Environmental Pathogen Monitoring programs for small dairy processing facilities

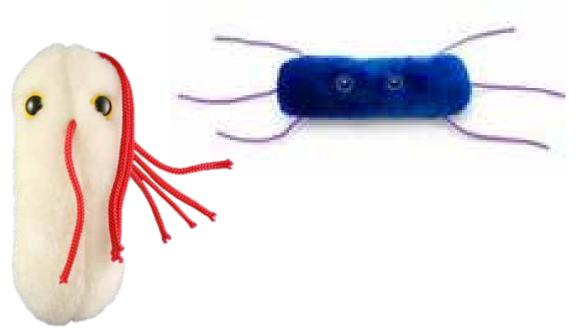
Sarah Beno November 11, 2016 Dairy Practices Council

Outline

- Dairy Pathogens
- Outbreak Detection
- Pathogen Environmental Monitoring Programs

Key Pathogens in Dairy

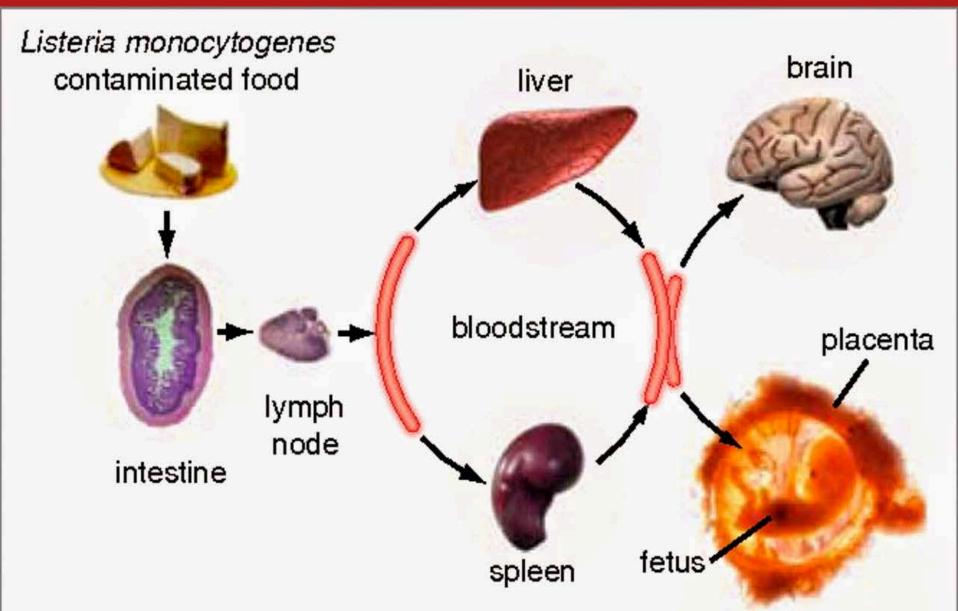
- Listeria monocytogenes
- Salmonella
- Cronobacter



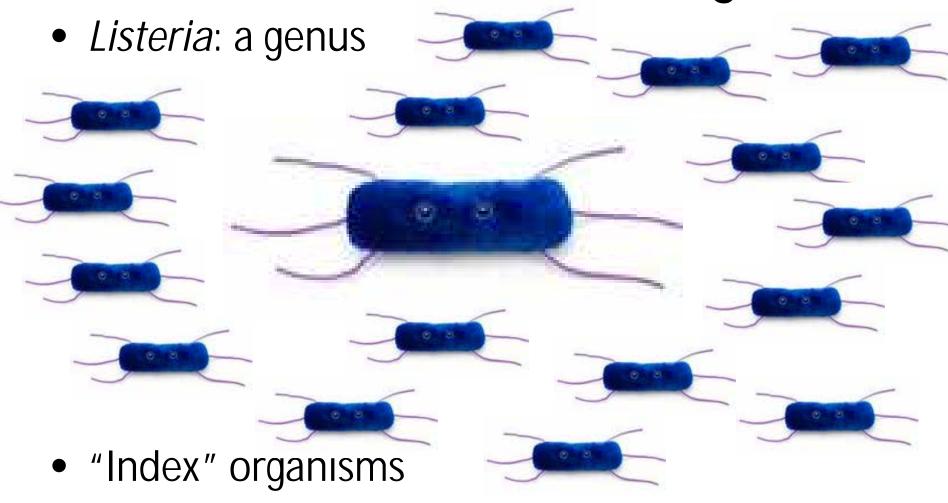


Listeria monocytogenes: Human Pathogen

- 1,300 cases/year in the United States
 - 255 deaths (~20% mortality rate)
- Most commonly affected:
 - Pregnant women
 - Elderly
 - Immunocompromised individuals
 - Infants
- Grows at refrigeration temperatures



Not all Listeria are pathogenic



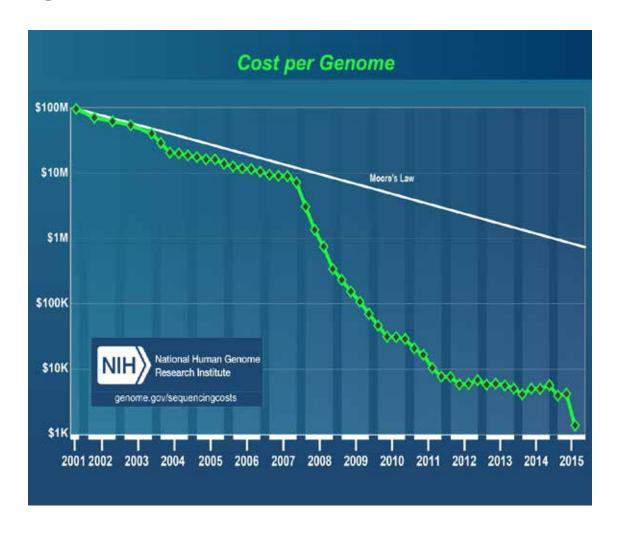
Listeria is everywhere

- 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

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The genome sequence revolution

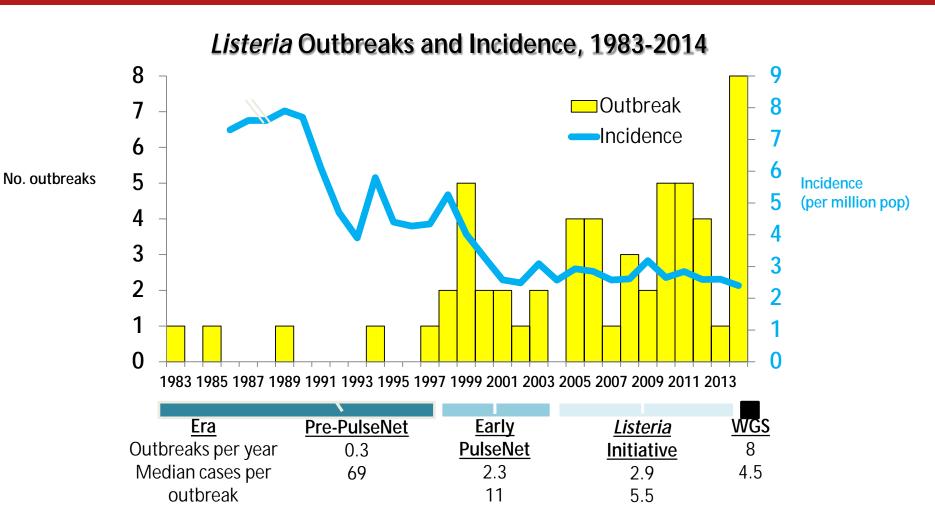


Multistate Outbreak of Listeriosis Linked to Soft Cheeses Distributed by Karoun Dairies, Inc.

Posted September 23, 2015 3:45 PM ET

- 24 people infected with one of the closely related *Listeria* strains have been reported from 9 states since August 8, 2010.
 - 22 people were hospitalized. Five illnesses were pregnancy-related; one resulted in a fetal loss. One death was reported from Ohio.
- FDA isolated *Listeria monocytogenes* from two environmental samples collected in September 2015 from the Central Valley Cheese, Inc. manufacturing facility in Turlock, California. Central Valley Cheese, Inc. manufactures cheese for Karoun Dairies. Whole genome sequencing showed that the two isolates are closely related genetically to isolates from ill people.

In addition, whole genome sequencing showed that 5 Listeria isolates collected in 2010 from the same facility were also closely related genetically to isolates from ill people.



Data are preliminary and subject to change

Outline

- Dairy Pathogens
- Outbreak Detection
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Importance of PEM

- Preventing recalls
 - Reduce risk of an outbreak
- Long term program improvements
- Verification of cleaning and sanitizing program
- Verification of hygienic/transition areas
- Required written records under FSMA

Seek and destroy

- Listeria contamination is often linked to the processing environment
- Implementing a pathogen environmental monitoring program allows tracking of the problem
 - Determining appropriate action toward positives will lead to better control

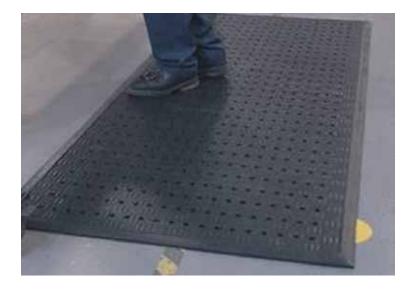
Seek and destroy targets Niches

- Niches
 - Drains, floor cracks, floor-wall junctures, hollow table legs, etc.
 - Used to identify sources
- Transfer points
 - Door handles, hands, wheels on a cart, etc.
 - Help determine how the pathogen is moving through the plant

Growth Niches

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





What are you sampling?



What about when you hear...

"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-athon)

ZONE 4

locker rooms, cafeteria, halls warehouse, loading dock

ZONE 3

phones, hand trucks, forklifts, walls, floor and drains

ZONE 2

nonproduct contact surfaces in close proximity to product (exterior of equipment, chill units, framework, equipment housing)

ZONE 1

product contact surfaces (slicers, conveyors, peelers, strip tables, utensils, racks, work tables, employee hands, dicers, pumps)

http://www.almonds.com/sites/default/files/content/attachments/pem_book.pdf

Testing Considerations

- Prioritize sampling sites
 - After kill step (pasteurization)
 - Before final packaging
- Focus attention on zones 2 & 3.
- Sites with historic positive results
- Reference plant layout, traffic patterns and hygienic zone areas to help determine additional sites

Number of Samples:

Considerations

- Based on facility size
- Aging facilities=increased risk=increased sampling
- Geography of facility
 - Proximity to hazards
- Budget
- Manpower

Methodology

- Sponge
 - used with gloves
 - most commonly used
- Handled sponge
- Crevice Swabs





Results

- Send samples to a certified lab
- Review results upon arrival
- Apply corrective actions
- Monitor trends



Follow-Up and Corrections

- Plant specific and may differ by zone
- Consider frequency of sampling vs. root cause analysis
- Intensified cleaning and sanitizing required
- Long-term trending and analysis
 - Pathogens
 - Index and indicator organisms

Corrective Actions

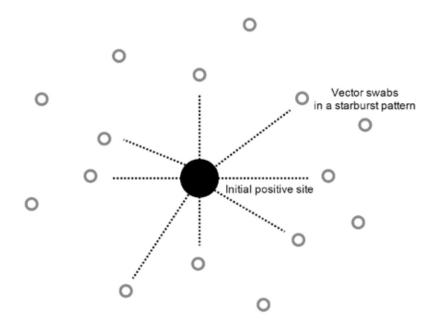
 Positive samples need follow-up: re-testing and vector swabbing

Shutting down problem areas

Written records

Vector Swabbing

Additional samples should be taken from environmental area that showed positive results



Managing a Problem Area

		May	June	July	July	August	September	October	November	December	January	February	March	April
Site#	Zone	5/22/14	6/17/14	7/1/14	7/9/14	8/11/14	9/16/14	10/8/14	11/13/14	12/10/14	1/13/15	2/4/15	3/11/15	4/21/15
1	3	Neg	Neg	NS	L. spp.	Neg	Neg	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg
2	3	Neg	Neg	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3	3	Neg	Neg	NS	L. mono	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
4	3	Neg	Neg	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5a	3	Neg	Neg	NS	Neg	Neg	L. mono	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5b	3	Neg	Neg	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5c	3	Neg	L. mono	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6	3	Neg	Neg	NS	Neg	Neg	Neg	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg
7	3	L. spp.	Neg	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
8	3	L. spp.	Neg	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
9	3	Neg	L. mono, L. sp	L. spp.	L. spp.	L. mono., L. s	NS	NS	NS	NS	NS	NS	NS	NS
9 9a	3	Neg NS	L. mono, L. sp	L. spp.	L. spp.	L. mono., L. s	NS Neg	NS Neg	NS Neg	NS Neg	NS Neg	NS Neg	NS Neg	NS Neg
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9a 10	3	NS Neg	NS Neg	NS NS	NS Neg	NS Neg	Neg Neg	Neg Neg	Neg Neg	Neg Neg	Neg Neg	Neg Neg	Neg Neg	Neg Neg
9a 10 11	3 3	NS Neg Neg	NS Neg L. spp.	NS NS Neg	NS Neg Neg	NS Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg Neg	Neg Neg L. spp.
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9a 10 11 12a 12b 13a 13b	3 3 3 3 3 3 3	NS Neg Neg Neg Neg Neg Neg Neg Neg	Neg L. spp. Neg Neg Neg Neg Neg Neg	NS Neg NS NS NS NS NS NS	NS Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Neg Neg Neg Neg	Neg Neg Neg Neg Neg Ns Ns	Neg Neg L. spp. Neg Neg Ns NS NS
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Date of Environmental Sampling/Swabbing: 5/15/2013
Site Found Positive: Circle one: (Listeria monocytogenes) or Listeria species or Salmonella
Date action taken: <u>5/23/2013</u>
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

Investigation of a Positive Finding

- Review infrastructure and equipment in the area
- Targeted cleaning
- Review records
- Corrective action
 - Location (zone)
 - Trends

Tracking Results

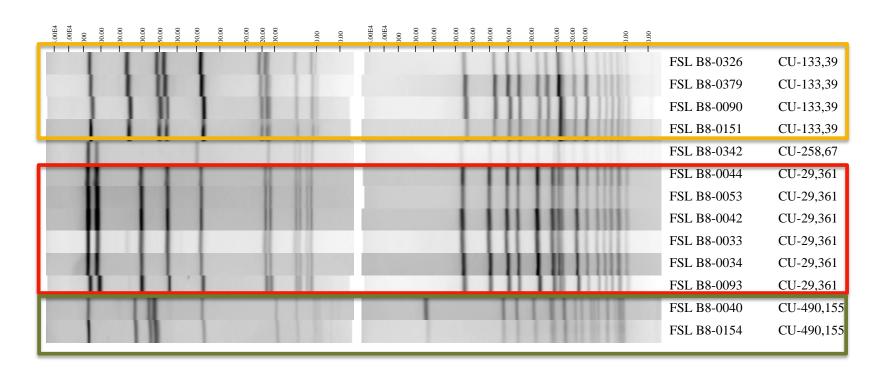
- Track and trend environmental data
- Collect and record data to provide actionable information
- Establish a baseline to monitor trends
- Detection of a pathogen in Zone 1 requires immediate action as product may be contaminated

DNA fingerprinting and persistence

- Pathogens can persist in the environment for years
- DNA fingerprinting can be used to identify sites where the pathogen may be persisting

DNA Fingerprinting

PFGE-AscI PFGE-ApaI



Persistence?

	May	June	July	August	September	October	November	December	January	February	March	April
Site #	5/22/14	6/19/14	7/22/14	8/11/14	9/15/14	10/9/14	11/19/14	12/9/14	1/14/15	2/11/15	3/11/15	4/13/15
1a	L. mono	L. spp.	L. mono	Neg	Neg	Neg	L. spp.	Neg	Neg	Neg	Neg	Neg
1b	L. spp.	L. spp.	Neg	Neg	Neg	Neg	L. spp.	L. mono	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3	L. mono & L.	I ann	Nor	Now	Nor	Nor	Now	Nor	I	Nor	Now	Now
4	Spp.	L. spp.	Neg Neg	Neg	Neg	Neg Neg	Neg Neg	Neg	L. spp.	Neg Neg	Neg Neg	Neg Neg
5	Neg Neg	Neg L. mono		Neg	Neg	ľ		Neg	Neg		Neg	
6	L. mono	L. mono	Neg L. mono	Neg Neg	Neg Neg	Neg Neg	Neg Neg	Neg L. mono	Neg Neg	Neg Neg	L. mono	Neg Neg
7	L. spp.	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	L. mono	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
8b	L. mono & L. spp.	Neg	Neg	Neg	L. spp.	Neg	Neg	L. spp.	Neg	Neg	Neg	Neg
8c	L. mono & L. spp.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
9	Neg	L. spp.	Neg	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg	L. spp.	Neg
10	Neg	L. spp.	Neg	Neg	Neg	L. spp.	Neg	Neg	Neg	Neg	L. spp.	Neg
15	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
16	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
17	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
18	NS	L. spp.	Neg	NS	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
19	NS	Neg	Neg	Neg	L. spp.	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg
24a	NS	NS	NS	NS	Neg	NS	NS	NS	NS	NS	NS	NS
24b	NS	NS	NS	Neg	NS	NS	NS	NS	NS	NS	NS	NS
24c	NS	Neg	NS	Neg	NS	NS	NS	NS	NS	NS	NS	NS



(A)													
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-F	D1)												
Raw salmon room, Drain (SB-F	D2)												
Raw salmon room, 3 floor mats													
RawSalmon room, mats- post cl	leaning												
Raw salmon room, platic pallet													
Raw Salmon room, pallet, post	cleaning												
Raw salmon room, pallet jacket													
E8: Apron, employee in raw are				1053A	-	1053A	1025A		1053A	-	1053A	-	
Incoming raw material packagir													
Finished Product Area	s												
E1: Trench Drain, processing	L.spp			116-693	L.spp	L.spp	L.spp		L.spp	L.spp		L.spp	
E2: Trench Drain, smoke roon													
Smoke room trench drain, in us													
E4: Cart wheels, for box trans								L.spp		L.spp			
E5: Floor, under conveyor beli						L.spp	L.spp	L.spp	L.spp	L.spp	L.spp		
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Finish room, floor mats #2													L.spj
Finish room, floor mats, reg. Cl	lean												шарр
Finish room, floor mats, reg. Cl													L.spp
Finish room, 1200 ppm Quat, w													L.spp
Finish room, 1200 ppm Quat, w													2
Bootdip valve cover, processin			L.spp										_
E6: Platform under Geba #1 sh			L.spp				L.spp	L.spp	L.spp				
E9: Sliding door handle, skinni		i.		Ė				1053A	L.spp			i.	
Food Contact Surfaces								10000	аларр				
E7: Gloved hands, finish prod.								_	-			-	
E10: Skinning machine	L.spp		L.spp				L.spp	L.spp	L.spp		Lenn		
E11: Geba #5 slicer			L.spp				L.spp	L.spp	L.spp		L.spp		
E11: Gena #5 sncer E12: 20/20 vac belt	L.spp		Ċ					i.	<u> </u>	i i	L.spp	i.	
E12. 20/20 VAC 09R	6 of 6	6 of 6	60f 6	L.spp 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	
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Park Product Samples 1800, 10 10 10 10 10 10 10	Tiant A2	2/26/01	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	121201	1/29/02	2/25/02			4/16/02		6/10/02	7/1/02			9/1//02	10/15/02	11/12/02	
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Raw Salmon room, pallet, pest cleaning E8: Agreen, employee in raw are to be a complex of the compl	RawSalmon room, mats- post cle	aning															1053A	1053A								
Rew salmon room, pallet jacket hondle Be Agreen, employee in raw and the control of the control	Raw salmon room, platic pallet														1053A	-			-	L.spp	-	-	-	-		-
Est Apron, employee in raw are to complete in raw and the complete in raw are to complete i	Raw Salmon room, pallet, post cl	leaning																								
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Finished Product Areas	E8: Apron, employee in raw are	1062D			1053A	-	1053A	1025A	-	1053A	-	1053A				-		-	-		-	-	-	-		
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Ext. Cart wheels, for box trans L.sp	E2: Trench Drain, smoke room					-														L.spp			L.spp			L. spp
Es: Floor, under conveyor belt L.spp	Smoke room trench drain, in use	,															1053A									
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Enish room, 1200 ppm Quat, weekend Bootdip valve cover, processin C. L.spp L	Finish room, floor mats, reg. Cle	an												L.spp												
Finish room, 1200 ppm Quat, weekend																										
Bootdip valve cover, processin - L.spp																										
E6: Platform under Geba #1 sh				L.spp																						
E9: Stiding door handle, skinnir. L.spp								L.spp	L.spp	L.spp																
Food Contact Surfaces E7: Gloved hands, finish prod. - - - - - - - - -		L.spp																								
E7: Gloved hands, finish prod. - - - - - - - - -																										
E10: Skinning machine																								1053		L. snn
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Research Paper

Development and Validation of Pathogen Environmental Monitoring Programs for Small Cheese Processing Facilities

SARAH M. BENO, MATTHEW J. STASIEWICZ,† ALEXIS D. ANDRUS, ROBERT D. RALYEA, DAVID J. KENT, NICOLE H. MARTIN, MARTIN WIEDMANN,* AND KATHRYN J. BOOR

Department of Food Science, Cornell University, 358 Stocking Hall, Ithaca, New York 14853, USA

MS 16-241: Received 1 June 2016/Accepted 18 August 2016

ABSTRACT

Pathogen environmental monitoring programs (EMPs) are essential for food processing facilities of all sizes that produce ready-to-eat food products exposed to the processing environment. We developed, implemented, and evaluated EMPs targeting Listeria spp. and Salmonella in nine small cheese processing facilities, including seven farmstead facilities. Individual EMPs with monthly sample collection protocols were designed specifically for each facility. Salmonella was detected in only one facility, with likely introduction from the adjacent farm indicated by pulsed-field gel electrophoresis data. Listeria spp. were isolated from all nine facilities during routine sampling. The overall Listeria spp. (other than Listeria monocytogenes) and L. monocytogenes prevalences in the 4,430 environmental samples collected were 6.03 and 1.35%, respectively. Molecular characterization and subtyping data suggested persistence of a given Listeria spp. strain in seven facilities and persistence of L. monocytogenes in four facilities. To assess routine sampling plans, validation sampling for Listeria spp. was performed in seven facilities after at least 6 months of routine sampling. This validation sampling was performed by independent individuals and included collection of 50 to 150 samples per facility, based on statistical sample size calculations. Two of the facilities had a significantly higher frequency of detection of Listeria spp. during the validation sampling than during routine sampling, whereas two other facilities had significantly lower frequencies of detection. This study provides a model for a science- and statistics-based approach to developing and validating pathogen EMPs.

Key words: Listeria; Persistence; Raw milk; Salmonella

Project Outline

- 2 years
- 9 processing facilities
- > 5,000 samples
- Zones 2-4
- Routine vs. Validation sampling

Listeria prevalence in small cheese processing facilities

TABLE 1. Summary of facilities sampled

							onmental samples tes (LS) and L. monocy	Environmental samples tested for Salmonella		
Facility ^a	Environment ^b	Approx production vol (lb milk/yr)	Processing schedule	No. of sampling times	Sampling period	Total no. of samples	No. (%) of LS samples ^d	No. (%) of LM samples	Total no. of samples	No. (%) of positive samples
A	Farmstead	874,000	Year round	24	Apr. 2013-Mar. 2015	917	29 (3.2)	12 (1.3)	156	0 (<0.6)
C	Farmstead	30,000	Year round	10	Apr. 2013-Jan. 2014	257	32 (12)	15 (5.8)	91	1 (1.1)
D	Farmstead	21,000	Seasonal	9	Apr. 2013-May 2014	202	3 (1.5)	2 (0.99)	82	0 (<1.2)
E	Farmstead	420,000	Year round	24	Apr. 2013-Mar. 2015	1,000	127 (12.7)	7 (0.7)	185	0 (<0.5)
F	Farmstead	NA^e	Seasonal	14	Apr. 2013-Oct. 2014	422	4 (0.9)	0 (<0.2)	74	0 (<1.4)
G	Farmstead	860,000	Year round	12	May 2014-Apr. 2015	596	25 (4.2)	8 (1.3)	NS ^f	NS
H	Stand alone	500,000	Year round	12	May 2014-Apr. 2015	259	35 (13.6)	13 (5.02)	NS	NS
I	Stand alone	35,000	Year round	12	May 2014-Apr. 2015	501	1 (0.2)	0 (<0.2)	NS	NS
J	Farmstead	70,000	Year round	12	June 2014-May 2015	276	11 (4.0)	3 (1.1)	NS	NS

^a Facility B dropped out of the study prior to the first sampling and therefore is not included in the facility list. Facility I is the Cornell University cheese processing facility.

^b Farmstead facilities are located within 100 m of a dairy farm that provides raw milk for processing.

^c Includes samples collected during routine and verification sampling.

^d LS numbers do include samples positive for L. monocytogenes.

NA, not available. Because the production volume for facility F number was not provided, we estimated that this facility processes <50,000 lb of milk per year.

f NS, not sampled.

How do you know your program is working?

Facility	Prevalence (from routine)	Prevalence (from validation)
А	5.12% (34/664)	1.33% (2/150)
Е	11.97% (88/735)	10% (6/60)
F	< 0.3% (0/334)	6% (3/50)
G	8.33 % (19/228)	2.35% (2/85)
Н	22.64% (24/106)	8% (2/50)
J	0.94 % (1/106)	14% (7/50)

Take Home Messages

- *Listeria* is a genus of 17 species. *L. monocytogenes* is a pathogen.
- We are detecting more outbreaks due to Whole Genome Sequencing
- Look for *Listeria*. Find it. Get rid of it.



Thank You!



Sarah Beno smb489@cornell.edu