

Case Study: Foodborne Outbreak (Cantaloupe)



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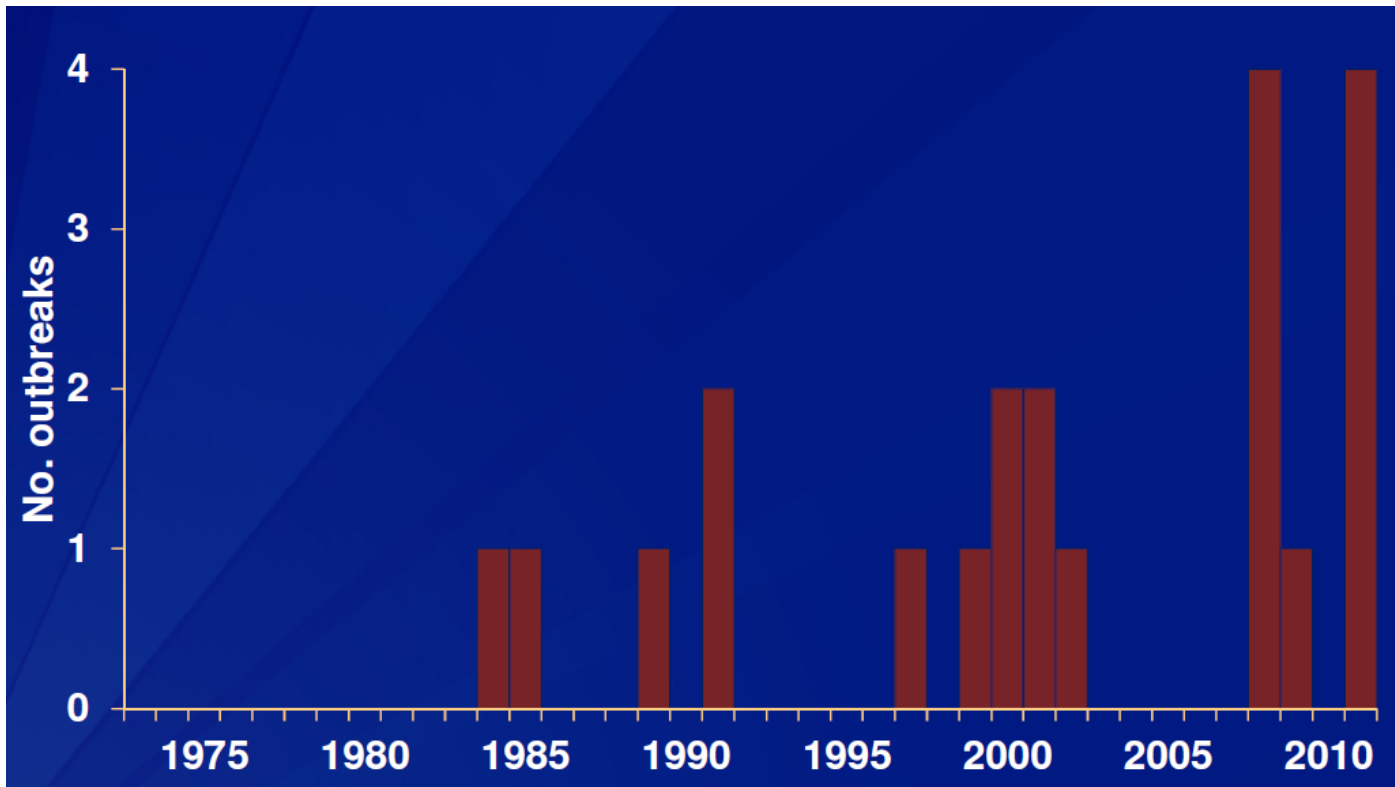
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Issues with Cantaloupe

- ❖ 23 outbreaks associated with Cantaloupes from 1985-2012 resulting in:
 - ❖ 1520 illnesses
 - ❖ 297 hospitalizations
 - ❖ 39 deaths (33 in 2011 *Listeria* outbreak, 3 in 2012 *Salmonella* outbreak)

Issues with Cantaloupe



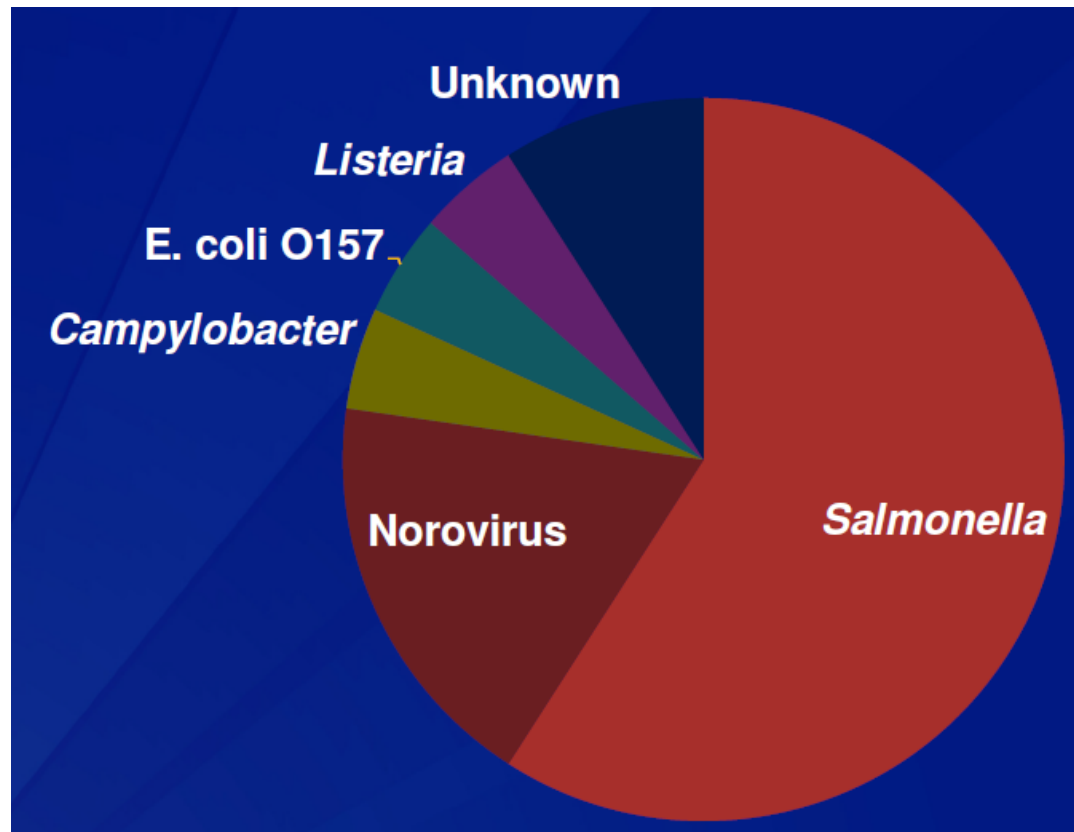
Gould 2012

Issues with Cantaloupe

	Cantaloupe	All Outbreaks
Hospitalizations	16%	3%
Deaths	3%	0.07%
Illnesses (Median)	23	20
Multistate Outbreaks	52%	1-2%
Imported food	33%	0.4%

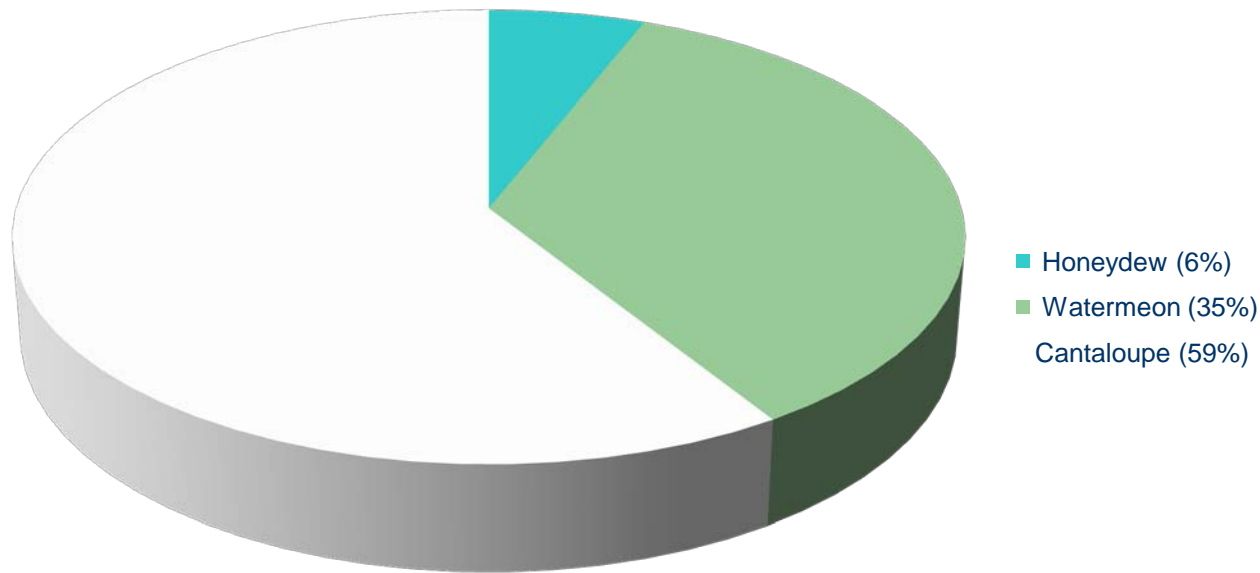
- ❖ Cantaloupe outbreaks more severe and widespread than other foodborne disease outbreaks

Issues with Cantaloupe



Gould 2012

Issues with Cantaloupe



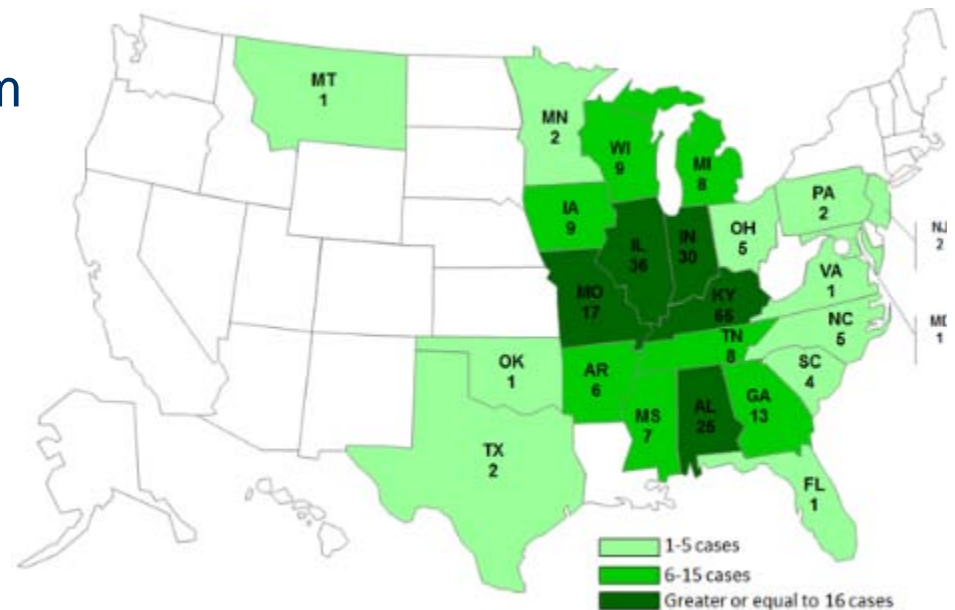
- ❖ More than half of Melon outbreaks are due to Cantaloupe
- ❖ 35 Melon outbreaks from 1973-2012

Issues with Cantaloupe

- ❖ Cantaloupe illnesses are associated more with women and older adults
- ❖ Many Cantaloupe outbreaks are linked to imported fruit
- ❖ Contamination occurs at all points along the farm to fork continuum

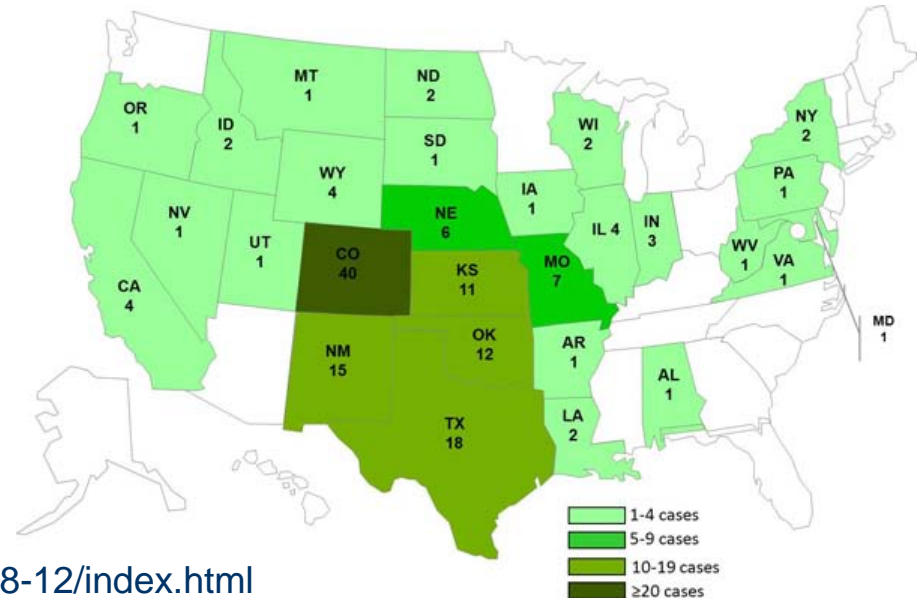
Issues with Cantaloupe

- ❖ 2012 Outbreak
 - ❖ 261 Illnesses
 - ❖ 3 deaths
 - ❖ Cases in 24 states
 - ❖ *Salmonella* Typhimurium
 - ❖ *Salmonella* Enteritidis



Issues with Cantaloupe

- ❖ 2011 Outbreak
 - ❖ 147 illnesses
 - ❖ 33 deaths, 1 miscarriage
 - ❖ Most deadly outbreak in 25 years
 - ❖ Cases in 28 states
 - ❖ *Listeria monocytogenes*



Background on *Listeria monocytogenes*

- ❖ 1911: recognized as animal pathogen
- ❖ 1926: *Bacterium monocytogenes*
- ❖ 1927: *Listerella monocytogenes*
- ❖ 1929: First reported as human pathogen
- ❖ 1940: *Listeria monocytogenes*
- ❖ 1980s: recognized as foodborne pathogen

Listeriosis

- ❖ Low incidence
- ❖ Human cases generally sporadic
- ❖ Source and route often unknown
- ❖ Susceptibility to infection: high risk populations
 - ❖ Pregnant women
 - ❖ Elderly (>60 years)
 - ❖ Newborns and infants
 - ❖ Immunocompromised
- ❖ Mortality
 - ❖ 15 % in typical foodborne outbreaks (Scallan *et al.* 2011):
cantaloupe outbreak: 22.4%

Symptoms

- ❖ Healthy individuals
 - ❖ Symptomless or "flu-like" condition
- ❖ Susceptible individuals
 - ❖ Meningitis
 - ❖ Stillbirth, abortion
 - ❖ Septicaemia
 - ❖ Endocarditis
 - ❖ Pneumonia
 - ❖ Urethritis
- ❖ More generic symptoms include fever, muscle aches, and diarrhea

Incidence in foods



- ❖ Meat and meat products (10 - 60%)
- ❖ Fish and fish products (20 - 30%)
- ❖ Salads and vegetables (10 - 20%)
- ❖ High risk foods: deli meat, raw milk, soft cheeses, seafood
- ❖ Low risk foods: fruits and vegetables?
 - ❖ 2009: sprouts
 - ❖ 2010: cut celery
 - ❖ 2011: cantaloupes

Timeline of Events: Multistate Outbreak of Listeriosis Linked to Whole Cantaloupes from Jensen Farms in Colorado—United States, 2011

Outbreak Identification and Source Implication

September 2
The Colorado Department of Public Health and Environment (CDPHE) notifies the Centers for Disease Control and Prevention (CDC) of seven ill persons with listeriosis (*Listeria* infection) reported since 8/29/11.

September 6
PulseNet defines outbreak strains in CO patients and identifies an ill person in NE and another in TX, each infected with an outbreak strain of *Listeria*. Initial interviews of 7 ill persons in CO with standard CDC questionnaire find all ate cantaloupe. Three ill persons report eating cantaloupe from one region in Southeastern CO ("Rocky Ford").

September 7
CDC begins coordinating the multistate investigation.

September 8
CDC asks states to use the supplemental questionnaire created by CDPHE to learn more about cantaloupe eaten by ill persons. Food and Drug Administration (FDA) begins investigation with initial information collected by CDPHE to identify sources of cantaloupe eaten by ill persons.

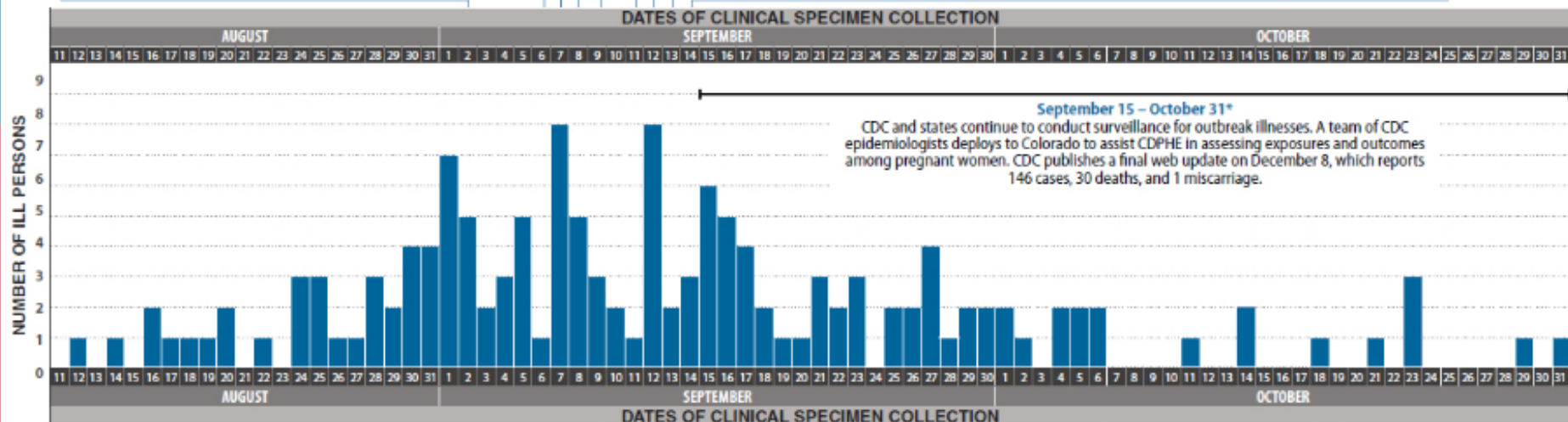
September 9
CDPHE announces that cantaloupes are the likely cause of illness and warns high-risk CO residents not to eat them.

September 11
Preliminary traceback investigation suggests that cantaloupe produced at Jensen Farms was sold at several retail locations where ill persons reported buying cantaloupe marketed as coming from one region in Southeastern CO.

September 12
CDC posts initial web announcement about the investigation, saying that the multistate outbreak of listeriosis is linked to cantaloupes marketed as coming from one region in Southeastern CO, and warning persons at high risk nationwide not to eat them.

September 13
FDA issues a statement that public health officials have discovered that most ill persons consumed cantaloupe purchased whole, most likely marketed as coming from one region in Southeastern CO.

September 14
CDC posts another web update about the investigation, including a warning that the multistate outbreak of listeriosis has been linked to cantaloupes from Jensen Farms.



Regulatory Actions, Recalls, and Results of Product Testing

September 5
Cantaloupes are collected by CDPHE for *Listeria* testing from the home of an ill person.

September 8
Cantaloupes are collected by CDPHE for *Listeria* testing from retail locations where ill persons reported buying cantaloupes.

September 9
Cantaloupes are collected by FDA for *Listeria* testing from retail locations where ill persons reported buying cantaloupes.

September 10
FDA and CDPHE visit Jensen Farms for an inspection and to collect environmental and product samples. The broker for Jensen Farms stops distribution of cantaloupes and tells grocery stores to remove Jensen Farms cantaloupes from shelves.

September 11
Cantaloupe samples collected by CDPHE on 9/5/11 from the home of an ill person and on 9/8/11 from 2 retail locations yield *Listeria*.

September 14
FDA announces that Jensen Farms has issued a voluntary recall of their whole cantaloupes.

September 16
A sample of cantaloupe collected by CDPHE from 2 retail location stores supplied by Jensen Farms yields an outbreak strain of *Listeria*.

September 19
Cantaloupes and environmental samples collected at Jensen Farms by FDA yield an outbreak strain of *Listeria*.

September 22
FDA, along with Colorado state and local officials, begins an environmental assessment at Jensen Farms.

September 23
Carol's Cuts of Kansas City, Kansas initiates a recall of fresh cut cantaloupe and cut mixed fruit containing cantaloupe because the cantaloupe was produced by Jensen Farms.

October 6
Fruit Fresh Up of Buffalo, New York initiates a recall of individual packages of fresh cut cantaloupe and cut mixed fruit containing cantaloupe because the cantaloupe was produced by Jensen Farms.

October 18
FDA issues a warning letter to Jensen Farms based on the presence of the outbreak strains of *Listeria* in environmental and cantaloupe samples taken on 9/10.

October 19
FDA announces findings of its environmental assessment at Jensen Farms.

* Excludes a newborn diagnosed with listeriosis with a specimen date in November whose mother was reported as a case earlier in the outbreak.

The FDA Report

- ❖ FDA identified the following factors as those that most likely contributed to the introduction, spread, and growth of *Listeria monocytogenes* in the cantaloupes:
 - ❖ There could have been low level sporadic *Listeria monocytogenes* in the field where the cantaloupe were grown, which could have been introduced into the packing facility
 - ❖ A truck used to haul culled cantaloupe to a cattle operation was parked adjacent to the packing facility and could have introduced contamination into the facility

The FDA Report

- ❖ Causes of the outbreak:
 - ❖ The packing facility's design allowed water to pool on the floor near equipment and employee walkways
 - ❖ The packing facility floor was constructed in a manner that made it difficult to clean
 - ❖ The packing equipment was not easily cleaned and sanitized; washing and drying equipment used for cantaloupe packing was previously used for postharvest handling of another raw agricultural commodity

The FDA Report

- ❖ Causes of the outbreak:
 - ❖ There was no pre-cooling step to remove field heat from the cantaloupes before cold storage
 - ❖ As the cantaloupes cooled there may have been condensation that promoted the growth of *Listeria monocytogenes*

Source Tracking Research

- ❖ There could have been low level sporadic *L. monocytogenes* in the field where the cantaloupe were grown, which could have been introduced into the packing facility
- ❖ A truck used to haul culled cantaloupe to a cattle operation was parked adjacent to the packing facility and could have introduced contamination into the facility

Source Tracking

- ❖ Automated Ribotyping analysis
 - ❖ Provides information about serotypes
 - ❖ Can determine lineage, provide clues about source
- ❖ Pulsed Field Gel Electrophoresis
 - ❖ Used by CDC to determine source of outbreaks
 - ❖ Patterns are placed in PulseNet
 - ❖ More discriminatory than Automated Ribotyping

Source Tracking

EcoRI

PvuII

DuPont ID Similarity	Label	DuPont ID	DuPont ID Label	RiboPrint™ Pattern					DuPont ID Similarity	Label	DuPont ID	DuPont ID Label	RiboPrint™ Pattern				
				1 kbp	5	10	15	50					1 kbp	5	10	15	50
0.98	ENV2011010 804-1 390-1	DUP-1030	Listeria monocytogenes							ENV2011010 804-1 390-1							
0.95	ENV2011010 804-2 390-2	DUP-1052	Listeria monocytogenes							ENV2011010 804-2 390-2							
0.92	ENV2011010- 805-3 390-3	DUP-1030	Listeria monocytogenes							ENV2011010- 805-3 390-3							
0.97	ENV2011010 805-4 390-4	DUP-1030	Listeria monocytogenes							ENV2011010 805-4 390-4							
0.95	ENV2011010 806-6 390-6	DUP-19169	Listeria monocytogenes							ENV2011010 806-6 390-6							
0.91	ENV2011010 807-7 390-7	DUP-9509	Enterococcus faecalis							ENV2011010 807-7 390-7							
0.96	ENV2011010 807-8 390-8	DUP-20238	Listeria monocytogenes							ENV2011010 807-8 390-8							

Source Tracking

- ❖ Among the *L. monocytogenes* environmental isolates obtained from the Colorado Department of Public Health and the Environment, we identified four ribotypes:
 - ❖ DUP-1030 (lineage II)
 - ❖ DUP-19169 (lineage II)
 - ❖ DUP-1052 (lineage I)
 - ❖ DUP-20238 (Unknown)

Source Tracking

- ❖ *L. monocytogenes* consist of at least 4 evolutionary lineages (I, II, III, and IV) with different but overlapping ecological niches
- ❖ Most *L. monocytogenes* isolates seem to belong to lineages I and II, and have serotypes more commonly associated with human clinical cases
- ❖ Lineage II strains are common in foods, are widespread in the natural and farm environments, and are also commonly isolated from animal listeriosis cases and sporadic human clinical cases

Source Tracking

- ❖ Most human listeriosis outbreaks are associated with lineage I isolates
- ❖ In many countries, lineage I strains are overrepresented among human isolates, as compared to lineage II strains
- ❖ Lineage III and IV strains are rare and predominantly isolated from animal sources

Source Tracking

- ❖ DUP-1030 was more frequently associated with animal cases ($26\pm 3\%$) than with human cases ($11\pm 4\%$)
- ❖ It was responsible for a human listeriosis outbreak in Carlisle in 1981
- ❖ DUP-1030 and DUP-19169 associated with foods
 - ❖ DUP-1030 found in smoked salmon
 - ❖ DUP-19169 found in semidry fermented sausage

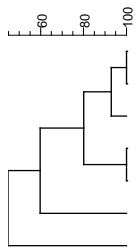
Source Tracking

- ❖ DUP-1052 is one of the most common ribotypes found among food isolates, and is commonly associated with human disease
- ❖ DUP-20238 not previously reported

PFGE Analysis

PFGE-Ascl

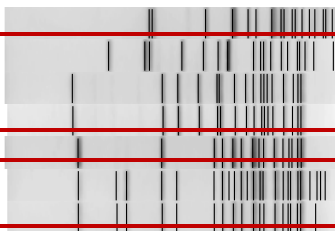
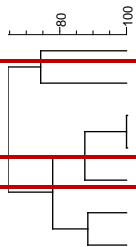
PFGE-Ascl



SourceState	TypeDetails	IsolatDate	Species	PFGE-Ascl-pattern	PFGE-Apal-pattern	Name
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0001	GX6A12.0001	ENV2011010805-3 (390-3)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0001	GX6A12.1776	ENV2011010805-4 (390-4)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0099	GX6A12.1776	ENV2011010804-1 (390-1)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0029	GX6A12.0069	ENV2011010806-6 (390-6)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0029	GX6A12.0069	ENV2011010807-8 (390-8)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0019	GX6A12.0227	ENV2011010804-2 (390-2)
CO	Cantaloupe	2011-09-08	Enterococcus faecalis			ENV2011010807-7 (390-7)

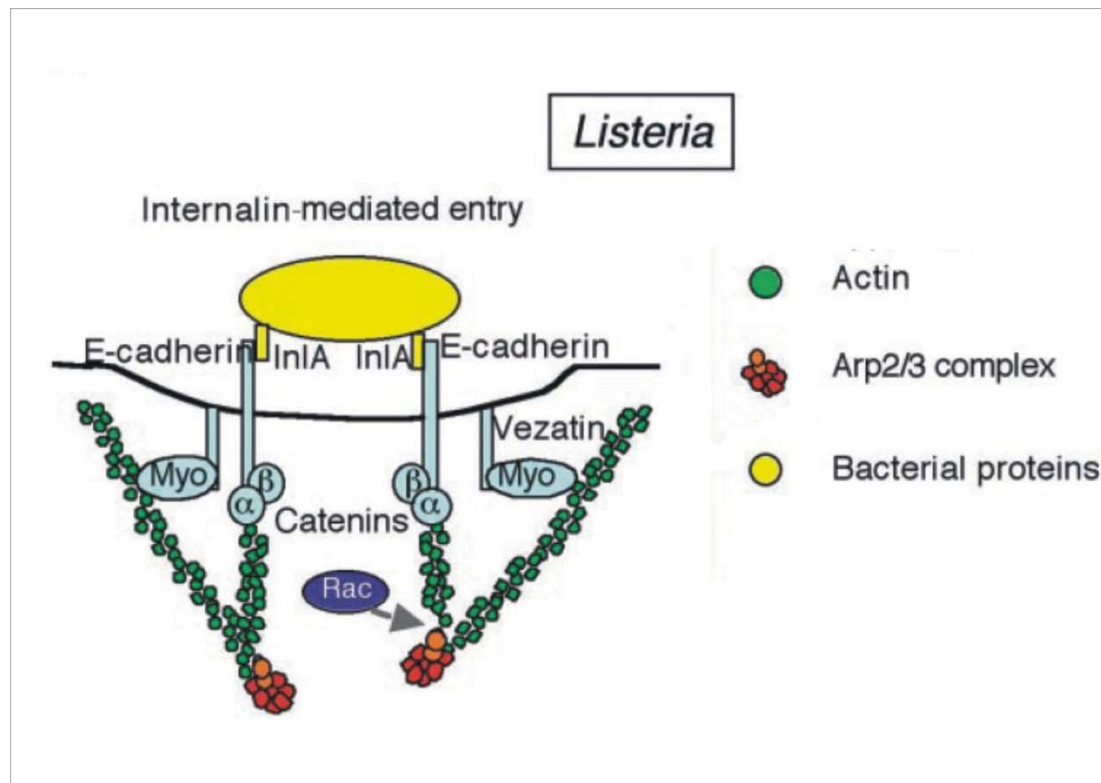
PFGE-Apal

PFGE-Apal



SourceState	TypeDetails	IsolatDate	Species	PFGE-Ascl-pattern	PFGE-Apal-pattern	Name
CO	Cantaloupe	2011-09-08	Enterococcus faecalis			ENV2011010807-7 (390-7)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0019	GX6A12.0227	ENV2011010804-2 (390-2)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0029	GX6A12.0069	ENV2011010806-6 (390-6)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0029	GX6A12.0069	ENV2011010807-8 (390-8)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0001	GX6A12.0001	ENV2011010805-3 (390-3)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0099	GX6A12.1776	ENV2011010804-1 (390-1)
CO	Cantaloupe	2011-09-08	Listeria monocytogenes	GX6A16.0001	GX6A12.1776	ENV2011010805-4 (390-4)

Virulence typing



Virulence typing

- ❖ In *L. monocytogenes*, attenuated virulence is associated with truncated forms of internalin A
- ❖ Attenuated strains have been characterized that have truncated forms of internalin A, from 47 to 60 kDa, instead of the commonly encountered 80-kDa internalin A
- ❖ Truncated forms are due to point mutations in *inIA*

Virulence Typing

gi 404238905_ISO	CTAATAACCCTAACAAAAGGTAGAGCGCATTATCGCTATCGCCAGTTGT 98	Isolate 3
gi 404837905_ISO	CTAATAACCCTAACAAAAGGTAGAGCGCATTATCGCTATCGCCAGTTGT 97	Isolate 4
gi 404237905_ISO	CTAATAACCCTAACAAAAGGTAGAGCGCATTATCGCTATCGCCAGTTGT 100	Isolate 1
gi 404297905_ISO	CTAA CAA T C C T A T C A A C A G G T A G A G C C A T T A T C G C T A T C C C A G T T G T A 100	Isolate 2
gi 404238905_ISO	GTATATTTGCGGAAGGTGGTGTAGTATTCCCGCCGTTATTTGTTGTAGGC 248	Isolate 3
gi 404837905_ISO	GTATATTTGCGGAAGGTGGTGTAGTATTCCCGCCGTTATTTGTTGTAGGC 247	Isolate 4
gi 404237905_ISO	GTATATTTGCGGAAGGTGGTGTAGTATTCCCGCC T T A T T T G T T G T A G G C 250	Isolate 1
gi 404297905_ISO	GTATATTTGCGGAAGGTGGTGTAGT G T T C C C T C C G T T A T T T G T A G T C G G C 250	Isolate 2
gi 404238905_ISO	CGCTATATTGGGCATATAAGGTGATGTTTTTAGCAGGCATTTTGCTAGTT 548	Isolate 3
gi 404837905_ISO	CGCTATATTGGGCATATAAGGTGATGTTTTTAGCAGGCATTTTGCTAGTT 547	Isolate 4
gi 404237905_ISO	CGCTATATTGGGCATATAAGGTGATGTTTTTAGCAGGCATTTTGCTAGTT 550	Isolate 1
gi 404297905_ISO	CGCTATATTG G C A T A T A A G G T G A T G T T T T A G C A G G C A T T T G C T A G T T 550	Isolate 2

Rapid, field-based diagnostics

FDA Environmental Swabs Positive Results

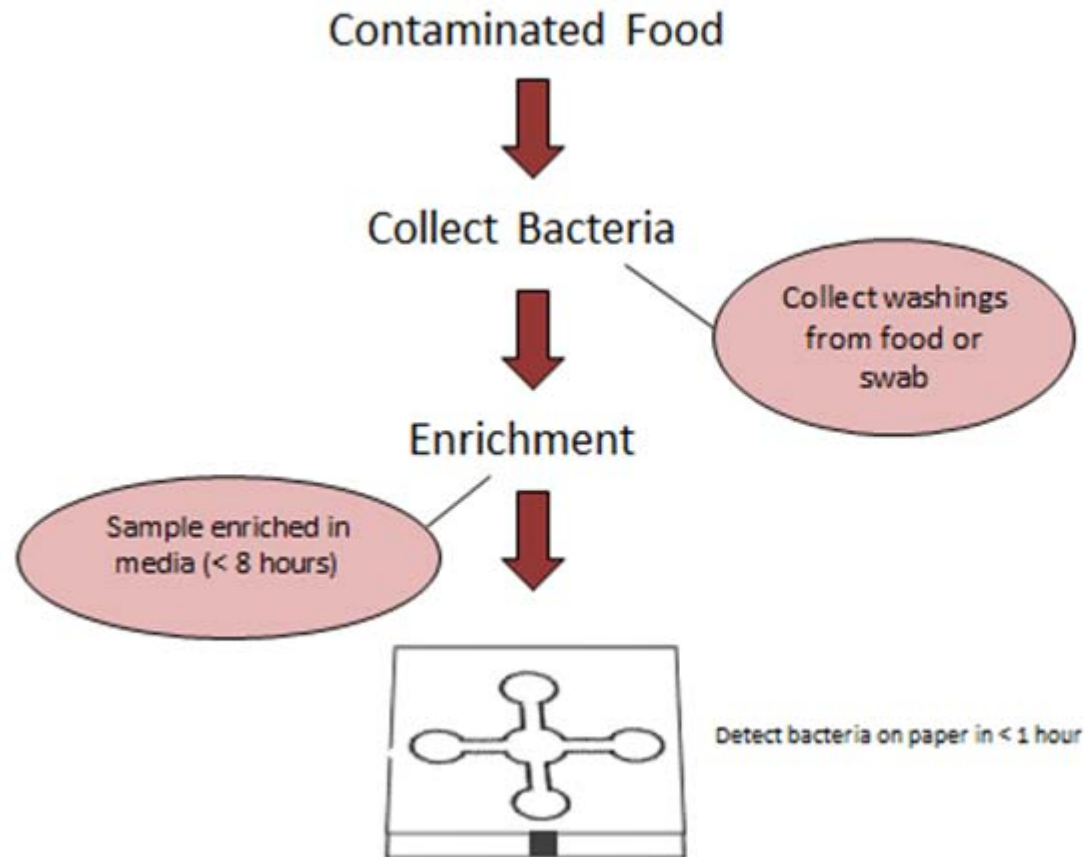
Processing Line	9 positive samples from the grading belt Swabs 21, 22, 23, 24, 26, 27, 29, 30 & 33
	2 positive samples from the conveyor Swabs 20 & 28
	1 positive sample from the felt rollers Swab 13
Packing Area	1 positive sample from the conveyor belt Swab 34

FDA Product Sample Results

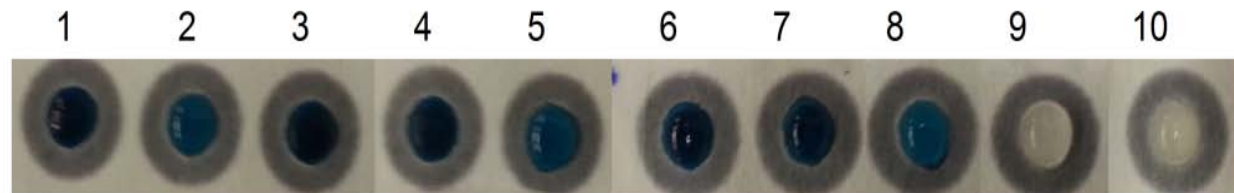
1 Cantaloupe Sample collected from cold storage
5 subs tested positive
(10 whole cantaloupes or "Subs")

Please refer to the section below for
[FDA's Sample Records and Results on Jensen Farms](#)

μ PAD Assay for *L. monocytogenes*



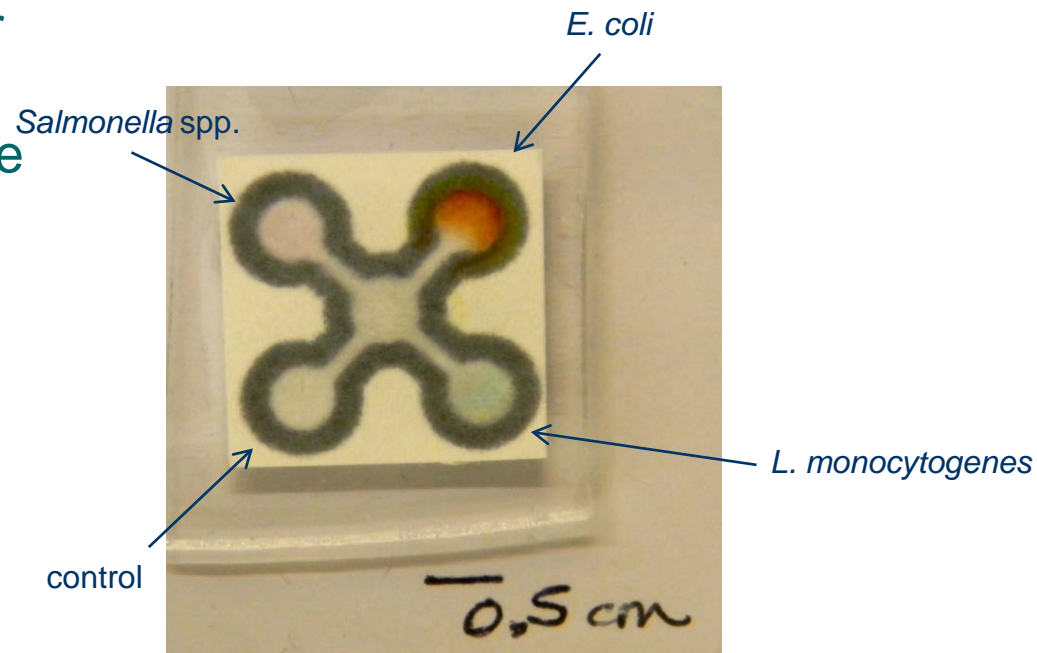
μ PAD Assay for *L. monocytogenes*



All *L. monocytogenes* tested were PIPLC positive (lanes 1-8), including two positive control strains (lanes 7 and 8). *Bacillus pumulis* and *Enterococcus faecalis* were PIPLC negative (lanes 9 and 10)

Multiplexed μ PAD Assay

- ❖ Goal: Detect three bacteria simultaneously
- ❖ Substrates spotted in outer test zones
- ❖ Solution containing all three enzymes in central sample well
- ❖ Cross-reactivity tested for each assay



Thanks to:

- ❖ Post-doctoral fellows

- ❖ Dr. Bledar Bisha
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- ❖ Shannon Coleman

- ❖ Wyoming Public Health Lab

- ❖ Wanda Manley

- ❖ Funding Sources

- ❖ USDA Specialty Crop Research Initiative
- ❖ Colorado State University Agricultural Experiment Station

Questions?

