Case Study: Foodborne Outbreak (Cantaloupe)

Lawrence Goodridge
Associate Professor
Department of Animal Sciences
Colorado State University
Lawrence.Goodridge@colostate.edu
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

<table>
<thead>
<tr>
<th></th>
<th>Cantaloupe</th>
<th>All Outbreaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalizations</td>
<td>16%</td>
<td>3%</td>
</tr>
<tr>
<td>Deaths</td>
<td>3%</td>
<td>0.07%</td>
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<tr>
<td>Illnesses (Median)</td>
<td>23</td>
<td>20</td>
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<tr>
<td>Multistate Outbreaks</td>
<td>52%</td>
<td>1-2%</td>
</tr>
<tr>
<td>Imported food</td>
<td>33%</td>
<td>0.4%</td>
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</tbody>
</table>

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

Gould 2012
Issues with Cantaloupe

Gould 2012
Issues with Cantaloupe

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

http://wwwn.cdc.gov/foodborneoutbreaks
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

http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/map.html
Issues with Cantaloupe

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

http://www.cdc.gov/salmonella/typhimurium-cantaloupe-08-12/index.html
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 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**
  Preliminary traceback investigation suggests that cantaloupe produced at Jensen Farms was sold at several retail locations where ill persons reported buying cantaloupe. Preliminary investigation suggests that multistate outbreak of *Listeria* is linked to cantaloupe marketed as coming from one region in Southeastern CO, and warning persons at high risk nationwide not to eat them.

- **September 12**
  CDC posts initial public announcement about the investigation, saying that the multistate outbreak of *Listeria* is linked to cantaloupe 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 public update about the investigation, including a warning that the multistate outbreak of *Listeria* has been linked to cantaloupe from Jensen Farms.

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**Regulatory Actions, Recalls, and Results of Product Testing**

- **September 5**
  Cantaloupes are collected by CDPHE for *Listeria* testing from retail locations where ill persons reported buying cantaloupes.

- **September 8**
  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 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 14**
  FDA announces that Jensen Farms has issued a voluntary recall of their whole cantaloupes.

- **September 15 – October 31**
  CDC and states continue to conduct surveillance for outbreak illnesses. A team of CDC epidemiologists deploys to Colorado to assist CDPHE in assessing exposures and outcomes among pregnant women. CDC publishes a final public update on December 8, which reports 146 cases, 30 deaths, and 1 miscarriage.

- **September 16**
  FDA announces that Jensen Farms has issued a voluntary recall of their whole cantaloupes.

- **September 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.

- **September 20**
  Fruit Fresh of Buffalo, New York issues a recall of individual packages of fresh cut cantaloupe and cut mixed fruit containing cantaloupe because the cantaloupe was produced by Jensen Farms.

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

- **October 19**
  FDA finds conclusions of its environmental assessment at Jensen Farms.

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*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

FDA 2011. Available at http://www.fda.gov/Food/FoodSafety/CORENetwork/ucm272372.htm#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.

FDA 2011. Available at http://www.fda.gov/Food/FoodSafety/CORENetwork/ucm272372.htm#report
Source Tracking

- Automated Riboprinting 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 Riboprinting
Source Tracking

### EcoRI

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<thead>
<tr>
<th>DuPont ID Similarity</th>
<th>Label</th>
<th>DuPont ID</th>
<th>DuPont ID Label</th>
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<td>DUP-1030</td>
<td>Listeria monocytogenes</td>
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<td>ENV2011010 807-8 390-8</td>
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### Pvull

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<td>ENV2011010 807-8 390-6</td>
<td>ENV2011010 807-8 390-6</td>
</tr>
</tbody>
</table>

RiboPrint™ Pattern | 1 kbp | 5 | 10 | 15 | 50
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±3%) than with human cases (11±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-AscI

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<thead>
<tr>
<th>SourceState</th>
<th>TypeDetails</th>
<th>IsolatDate</th>
<th>Species</th>
<th>PFGE-AscI-pattern</th>
<th>Name</th>
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<tbody>
<tr>
<td>CO</td>
<td>Cantaloupe</td>
<td>2011-09-08</td>
<td>Listeria monocytogenes</td>
<td>GX6A16.0001</td>
<td>ENV2011010805-3 (390-3)</td>
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<tr>
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<td>2011-09-08</td>
<td>Listeria monocytogenes</td>
<td>GX6A16.0001</td>
<td>ENV2011010805-4 (390-4)</td>
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#### PFGE-ApaI

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<th>SourceState</th>
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</tr>
</tbody>
</table>
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 *inlA*

Virulence Typing
**Rapid, field-based diagnostics**

<table>
<thead>
<tr>
<th>FDA Environmental Swabs Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Processing Line</strong></td>
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<tr>
<td>9 positive samples from the grading belt</td>
</tr>
<tr>
<td>Swabs 21, 22, 23, 24, 26, 27, 29, 30 &amp; 33</td>
</tr>
<tr>
<td>2 positive samples from the conveyor</td>
</tr>
<tr>
<td>Swabs 20 &amp; 28</td>
</tr>
<tr>
<td>1 positive sample from the felt rollers</td>
</tr>
<tr>
<td>Swab 13</td>
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<tr>
<td><strong>Packing Area</strong></td>
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<tr>
<td>1 positive sample from the conveyor belt</td>
</tr>
<tr>
<td>Swab 34</td>
</tr>
</tbody>
</table>

**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

FDA 2011. Available at http://www.fda.gov/Food/FoodSafety/CORENetwork/ucm272372.htm#report
μPAD Assay for *L. monocytogenes*
μPAD 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 μPAD 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

![Image of the μPAD Assay result with labels for E. coli, Salmonella spp., L. monocytogenes, and control. The test zones are labeled with red, green, and blue colors, respectively.]
Thanks to:

- Post-doctoral fellows
  - Dr. Bledar Bisha
  - Dr. Jeff Chandler
- Students
  - Alma Perez-Mendez
  - Shannon Coleman
- Wyoming Public Health Lab
  - Wanda Manley
- Funding Sources
  - USDA Specialty Crop Research Initiative
  - Colorado State University Agricultural Experiment Station
Questions?