7/23/02	8/20/02	9/17/02	10/15/02	11/12/02	12/10/02
														1-L.spp			2-L.spp			1-L.spp					1-L.spp
Raw Product Samples	1062D	1060A	-	-	-	-	-	-	-	L.spp	-	L.spp		1025A	-		1053C	-	-	1039C	-	-	-	-	1039C
	1 of 6	1 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	3 of 6	6 of 6	1 of 6		1 of 6	6 of 6		1 of 6	6 of 6	6 of 6	1 of 6	6 of 6	6 of 6	6 of 6	6 of 6	1 of 6
Raw/In-Process Areas																									
E3: Floor drain, raw salmon ro	1053A	-	-	-	-	-	-	-	-	L.spp	L.spp	L.spp		1053A	-		-	-	-	-	-	-	-	-	-
Salmon receiving floor drain				-	-	L.spp	1053A	-	-	L.spp	-	-		L. spp	-		-	-	-	L.spp	L.spp	L.spp	-	-	-
Raw salmon room, Drain (SB-FD1)														1053A	1053A		-	-	1053a	1053A	-	1053A	1062A	L.spp	1053A
Raw salmon room, Drain (SB-FD2)														1053A	1053A		1053A	-	L.spp	1053A	1053A	L.spp			-
Raw salmon room, 3 floor mats														1053A	1053A			1053A	1053A	1053A	1053A	1053A	1053A	1053A	1053A
RawSalmon room, mats- post cleaning																1053A	1053A								
Raw salmon room, platic pallet														1053A	-			-	L.spp	-	-	-	-	-	-
Raw Salmon room, pallet, post cleaning																-	-								
Raw salmon room, pallet jacket handle														-	-		-	-	-	-	-	-	-	-	-
E8: Apron, employee in raw are	1062D	-	-	1053A	-	1053A	1025A	-	1053A	-	1053A	-		-	-		-	-	-	-	-	-	-	-	-
Incoming raw material packaging												-		-	-		-	-	-	-	-	L.spp	-	L.spp	-
Finished Product Areas																									
E1: Trench Drain, processing r	L.spp	-	-	116-693	L.spp	L.spp	L.spp	-	L.spp	L.spp	-	L.spp		1042C	L.spp		1042C	L.spp	-	-	L.spp	L.spp	L.spp	L.spp	L. spp
E2: Trench Drain, smoke roon	-	-	-	-	-	-	-	-	-	-	-	-		-	-		-	-	L.spp	-	-	L.spp	-	-	L. spp
Smoke room trench drain, in use																1053A	-	-	-	-	-	-	-	-	-
E4: Cart wheels, for box transp	L.spp	-	-	-	-	-	-	L.spp	-	L.spp	-	-		-	-		-	-	-	-	-	L.spp	L.spp	-	L. spp
E5: Floor, under conveyor belt	L.spp	-	-	-	-	L.spp	L.spp	L.spp	L.spp	L.spp	L.spp	-		-	-		-	-	-	-	-	-	1053	1053	-
Finish Room, floor mats #1													L.spp												
Finish room, floor mats #2													L.spp												
Finish room, floor mats, reg. Clean														L.spp	L.spp	L.spp	L.spp	L.spp	L.spp	L.spp	L.spp	1042B	L.spp	L.spp	L. spp
Finish room, floor mats, reg. Clean													L.spp												
Finish room, 1200 ppm Quat, weekend													L.spp												
Finish room, 1200 ppm Quat, weekend													-												
Bootdip valve cover, processin	-	-	L.spp																						
E6: Platform under Geba #1 sli	-	-	-	-	-	-	L.spp	L.spp	L.spp	-	-	-		-	-		-	-	-	-	-	-	-	-	-
E9: Sliding door handle, skinni	L.spp	-	-	-	-	-	-	1053A	L.spp	-	-	-		-	-		-	-	-	L.spp	-	-	-	-	-
Food Contact Surfaces																									
E7: Gloved hands, finish prod.	-	-	-	-	-	-	-	-	-	-	-	-		-	-		-	-	-	-	-	-	1053	-	L. spp
E10: Skinning machine	L.spp	-	L.spp	-	-	-	L.spp	L.spp	L.spp	-	L.spp	-		L.spp	-		-	-	-	-	-	L.spp	-	L. spp	-
E11: Geba #5 slicer	L.spp	-	-	-	-	-	-	-	-	-	L.spp	-		-	-		-	-	-	-	-	-	1053	-	-
E12: 20/20 vac belt	-	-	-	L.spp	-	-	-	-	-	-	-	-		-	-		-	-	-	-	-	-	-	-	-
	6 of 6	6 of 6	6of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6		6 of 6	6 of 6		6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	1 of 6	6 of 6
Finished Product Sample	-	-	-	-	-	-	-	-	-	-	-	-		-	-		-	-	-	-	-	-	-	1039A	-

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-

25 cases, 6 deaths

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

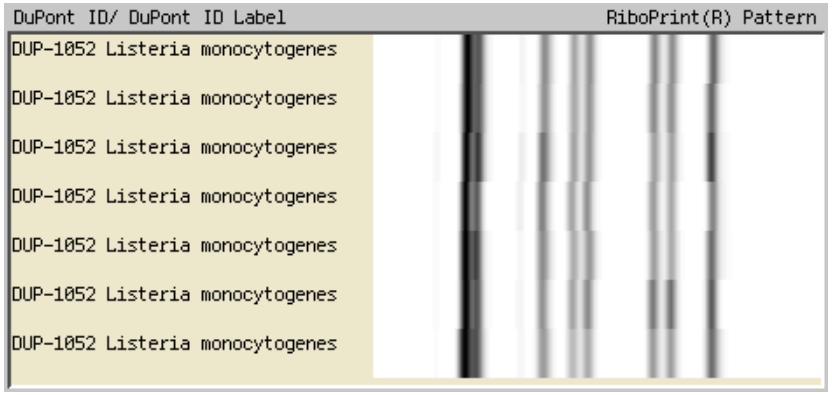
Persistence in processing equipment

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

209 cases

Information as of May 13, 2008 (FINAL Update)

Click Here for Advice to Consumers **28 cases**

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*

Cornell University
College of Agriculture and Life Sciences

Food Safety News
Breaking news for everyone's consumption

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.

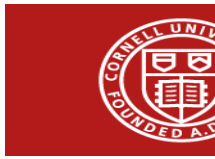
"Inspection of the cheese-making facility revealed that substantial sanitation deficiencies during the cheese-making process itself, after the milk was pasteurized, likely led to contamination," the agency's April 4 Morbidity and Mortality Weekly report stated.

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



Cornell University
College of Agriculture and Life Sciences

ELSEVIER

Food Microbiology 24 (2007) 433–443

FOOD MICROBIOLOGY

www.elsevier.com/locate/fm

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[a], Ifigenia Geornaras[a], Patricia A. Kendall[b], John A. Scanga[a], John N. Sofos[a,*]

International Journal of Food Microbiology 179 (2014) 1–9

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

ELSEVIER

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

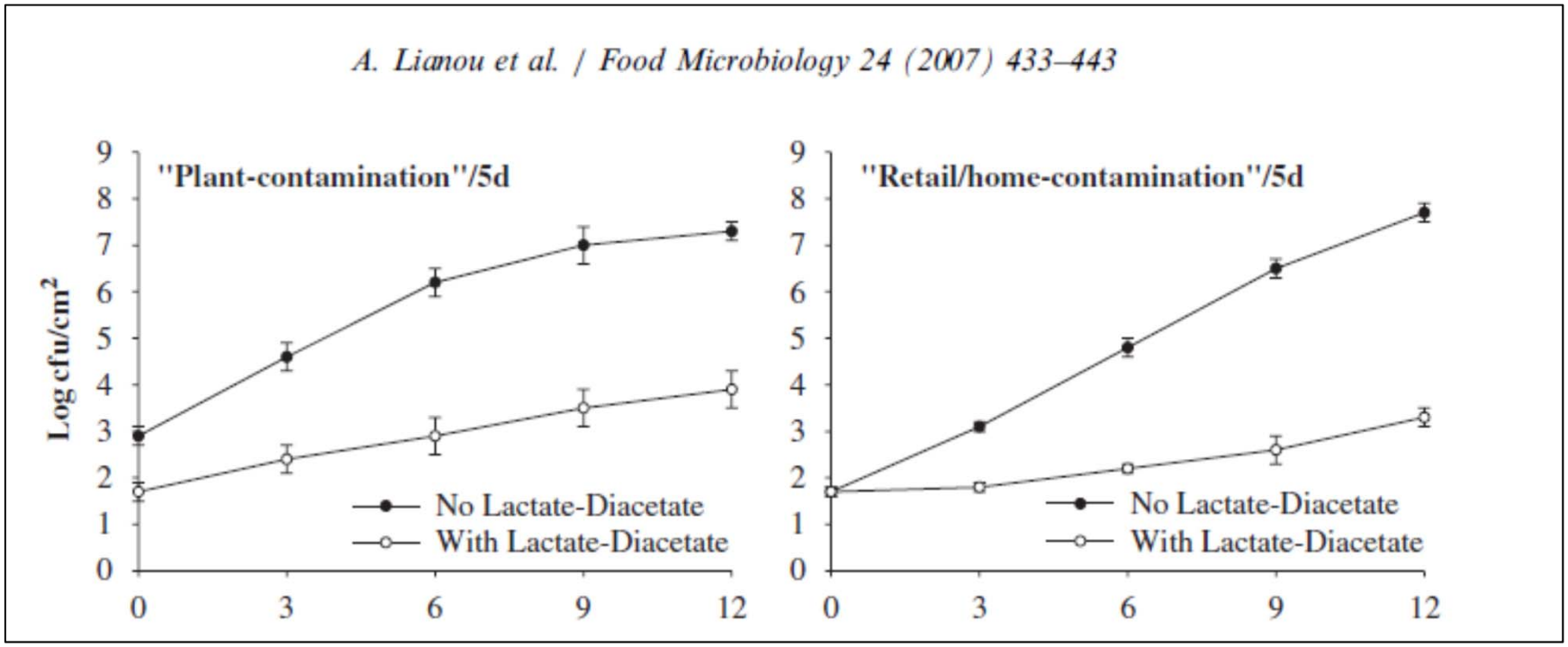
CrossMark

Jihun Kang [a], Matthew J. Stasiewicz [a], Dillon Murray [a,c], Kathryn J. Boor [a], Martin Wiedmann [a], Teresa M. Bergholz [a,b,*]

Journal of Food Protection, Vol. 72, No. 5, 2009, Pages 978–989

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

ABANI K. PRADHAN,[1]* RENATA IVANEK,[1] YRJÖ T. GRÖHN,[1] IFIGENIA GEORNARAS,[2] JOHN N. SOFOS,[2] AND MARTIN WIEDMANN[3]


A. Lianou et al. / Food Microbiology 24 (2007) 433–443
"Plant-contamination"/5d
"Retail/home-contamination"/5d
Log cfu/cm²
No Lactate-Diacetate
With Lactate-Diacetate
0
1
2
3
4
5
6
7
8
9
0
3
6
9
12

Listeria monocytogenes interventions

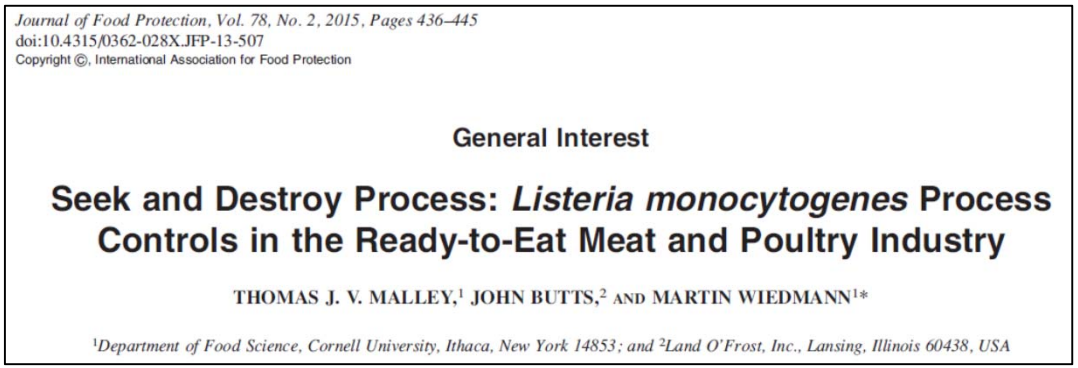
- **Prevent post kill step re-contamination of products (at plants as well as at retail)**
  - Sanitary equipment design
  - Environmental testing
  - Appropriately designed and implemented SSOPs (sanitation standard operating procedures)
  - Post-Lethality Treatment of Product
- Prevent growth:
  -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



Journal of Food Protection, Vol. 78, No. 2, 2015, Pages 436–445
doi:10.4315/0362-028X.JFP-13-507

General Interest

Seek and Destroy Process: *Listeria monocytogenes* Process Controls in the Ready-to-Eat Meat and Poultry Industry

THOMAS J. V. MALLEY,[1] JOHN BUTTS,[2] AND MARTIN WIEDMANN[1]*

[1]*Department of Food Science, Cornell University, Ithaca, New York 14853; and* [2]*Land O'Frost, Inc., Lansing, Illinois 60438, USA*

Designing environmental sampling plans

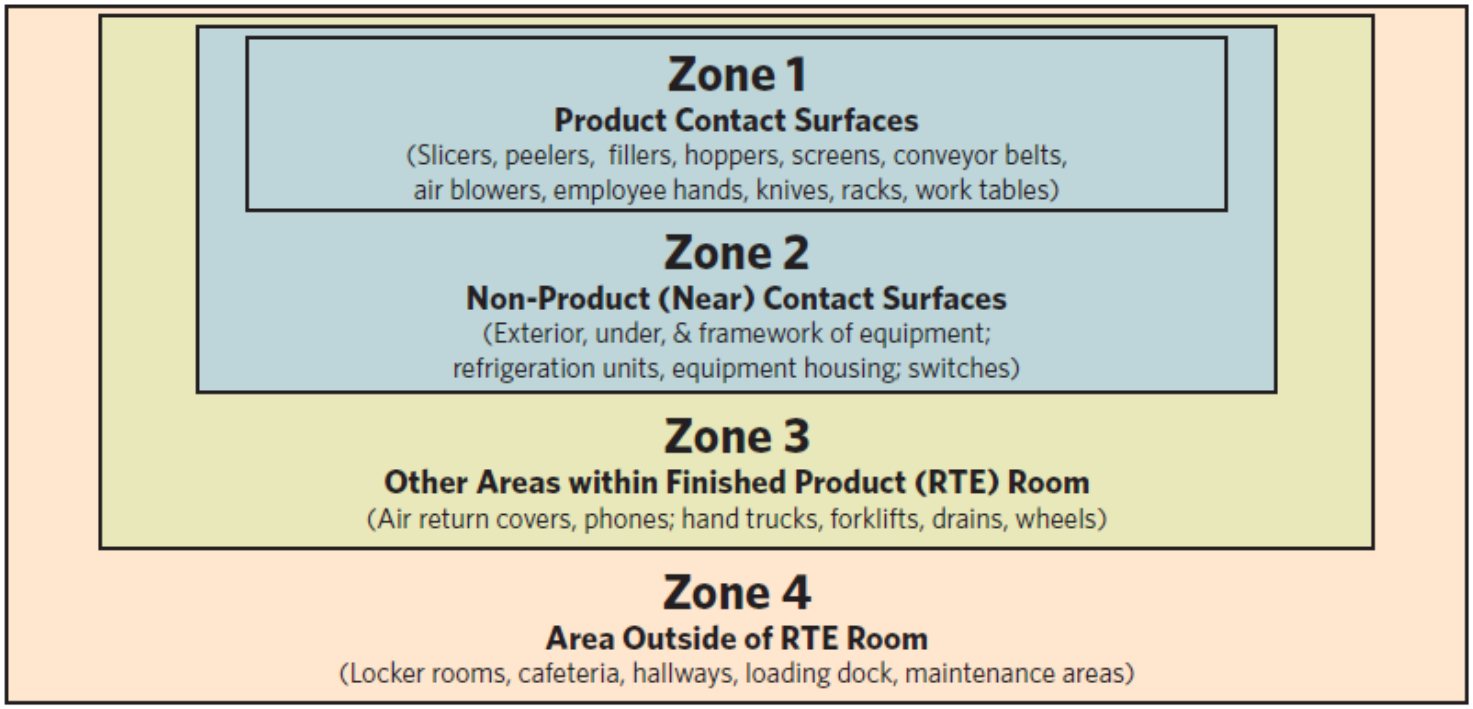
- Effective environmental sampling plans can prevent food contamination before it occurs
- Sampling plans need to be developed individually for each plant
  - Layout, production schedules, facility design
- For many products *Listeria* and *Salmonella* as key targets
  - Environmental sampling for other 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.

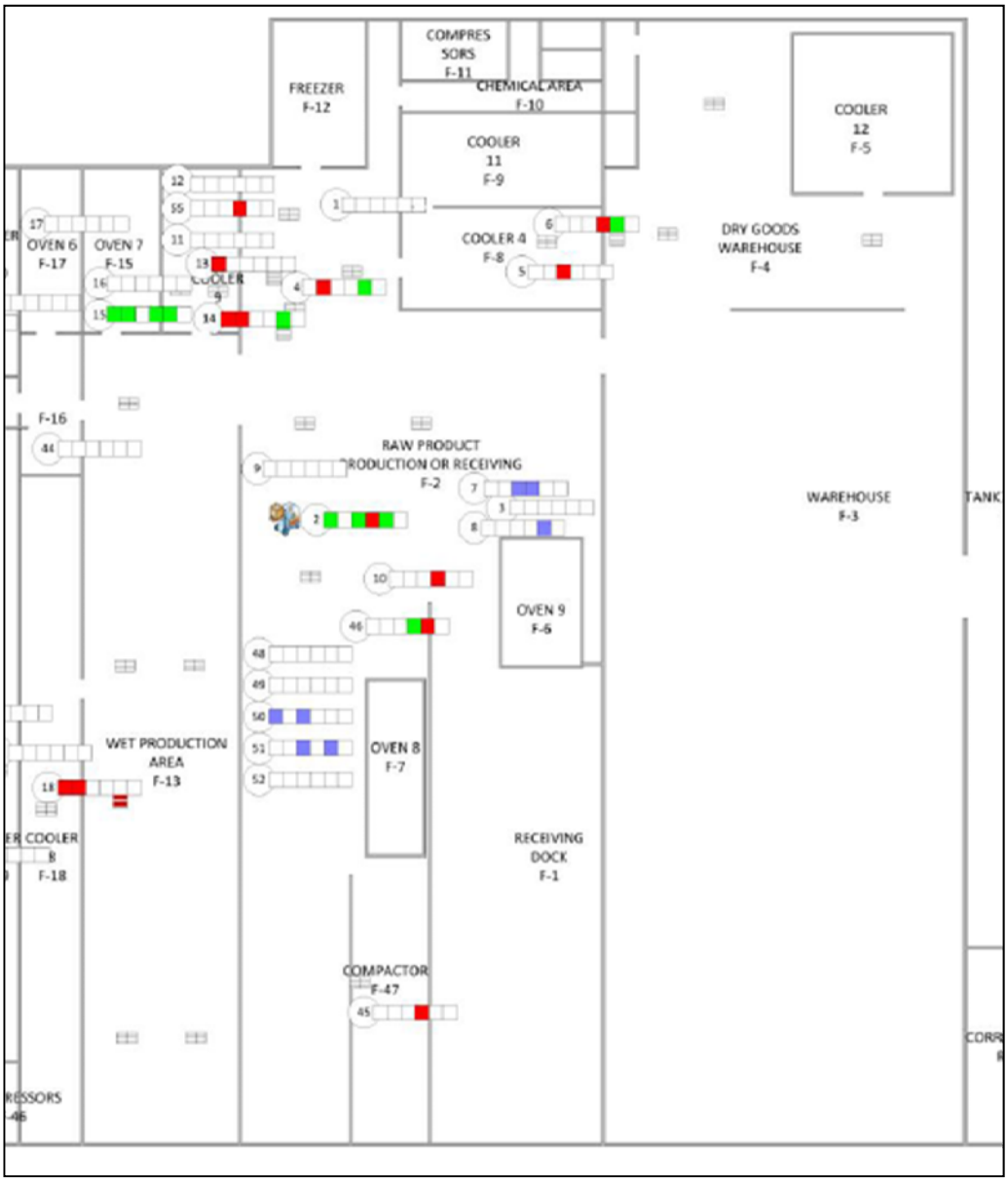
http://www.usdairy.com/trends-and-initiatives/industry-focus/food-safety

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

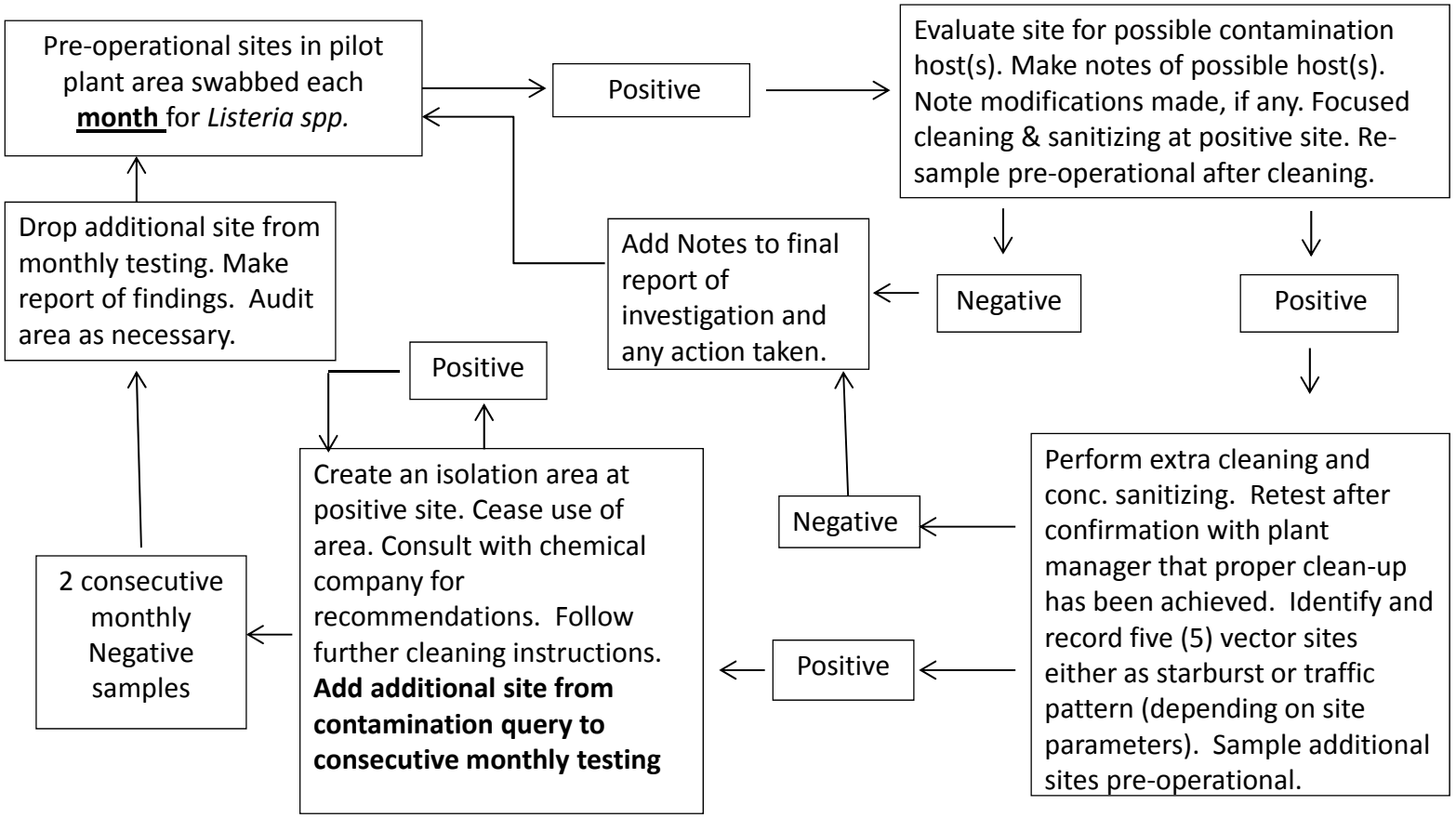
- Review testing results every time results are reported
  - This should include review of at least last 4-8 sampling results to identify trends (e.g., site that has positives with intervening negatives)
  - Take corrections on each positive sample and **document action**
- Organize testing results in 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


COMPRES
SORS
F-11
FREEZER
F-12
CHEMICAL AREA
F-10
COOLER
12
F-5
COOLER
11
F-9
DRY GOODS
WAREHOUSE
F-4
COOLER 4
F-8
OVEN 6
F-17
OVEN 7
F-15
COOLER
9
F-16
RAW PRODUCT
PRODUCTION OR RECEIVING
F-2
WAREHOUSE
F-3
TANK
OVEN 9
F-6
OVEN 8
F-7
WET PRODUCTION
AREA
F-13
COOLER
8
F-18
RECEIVING
DOCK
F-1
COMPACTOR
F-47

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



Need to have specific and separate written records for corrections

Corrective Action:

Corrective Action Record for "Name of Plant"

Date of Environmental Sampling/Swabbing: Plant A 5/15/2013

Site Found Positive: 23 Circle one: *Listeria monocytogenes* or *Listeria* species

Date action taken: 5/23/2013

Detailed description of action taken on positive site:

Thorough 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 tru prevalence
- Outside experts perform sampling

Prevalence data – routine sampling and validation

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

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