Making *Salmonella* Count
Public Health Perspectives and Laboratory Testing Implementation

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SALMONELLA

Gram negative, rod shaped, motile, facultative intracellular pathogen

Taxonomy

- Two species *Salmonella enterica* (i.e., six subspecies) & *Salmonella bongori*
- > 2,500 serotypes
- Refer to as genus & serotype (e.g., *Salmonella* Typhi)

Widespread occurrence in gastrointestinal tract

- Humans, other animals, birds, reptiles some insects
- Particularly swine and poultry

Ubiquitous in environment (soil & water)
Salmonella serotypes show host specificity

- *Salmonella* Pullorum (poultry)
- *Salmonella* Dublin (cattle)
- *Salmonella* Arizonae (reptiles)

Host specificity mediated by species-specific fimbriae (*lpf*) which are important for tissue tropism to M cells
**NON-TYHPOIDAL SALMONELLOSIS**

Consumption of raw or undercooked foods (often of animal origin) contaminated by animal feces or raw eggs (*Salmonella* Enteritidis)  
Unwashed hands of food handler  
Diarrhea, fever, abdominal cramps onset 12-72 h following consumption of contaminated food  
Illness lasts 4 to 7 d & usually resolves without antibiotic treatment  
  
• *Salmonella* infection may disseminate from intestine to bloodstream & other tissues  
• Reiter’s syndrome (joint pain, eye irritation, & painful urination)
INCIDENCE OF FOODBORNE ILLNESS

• Centers for Disease Control & Prevention, Division of Foodborne, Waterborne and Environmental Diseases
  • Monitoring of laboratory confirmed cases (FoodNet data)
  • Estimates derived from statistical modeling with inputs and uncertainty measurements
    • Underreporting and under-diagnosing
FOODNET HAS SITES IN 10 STATES
ESTIMATED INCIDENCE OF FOODBORNE ILLNESS IN THE U.S. EACH YEAR

Summary of domestically acquired foodborne illness attributed to 31 major pathogens

• 9.4 million illnesses
• 55,961 hospitalizations
• 1,351 deaths
• *Toxoplasma gondii*, *Salmonella*, and *Listeria monocytogenes* responsible for >70% of deaths due to major foodborne pathogens

Summary of domestically acquired foodborne illness attributed to unspecified agents

• 38.4 million illnesses
• 71,878 hospitalizations
• 1,686 deaths


(Scallan et al., 2011a and b)
RELATIVE RATE OF CULTURE CONFIRMED INFECTIONS

The graph shows the relative rate of culture confirmed infections over a period from 1996 to 2012. The infections tracked include Vibrio, Campylobacter, Salmonella, Listeria, and STEC O157. The relative rate is plotted on a log scale, and the years are indicated on the x-axis.

- Vibrio: The line for Vibrio shows a steady increase from 1996, peaking around 2012.
- Campylobacter: This infection rate also shows a steady increase, similar to Vibrio, with a peak around 2012.
- Salmonella: The Salmonella infection rate is relatively stable, with minor fluctuations over the years.
- Listeria: The Listeria infection rate shows a slight increase overall, with some fluctuations, peaking in 2012.
- STEC O157: The line for STEC O157 is the most volatile, showing significant changes over the years with a peak around 2012.

The graph highlights the increasing trend in the relative rate of these infections over time.
RELATIVE RATE OF CULTURE CONFIRMED INFECTIONS: NEW BASELINE DATA
HACCP Percent of Total Positive Serotypes
Ground Beef, CY-2014

- Montevideo: 22.4%
- Dublin: 12.1%
- Cerro: 9.5%
- Muenchen: 6.9%
- Newport: 8.6%
- Muenster: 4.3%
- Anatum: 5.2%
- Other: 31.0%
**Table 1a.** Culture-confirmed human *Salmonella* infections reported to LEDS, with the 20 most frequently reported serotypes listed individually, United States, 2014

<table>
<thead>
<tr>
<th>Rank</th>
<th>Serotype</th>
<th>Number reported</th>
<th>Percent</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enteritidis</td>
<td>8,895</td>
<td>20.0</td>
<td>2.79</td>
</tr>
<tr>
<td>2</td>
<td>Typhimurium</td>
<td>5,041</td>
<td>11.3</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>Newport</td>
<td>4,437</td>
<td>10.0</td>
<td>1.39</td>
</tr>
<tr>
<td>4</td>
<td>Javiana</td>
<td>2,704</td>
<td>6.1</td>
<td>0.85</td>
</tr>
<tr>
<td>5</td>
<td>1,4,[5],12:i:-</td>
<td>2,189</td>
<td>4.9</td>
<td>0.69</td>
</tr>
<tr>
<td>6</td>
<td>Heidelberg</td>
<td>1,430</td>
<td>3.2</td>
<td>0.45</td>
</tr>
<tr>
<td>7</td>
<td>Infantis</td>
<td>1,357</td>
<td>3.1</td>
<td>0.43</td>
</tr>
<tr>
<td>8</td>
<td>Saintpaul</td>
<td>980</td>
<td>2.2</td>
<td>0.31</td>
</tr>
<tr>
<td>9</td>
<td>Muenchen</td>
<td>873</td>
<td>2.0</td>
<td>0.27</td>
</tr>
<tr>
<td>10</td>
<td>Montevideo</td>
<td>841</td>
<td>1.9</td>
<td>0.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rank</th>
<th>Serotype</th>
<th>Number reported</th>
<th>Percent</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Oranienburg</td>
<td>728</td>
<td>1.6</td>
<td>0.23</td>
</tr>
<tr>
<td>12</td>
<td>Thompson</td>
<td>626</td>
<td>1.4</td>
<td>0.20</td>
</tr>
<tr>
<td>13</td>
<td>Braenderup</td>
<td>610</td>
<td>1.4</td>
<td>0.19</td>
</tr>
<tr>
<td>14</td>
<td>Mississippi</td>
<td>532</td>
<td>1.2</td>
<td>0.17</td>
</tr>
<tr>
<td>15</td>
<td>Typhi</td>
<td>527</td>
<td>1.2</td>
<td>0.17</td>
</tr>
<tr>
<td>16</td>
<td>Bareilly</td>
<td>381</td>
<td>0.9</td>
<td>0.12</td>
</tr>
<tr>
<td>17</td>
<td>Paratyphi B var. L(+) tartrate +</td>
<td>335</td>
<td>0.8</td>
<td>0.11</td>
</tr>
<tr>
<td>18</td>
<td>Poona</td>
<td>322</td>
<td>0.7</td>
<td>0.10</td>
</tr>
<tr>
<td>19</td>
<td>Berta</td>
<td>318</td>
<td>0.7</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>Agona</td>
<td>307</td>
<td>0.7</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Background

- *Salmonella* is not an adulterant for raw meat and poultry.*
- However, must meet established performance standards.
- Whether or not an individual develops salmonellosis is based on several factors such as the immune status of the individual, the amount of product consumed, and the *concentration* of *Salmonella* in the product.
**Background**

*Salmonella* prevalence is fairly high in FSIS sampling:

<table>
<thead>
<tr>
<th>Species</th>
<th>Product</th>
<th>Pathogen</th>
<th>Current Period July 1, 2018 - June 30, 2019</th>
<th>Historical Calculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Beef</td>
<td>RGB Components</td>
<td><em>Salmonella</em> spp.</td>
<td>Number of Samples 1,213</td>
<td>Number of Positives 85</td>
</tr>
<tr>
<td></td>
<td>Manufacturing Trim</td>
<td><em>Salmonella</em> spp.</td>
<td>3,968</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>Bench Trim</td>
<td><em>Salmonella</em> spp.</td>
<td>1,372</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Raw Ground</td>
<td><em>Salmonella</em> spp.</td>
<td>10,663</td>
<td>201</td>
</tr>
<tr>
<td>Raw Pork</td>
<td>Intact Cuts</td>
<td><em>Salmonella</em> spp.</td>
<td>1,330</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>Non-intact Cuts</td>
<td><em>Salmonella</em> spp.</td>
<td>1,165</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Comminuted</td>
<td><em>Salmonella</em> spp.</td>
<td>1,652</td>
<td>351</td>
</tr>
<tr>
<td>Raw Chicken</td>
<td>Whole Carcasses</td>
<td><em>Salmonella</em> spp.</td>
<td>8,990</td>
<td>424</td>
</tr>
<tr>
<td></td>
<td>Quarter or Half Carcasses</td>
<td><em>Salmonella</em> spp.</td>
<td>91</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Parts - Legs, Breasts, Wings</td>
<td><em>Salmonella</em> spp.</td>
<td>8,431</td>
<td>814</td>
</tr>
<tr>
<td></td>
<td>Other Parts</td>
<td><em>Salmonella</em> spp.</td>
<td>305</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Comminuted</td>
<td><em>Salmonella</em> spp.</td>
<td>1,965</td>
<td>467</td>
</tr>
<tr>
<td></td>
<td>Mechanically Separated</td>
<td><em>Salmonella</em> spp.</td>
<td>112</td>
<td>89</td>
</tr>
<tr>
<td>Raw Turkey</td>
<td>Whole Carcasses</td>
<td><em>Salmonella</em> spp.</td>
<td>1,872</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Comminuted</td>
<td><em>Salmonella</em> spp.</td>
<td>1,539</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td>Mechanically Separated</td>
<td><em>Salmonella</em> spp.</td>
<td>105</td>
<td>53</td>
</tr>
</tbody>
</table>
Background

• Just because *Salmonella* prevalence is high, it does not necessarily mean that the concentrations are high.

• *Salmonella* quantification techniques aim to identify product lots with elevated concentrations of *Salmonella* that will pose an elevated public health risk.

|-----------|-----|-----------|------|---|-----------|-----|-----------|-----|
Deliverables

• Understand what decisions have to be made prior to implementing a quantification-based *Salmonella* management program.

• Understand the methodology that laboratories use to perform routine testing and the process of validating this testing.
Implementation Decisions

- What level should you target? What is your risk?

\[ \text{Attack Rate} = \frac{\text{Number of Exposed People Who Are Sick}}{\text{Total Number of People Exposed}} \]

Log = Factor of 10
1.0 Log = 10
2.0 Log = 100
3.0 Log = 1000
4.0 Log = 10000
Implementation Decisions

• Sample types?

• Frequency of sampling?
Implementation Decisions

- What sample size do you test?
Implementation Decisions

• How do you dispose of positive product?

• Budget for product disposition?
Laboratory Methodology

• Old Approaches: Cultural Methods
  – Most Probable Number (MPN)
  – Test +/- on sets of tubes with different quantities of the original sample

• Direct Plating
  – Plate directly to selective media
  – Issues with background organisms
New Approaches: Limits Testing

Bend rules on traditional +/- assays by using them in a semi-quantitative manner.

Traditional +/- assays are designed to determine if there is any *Salmonella* present (i.e. 1 CFU/375 g sample).

With limits testing you shorten incubation time to catch only those samples that have high enough concentrations of *Salmonella* starting out (i.e. as high as 10 CFU/g)
Laboratory Methodology

If you test at 8 hours you will get positives for all three starting concentrations.

Limit of Detection for +/- Assay
Laboratory Methodology

If you test at 3 hours you will only get positives for those samples with concentrations over 100 CFU/g

Limit of Detection for +/- Assay
Validation Process

• Grow *Salmonella* in liquid culture.

• Enumerate and store in refrigerator overnight.
Validation Process

• Use plate counts to adjust culture concentration.

• Inoculate meat samples.
Validation Process

• Enrich meat samples.

• Incubate meat samples and pull every hour for testing from 2-6 hours
Validation Process

• Test on testing platform.

• Evaluate results and determine time point for threshold testing.
Validation Process

• Verify starting concentration with plate counts plated after inoculation of the samples.
Current Implementation

There are two beef producers that we are aware of, each with a different form of testing that are currently doing *Salmonella* threshold testing.

**Approach 1:**
Client is currently using their *E. coli* O157:H7 enrichments to test for *Salmonella* level at 10 CFU/g at 4 hours of enrichment.

**Approach 2:**
Client is currently using their *E. coli* O157:H7 enrichments to test for *Salmonella* level of 1 CFU/g at 5 hours of enrichment.
Thank You!

QUESTIONS?