Use of Whole Genome Sequencing in a *Salmonella* Enteritidis Outbreak Associated with Chicken Kiev

Carlota Medus, PhD, MPH
Epidemiologist Principal
Foodborne Diseases Unit
Background

• The Minnesota Department of Health (MDH) routinely subtypes all clinical *Salmonella* Enteritidis (SE) isolates by pulsed-field gel electrophoresis (PFGE) using 2 enzymes

• In 2014, MDH started subtyping all clinical SE isolates using whole genome sequencing (WGS) prospectively
  – Isolates sequenced at MDH, bioinformatics at Wadsworth Center/New York State Department of Health, Albany, NY
Background

During the week of August 25, 2014

• Two SE isolates with indistinguishable 2-enzyme PFGE patterns JEGX01.0004/JEGA26.0002 were identified

• On August 29, the two isolates were determined to be indistinguishable by WGS (0 SNPs different)

• By October 8, 8 SE isolates that indistinguishable by PFGE and WGS were identified
Minnesota Approach to Investigation of PFGE Clusters

- All reported *Salmonella* cases are interviewed even before a cluster is identified
- Collect details on specific exposures
  - Point of sale or service
  - Food brands, variety names
- Used in generating hypotheses for commercially distributed food outbreaks
Minnesota Approach to Investigation of PFGE Clusters: Dynamic Cluster Investigation Model
Methods

• Case interviews were conducted by phone by Team Diarrhea, MDH’s student team

• Dynamic interviewing approach
Methods

• Case definition:
  – A person with an SE isolate with PFGE patterns JEGX01.0004/JEGA26.0002 and
  – Closely related (<5 SNPs from another isolate in the cluster) by WGS
Data Analysis

- Compared consumption of suspect outbreak vehicle to data from *E. coli* O157 cases in MN, 2009-2013 as a proxy for background consumption
- Compared consumption of suspect outbreak vehicle among WGS matches (met case definition) to PFGE matches that did not match by WGS (did not meet case definition)
Methods

• Food specimens were collected from three grocery stores and two case-households

• Tested by the Minnesota Department of Agriculture (MDA)
  – PFGE by MDH
  – WGS by MDH and Wadsworth Center/New York State Department of Health, Albany, NY
All *Salmonella* Enteritidis JEGX01.0004/JEGA26.0002 Isolates Received August-September 2014 (n=19)
All *Salmonella* Enteritidis JEGX01.0004/JEGA26.0002 Isolates Received August-September 2014 (n=19)
Results

- 8 cases in 7 households
- Dates of illness onset: August 14 – September 16, 2014
- 1 hospitalized, no deaths
Results

• 6 of 7 (86%) ate chicken Kiev or A La Kiev
  – 3 reported brand A, 2 reported store brand but described packaging consistent with brand A

• 1 of 7 (14%) ate “fried chicken reheated at home” - excluded from statistical analyses
Results

Brand A chicken A La Kiev is

• Frozen

• Raw

• Breaded

• Pre-browned

• Stuffed: butter, spices and chives
Results

• 4 of 5 (80%) cooked the stuffed chicken in the oven, only 1 cooked it in the microwave

• 3 of 4 (75%) knew the chicken was raw
All _Salmonella_ Enteritidis JEGX01.0004/JEGA26.0002 Isolates Received August-September 2014 (n=19)
Results

• Comparison to background consumption:
  – 6 of 6 cases ate chicken Kiev vs. 3% background consumption rate of any stuffed chicken products; p < 0.001

• Comparison of cases vs. PFGE matches that were different by WGS:
  – 6 of 6 cases vs. 0 of 8 ate chicken Kiev; p < 0.001
Food Testing Results

- 26/30 (87%) Brand A chicken Kiev samples from retail positive for *Salmonella*
  - 9 SE, 8 matched the outbreak PFGE
  - 17 S. Schwarzengrund

- 7/11 (64%) Brand A samples from cases’ homes positive for *Salmonella*
  - 6 SE, all matched the outbreak PFGE
  - 1 S. Schwarzengrund
Food Testing Results

- 13 SE food isolates were 0-2 SNPs from the case-isolates by WGS; 2 were >30 SNPs from the outbreak sequence
News Release

FOR IMMEDIATE RELEASE: October 23, 2014

Salmonella cases linked to raw, frozen chicken entrees
Consumers urged to cook raw chicken products thoroughly

ST. PAUL, Minn. – State health and agriculture officials said today that six recent cases of salmonellosis in Minnesota have been linked to raw, frozen, breaded and pre-browned, stuffed chicken entrees. The implicated product is Antioch Farms brand A La Kiev raw stuffed chicken breast with a U.S. Department of Agriculture stamped code of P-1358. This product is sold at many different grocery store chains.

Investigators from the Minnesota Department of Health (MDH) and the Minnesota Department of Agriculture (MDA) determined that six cases of Salmonella infection from August and September 2014 were due to the same strain of Salmonella Enteritidis. One person was hospitalized for their illness.

“Our DNA fingerprinting found that the individuals were sickened by the same strain of Salmonella,” said Dr. Carlota Medus, epidemiologist for the Foodborne Diseases Unit at MDH. “The Minnesota Department of Agriculture collected samples of the same type of product from grocery stores and the outbreak strain of Salmonella was found in packages of this product.”

The salmonellosis cases of salmonellosis in Minnesota linked to the same type of product from 1998.
News Release

Illinois Firm Recalls Chicken Products Due to Possible Salmonella Enteritidis Contamination

Class I Recall
Health Risk: High

En Español

Congressional and Public Affairs
Benjamin Bell
(202) 720-9113

WASHINGTON, October 24, 2014 – Aspen Foods Division of Koch Meats, a Chicago, Ill., based establishment, is recalling 28,980 pounds of chicken products that may be contaminated with a particular strain of Salmonella Enteritidis, the U.S. Department of Agriculture’s Food Safety and Inspection Service (FSIS) announced today. FSIS requested Aspen Foods conduct this recall because this product is known to be associated with a specific illness cluster.

The recalled product includes partially prepared chicken products sold by retailers under the Antioch Farms brand name, with “sell by” dates of October 1, 2015 and October 7, 2015. The products subject to recall bear the establishment number “P-1358” inside the USDA mark of inspection. The chicken products were produced on July 2, 2014 and July 8, 2014. These products were shipped to retail stores and distribution centers in Minnesota.
Product Distribution
Case Finding in Other States

- Reached out to states that had received the recalled product
- Several states provided case-interview data, some re-interviewed cases that matched by PFGE during the outbreak period
  - ME, ND, NH, and WI found cases that reported stuffed chicken consumption, but different brand, or brand unknown
- IL, MT, NH, WI, WY sent isolates for WGS
  - 1 isolate from WI was 4 SNPs from a Kiev sample, but the case did not report eating it
Past Outbreaks

- There were 6 outbreaks associated with stuffed chicken products from 1998-2008.
- In those outbreaks 71% to 100% of the cases cooked the products in the microwave.
- FSIS published label guidance on March, 2006
  - "The principal display panel of labels should bear prominent and clear terms that convey that the product contains raw or uncooked poultry, i.e., ‘raw,’ ‘uncooked,’ or ‘not ready to eat.’"
  - Validate cooking instructions, and
  - Specify to cook to an internal temperature of 165° F.
<table>
<thead>
<tr>
<th>Year</th>
<th>Pathogen</th>
<th>PFGE</th>
<th>Plant number</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>S. Typhimurium</td>
<td>TM123</td>
<td>P-2375</td>
<td>33</td>
</tr>
<tr>
<td>2005</td>
<td>S. Heidelberg</td>
<td>SH60</td>
<td>P-1358</td>
<td>4</td>
</tr>
<tr>
<td>2005-2006</td>
<td>S. Enteritidis</td>
<td>SE43</td>
<td>P-1358, P-2375, P-273</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JEGX01.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>S. Typhimurium</td>
<td>TM2C</td>
<td>P-1358</td>
<td>3</td>
</tr>
<tr>
<td>2008</td>
<td>S. Enteritidis</td>
<td>SE43B18</td>
<td>P-2375</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JEGX01.0005/JEGA26.0004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>S. 4, 12:i:-</td>
<td>TM932TMB44</td>
<td>P-2375, P-273, other</td>
<td>16</td>
</tr>
<tr>
<td>2014</td>
<td>S. Enteritidis</td>
<td>SE1B1</td>
<td>P-1358</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JEGX01.0004/JEGA26.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>S. Enteritidis</td>
<td>SE43B72</td>
<td>P-1358</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JEGX01.0005/JEGA26.0037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2015</td>
<td>S. Enteritidis</td>
<td>SE1B239</td>
<td>P-273, P-4230</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JEGX01.0004/JEGA26.0203</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LABEL CHANGES
March, 2006
Outbreak described here
Discussion

- WGS was extremely useful:
  - Increased the specificity of the case definition; easier to implicate the outbreak vehicle
  - PFGE was used to identify potential cases during the investigation (did not wait for WGS results)
Discussion

- This was the 7th outbreak associated with these products
- High proportion of products tested positive for *Salmonella*
- Unlike previous outbreaks, a high proportion of cases report following label cooking instructions
- Changing label language was insufficient to prevent illnesses from these products
# Acknowledgments

Coauthors: Angie Taylor\(^1\), Carrie Rigdon\(^2\), Alida Sorenson\(^2\), Victoria Lappi\(^1\), William Wolfgang\(^3\), Dave Boxrud\(^1\), Kirk Smith\(^1\)

\(^1\)Minnesota Dept. of Health, Saint Paul, MN  
\(^2\)Minnesota Dept. of Agriculture, Saint Paul, MN  
\(^3\)Wadsworth Center/New York State Department of Health, Albany, NY

<table>
<thead>
<tr>
<th>New York State Dept. of Health</th>
<th>MDA laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pascal LaPierre</td>
<td>Margaret Parins</td>
</tr>
<tr>
<td>Michael Palumbo</td>
<td>Christie Radtke</td>
</tr>
<tr>
<td>The Wadsworth Center Public Health Genomics Initiative for bioinformatics support.</td>
<td>Matt Forstner</td>
</tr>
<tr>
<td>MDH Foodborne Diseases Unit</td>
<td>Jeff Wymer</td>
</tr>
<tr>
<td>Josh Rounds</td>
<td>Bryanne Shaw</td>
</tr>
<tr>
<td>Dana Eikmeier</td>
<td></td>
</tr>
<tr>
<td>Team Diarrhea</td>
<td></td>
</tr>
<tr>
<td>States colleagues</td>
<td>CDC, FSIS</td>
</tr>
</tbody>
</table>

Epidemiologists in IL, ME, MT, ND, NH, WI, and WY
Acknowledgments

Team Diarrhea
Raw Stuffed Chicken Breast
Breaded, Boneless Breast of Chicken with Rib Meat

A La Kiev
Filled with Butter, Spices and Chives

KEEP FROZEN
NET WT 5 OZ (142g)
RAW PRODUCT:

BEST IF USED BY 100215 - P-1358
SERVING SUGGESTED: 1-2 Osloed Patty or 1-1/2 Patties

FOR FOOD SAFETY, COOK TO A MINIMUM INTERNAL TEMPERATURE OF 165°F MEASURED BY A MEAT THERMOMETER.