



Cornell University
College of Agriculture and Life Sciences

Environmental Pathogen Monitoring programs for small dairy processing facilities

Sarah Beno

November 11, 2016

Dairy Practices Council



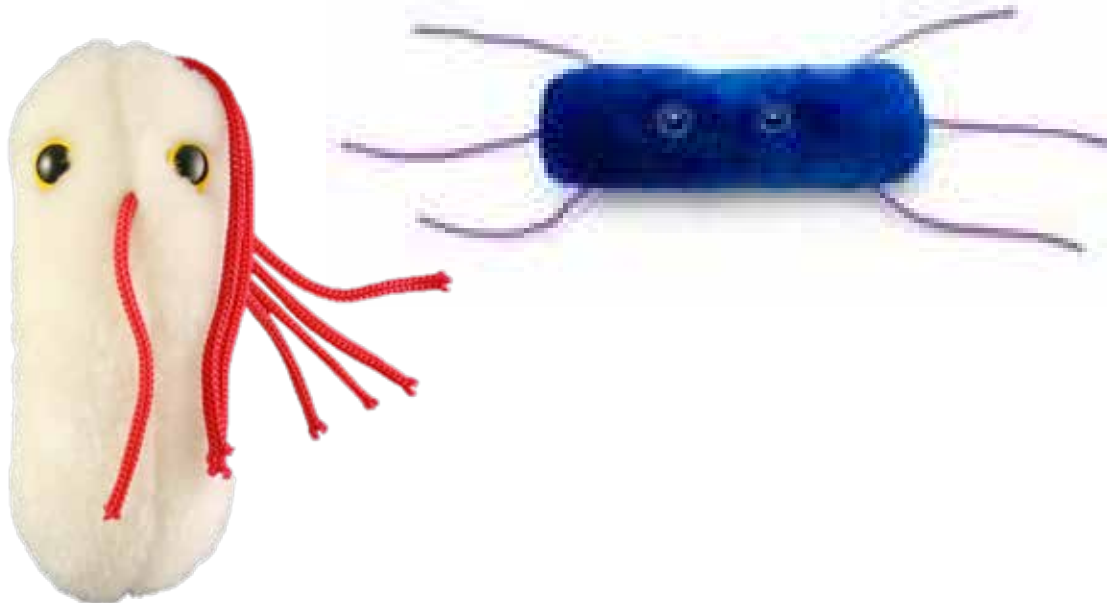
Outline

- 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





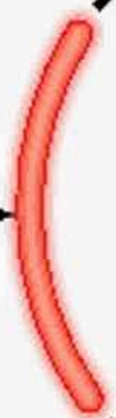
Listeria monocytogenes
contaminated food



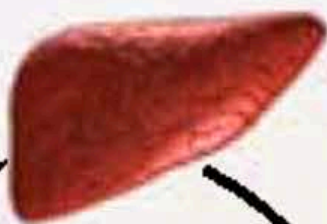
intestine



lymph
node



bloodstream



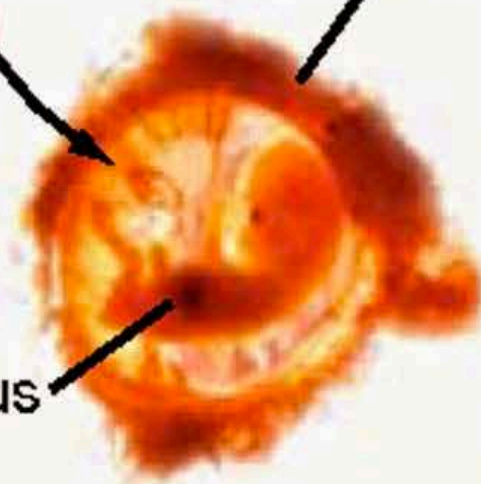
liver



spleen



brain



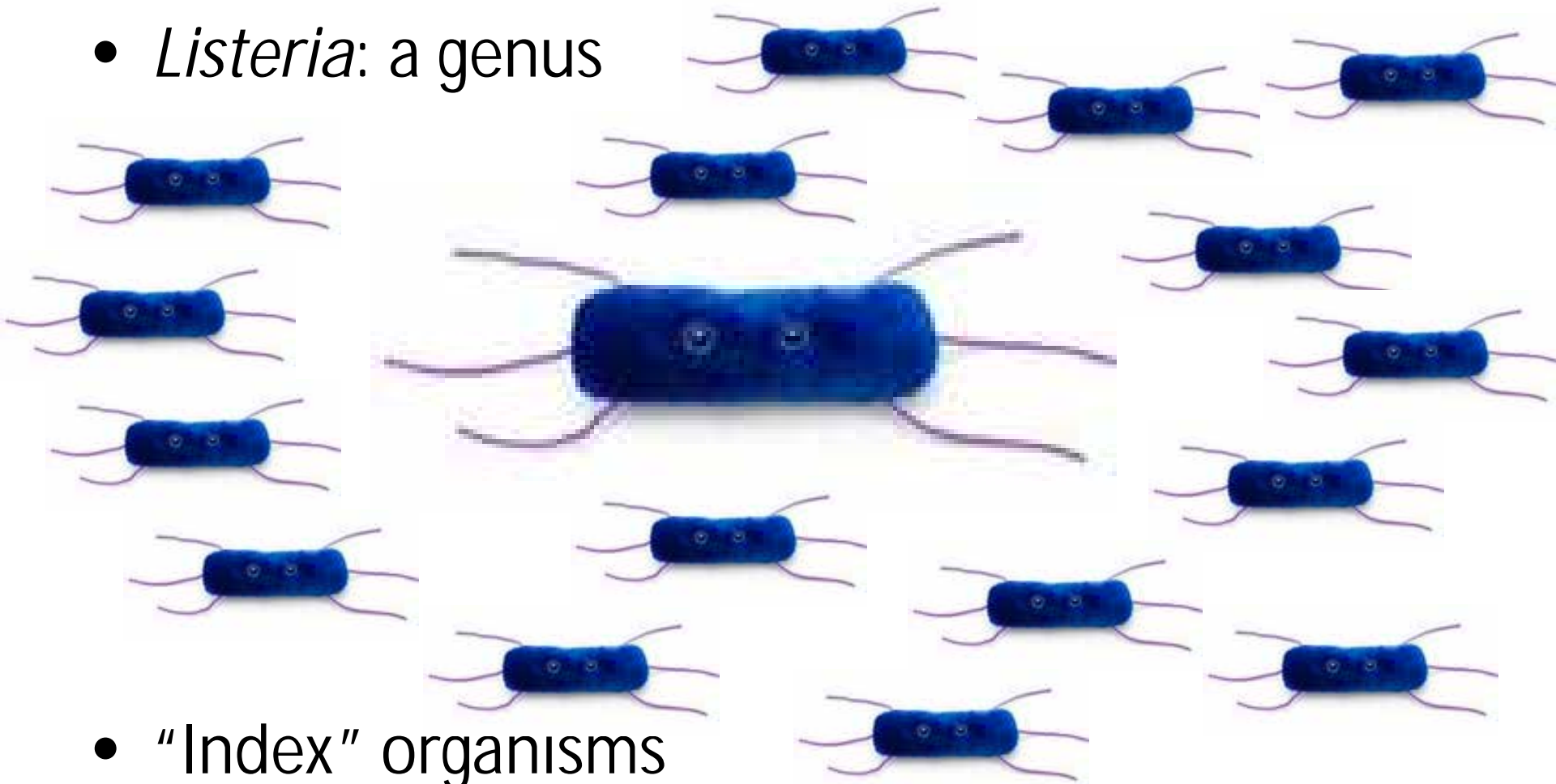
placenta

fetus



Not all *Listeria* are pathogenic

- *Listeria*: a genus



- “Index” organisms



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

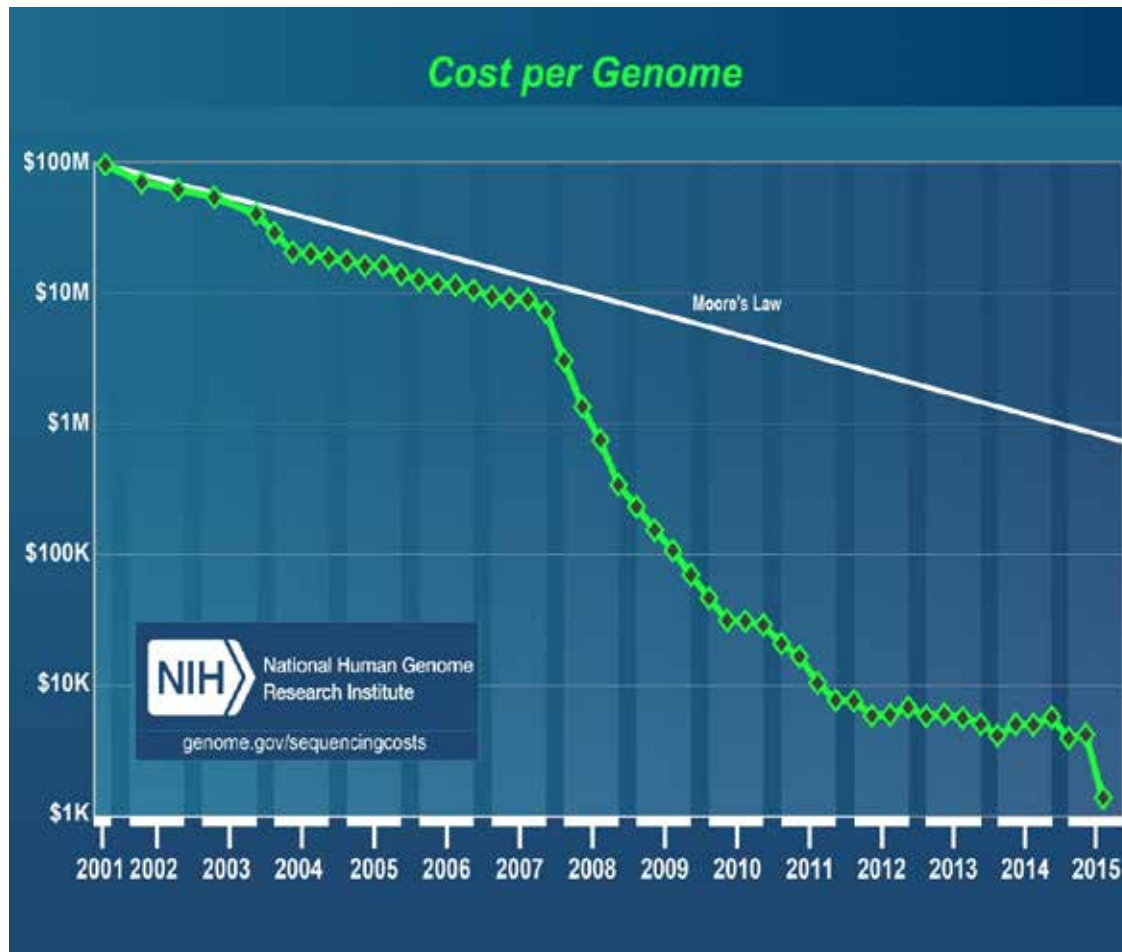


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The genome sequence revolution





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

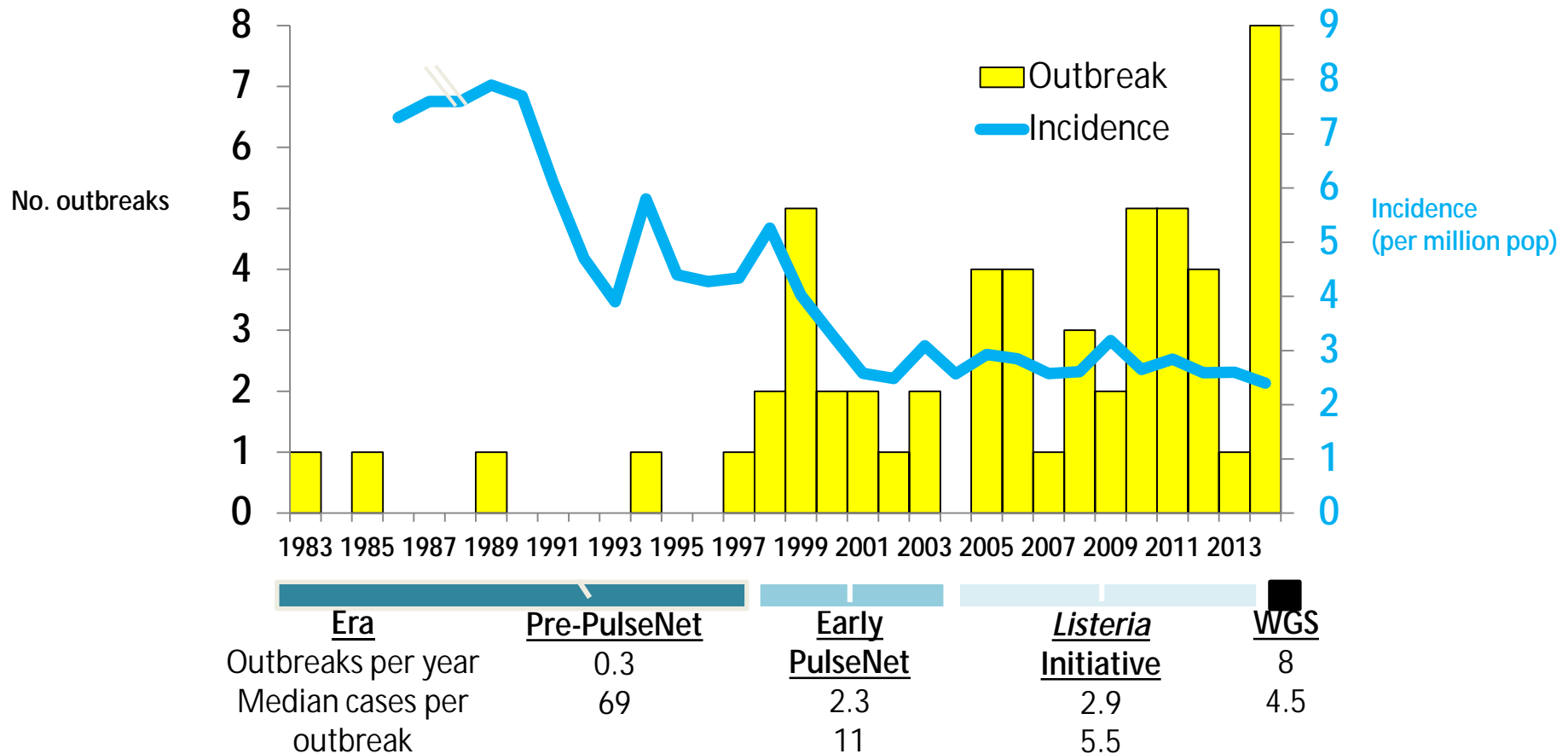
Posted September 23, 2015 3:45 PM ET

- 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.



Listeria Outbreaks and Incidence, 1983-2014



Data are preliminary and subject to change



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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



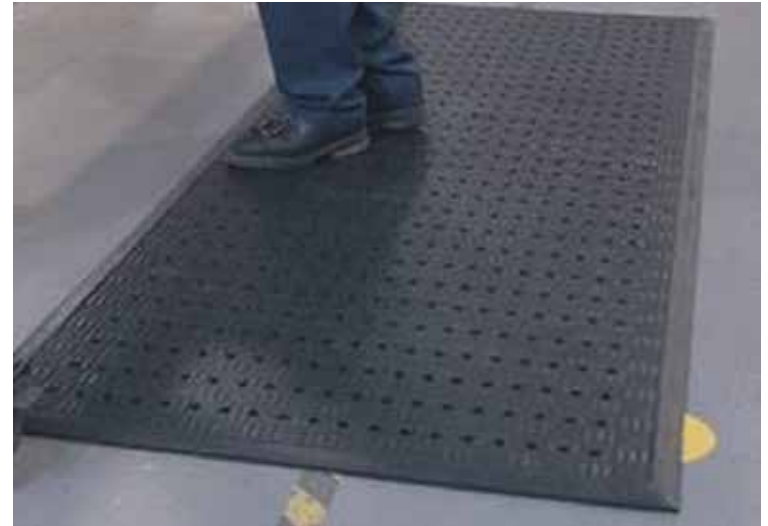
Seek and destroy targets Niches

- 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.





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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-a-thon)



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)



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



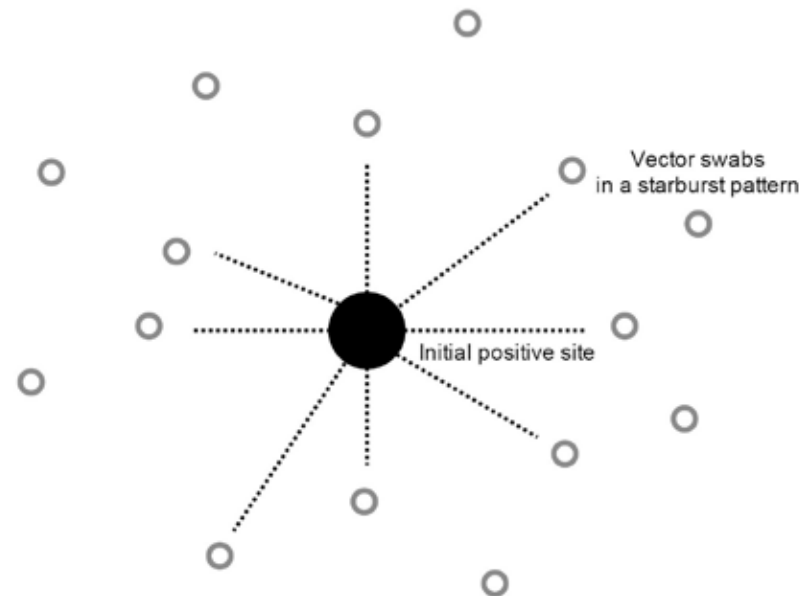
Corrective Actions

- 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

[illegible]



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

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

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



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

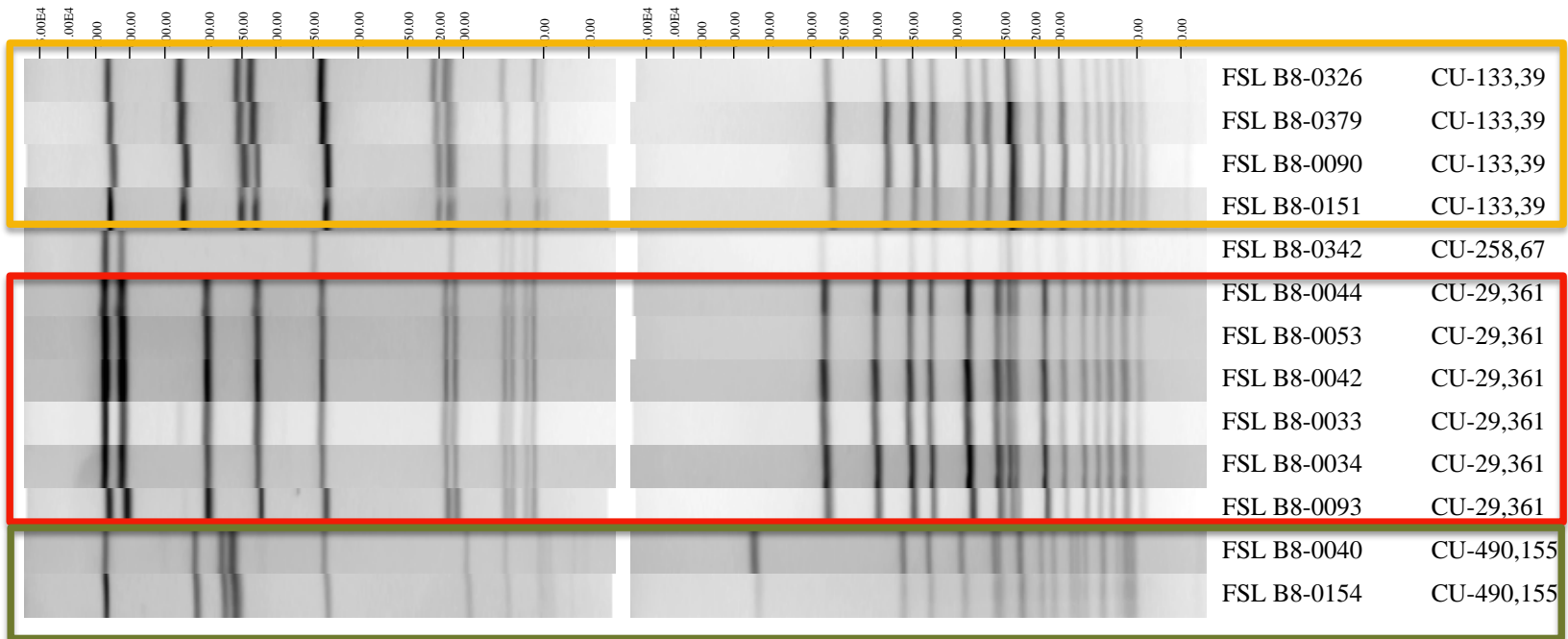
- Pathogens can persist in the environment for years
- DNA fingerprinting can be used to identify sites where the pathogen may be persisting



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. spp.	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg	L. spp.	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5	Neg	L. mono	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6	L. mono	L. mono	L. mono	Neg	Neg	Neg	Neg	L. mono	Neg	Neg	L. mono	Neg
7	L. spp.	L. spp.	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
8a	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

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, plastic 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 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 trans 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

-

Boothip valve cover, processing

- - L.spp

E6: Platform under Geba #1 sh - - - - - L.spp L.spp L.spp - - -

E9: Sliding door handle, skinn L.spp - - - - - 1053A L.spp - - -

Food Contact Surfaces

E7: Gloved hands, finish prod. l - - - - - - - - - - -

E10: Skinning machine L.spp - L.spp - - - L.spp L.spp L.spp - L.spp -

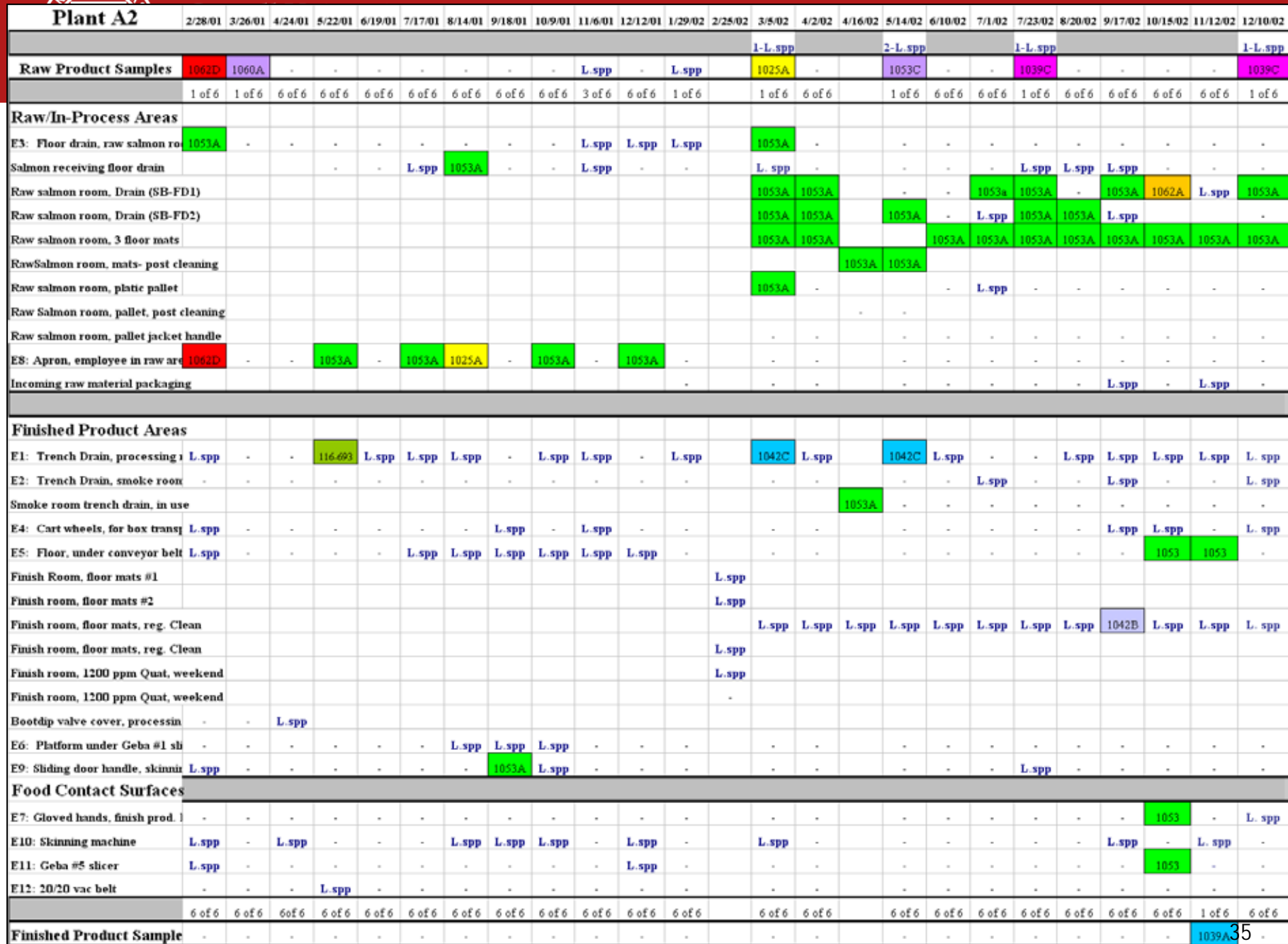
E11: Geba #5 slicer L.spp - - - - - - - - L.spp -

E12: 20/20 vac belt - - - L.spp - - - - - - - -

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Finished Product Sample

- - - - - - - - - - - -





Research Paper

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

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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

Facility ^a	Environment ^b	Approx production vol (lb milk/yr)	Processing schedule	No. of sampling times	Sampling period	Environmental samples tested for <i>Listeria</i> spp. (LS) and <i>L. monocytogenes</i> (LM)			Environmental samples tested for <i>Salmonella</i>	
						Total no. of samples ^c	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*.

^e 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)
A	5.12% (34/664)	1.33% (2/150)
E	11.97% (88/735)	10% (6/60)
F	<0.3% (0/334)	6% (3/50)
G	8.33% (19/228)	2.35% (2/85)
H	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.





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Thank You!



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