Shiga Toxigenic *E. coli*: a fully emerged, still underappreciated pathogen

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Disclosures

• Speaking fees/honorarium – Meridian Biosciences
• Honorarium – Biofire Diagnostics
• Research reagents – BioGX
• Research reagents - Alere
Objectives

- Summarize the key features of STEC and the illnesses associated with infection
- Evaluate their laboratory methods to determine if their current practice meets the guidelines and recommendations for testing
- Evaluate their laboratory’s ability to detect emerging strains of STEC
Pathogenic *E. coli*

- Enteropathogenic *E. coli* (EPEC)
- Uropathogenic *E. coli* (UPEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC)
- Extraintestinal pathogenic (ExPEC)
- Enteroaggregative *E. coli* (EAggEC)
- Shigatoxigenic *E. coli* (STEC/EHEC/VTEC)
What’s in a name?

• STEC = shiga toxigenic *E. coli*
  – Toxin name is shiga-like toxin

• VTEC = verotoxigenic *E. coli*
  – Attributed to early cytotoxic effects seen in Vero cells (antiquated name)

• EHEC = enterohaemorrhagic *E. coli*
  – Attributed to blood loss from the bowel
STEC

• First isolated in 1982

• Infectious dose 10-50 cfu
  – 3-8 day incubation period (dose dependent?)

• Media-worthy: several prominent foodborne disease outbreaks worldwide
  – Multiple deaths, significant healthcare dollars

• Conventionally serotyped by:
  – “O” lipopolysaccharide and “H” flagellar antigens
What is STEC?

- Normal *E. coli*...
  - Considered of ungulate origin
  - Two phage-encoded shiga-like toxins
    - Stx1 – analogous to *Shigella dysenteriae* shiga toxin
    - Stx2 – unique toxin to STEC (~50% similar to Stx1)
  - LEE pathogenicity island
    - *eae* (Intimin)
    - TIR (transmembrane intimin receptor)
    - Type 3 secretion system
    - Attachment/Effacement lesions
Pathogenicity

T3SS
Intimin
TIR

Actin Filaments

Stx1
Stx2

Circulation

DEPARTMENT OF PATHOLOGY
Shiga toxin function

• Toxins enter circulation → kidney

• Subunit B binds globotriaosylceramide (Gb₃) receptor
  – Kidney >>> intestinal epithelium
  – Bovine lack vascular receptors

• Subunit A cytotoxic to cells
  – Inactivates ribosomes → renal cell death
Hemolytic Uremic Syndrome (HUS)

- Glomerular endothelial cells swell & detach
- 2º activation of platelet & coagulation cascade initiated
  - Fibrin deposition $\rightarrow$ narrowing capillaries $\rightarrow$ RBC shearing
    $\Rightarrow$ microangiopathic hemolytic anemia
  - Platelet consumption $\rightarrow$ thrombocytopenia
  - Restricted blood flow $\rightarrow$ renal failure
Patient symptoms

• Severe acute diarrhea +/- blood
• Afebrile
• ~8% of infections lead to HUS

• More complications in elderly & children
  – 10-15% of Peds develop HUS
  – Majority of patients >65 are hospitalized

• Children often require dialysis
• Early/aggressive IV isotonic volume expansion
Therapy/Management

• Antibiotic therapy is controversial
• Phage induction by Quinolones, TMP-SXT, β-lactams
  – S.O.S. response induces phage promoter expression
    = ↑ Stx production
• Antimotility agents may increase disease severity
• Supportive care is the gold standard
• Standard contact precautions
STEC - serotypes

• 150+ disease assoc. serotypes
  – O157:H7 most commonly reported in USA
  – O26:H11 second most
  – Other virulent types also reported

• Typing for outbreak investigations

• Testing for toxin is the logical progression toward proper testing & comprehensive reporting
Perceived degree of severity

- O157 Stx2
- O157 Stx1/2
- Non-O157 Stx2
- Non-O157 Stx1/2
- Stx1 any serogroup

- Stx2 +
- Stx 1/2
- Stx1

- Serogroup correlation may still be a biased phenomenon
New guidelines

- 2009 MMWR guidelines: CDC/APHL recommend toxin detection plus culture for O157
  - Adoption has been slow and voluntary
- CAP - none
- Dec. 2012: JCAHO mandates shiga toxin detection for all stool specimens submitted for diarrheal illness
EFFECTIVE July 1, 2013

Element of Performance for QSA.04.06.01
A 6. All stool specimens from patients diagnosed with acute community-acquired diarrhea are simultaneously cultured for O157 Shiga toxin-producing Escherichia coli (STEC) on selective and differential agar and assayed for non-O157 STEC with a test that detects Shiga toxins or the genes encoding these toxins.

This is NOT voluntary, and typically CAP follows JCAHO…so stay tuned!
Lab testing

- Culture

- EIA or Immunochromatographic detection of toxin

- PCR for stx1 and stx2 genes
Culture

- Culture conditions select for O157
  - Sorbitol MacConkey +/- cefixime & tellurite (CT-SMAC)
  - CHROMagar® O157
  - Rainbow® O157 agar
- Results in <24 hours
- Cannot detect other STEC
- May detect shiga toxin negative O157 *E. coli*
- Can fail to recover O157 STEC
- Further contribute to a reporting bias when used alone
EIA/Immunochromatography

- Detects toxin in sample
- High sensitivity
- No serogroup bias
- Cross react w/ *S. dysenteriae* toxin
- May not detect toxin subtypes
e.g. Stx2<sub>d</sub> & Stx2<sub>e</sub>*
- Variable specimen type

*Willford et al. J Food Prot, 2009*
Test performance

- Agreement with reference methods (per package inserts/510(k))

<table>
<thead>
<tr>
<th></th>
<th>Immunocard® STAT! EHEC¹</th>
<th>Duopath® Verotoxin¹</th>
<th>ProSpecT® EHEC²</th>
<th>PremierTM EHEC²</th>
<th>Shiga Toxin Quik Chek®²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Stool</td>
<td>-</td>
<td>-</td>
<td>87%</td>
<td>78.9/95.8%</td>
<td>98%/99.9%</td>
</tr>
<tr>
<td>Broth Enrichment</td>
<td>89.1/99.7%</td>
<td>-</td>
<td>92/100%</td>
<td>100/97.9%</td>
<td>-</td>
</tr>
<tr>
<td>Colony testing</td>
<td>100/99.7%</td>
<td>99.5/97.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

¹ Premier EHEC as reference method
² Cytoxic culture as reference method
EIA vs Immunochromatography

Factors to consider

- Laboratory model
  - Central vs satellite
- Cost
- Test volume
- Specimen preferred (enriched or raw?)
Enriched stool vs raw?

• Quik Chek claims equivalent performance to enriched stool using direct from stool testing on LFA
  – Performed Quik Chek on all Pos. EIA specimens

• N= 5687 EIA performed: June 18, 2013 – July 16, 2014
  – 26 EIA positives (0.46% positivity)
  – 19 LFA positives

• 5 discrepant specimens arbitrated by PCR
# Discrepant resolution

<table>
<thead>
<tr>
<th>EIA enriched</th>
<th>Quik Chek</th>
<th>PCR</th>
<th>EIA repeat enriched</th>
<th>Quik Chek enriched</th>
<th>PCR enriched</th>
<th>Final resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Negative for Shiga toxin, EIA false Pos</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>Stx2</td>
<td>+</td>
<td>Stx2</td>
<td>Stx2</td>
<td>Positive for Stx2, STEC</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>Stx2</td>
<td>Positive for Stx2, STEC</td>
</tr>
<tr>
<td>+</td>
<td>Stx1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>O157+</td>
<td>Positive for Stx1, O157 STEC</td>
</tr>
<tr>
<td>+</td>
<td>Stx2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Stx2</td>
<td>Positive for Stx2, STEC</td>
</tr>
</tbody>
</table>

Couturier et al. Manuscript in preparation, Poster presentation ASM 2015
Conclusion

• Stool enrichment with EIA detected 4 additional positive specimens that LFA could not detect
  – EIA detected one false positive, however LFA was not applied to all specimens received

• 25 true STEC positive specimens during our study period were detected by EIA using manufacturer’s recommendations, only 21 detected by LFA

• At such a low prevalence (0.46%) this is an unacceptable performance loss; enrichment is recommended
PCR

- Historically non-standardized
- Flexible throughput
- Better performance from enrichment culture
- Can screen single colonies
- Detect and identify each shiga toxin gene
  - Can design to detect stx1/2 subtypes
- Indirect evidence of toxin
  - Expression?
FDA cleared methods for stx1/2

- Luminex™ Gastointestinal Pathogen Panel
- Prodesse® Progastro® SSCS
- BioFire® FilmArray™ Gastrointestinal Panel
- Nanosphere Verigene® Enteric Pathogens Test
- BD MAX™ Enteric Bacterial Panel
Luminex™ Gastointestinal Pathogen Panel

- Detect 11 GI pathogens
- Frozen stool: samples cannot be cultured
- High throughput, batch runs, complicated assay, 5+ hour TAT
Published studies

  – 94% (28/30) sensitivity vs EIA and LDT PCR

• Claas et al. *J. Microbiol Biotechnol* 2013
  – 901 stools
  • STEC detection
    – 15/16 vs culture for O157
    – 8/8 vs EIA
      » 1 False positive by Luminex
      » 5 False negative by Luminex (unresolved)
Prodesse® Progastro® SSACS

- Detect *Salmonella*, *Shigella*, *Campylobacter*, and *stx1* & *stx2*
- Frozen stool or Cary Blair preserved stool (allows for culture)
- High throughput, complicated assay, batch runs, 4 hour TAT

Extraction: Biomerieux NucliSENS easyMAG system

Amplification: Cepheid Smart Cycler II
Published study

  - 100% specificity (17/17), 99.9% specificity
    - 1 false positive stx (initially 9…8 resolved)
    - EIA/Cx only 54% sensitive compared to sequence confirmed specimens
BioFire® FilmArray™ Gastrointestinal Panel

- Detects 22 pathogens/targets
- Cary Blair stool: can be cultured
- Low throughput, random access, very simple method, ~1 hour TAT
Published study

• Buss et al. J. Clin Micro. 2015. 53(3).
• Detected 38 STEC versus 33 on comparator PCR method
  – “5 false positives” (N=1556 samples total)
BD MAX™ Enteric Bacterial Panel

- Detects *Salmonella*, *Shigella*, *Campylobacter*, and *stx1* & *stx2*
- Fresh unpreserved stool or Cary Blair: can be cultured
- Variable throughput, simple method, ~2.5 hour TAT
Published study

- Detected 85 STEC versus comparator PCR method or EIA/culture
  - 8 false positives after resolution (N=2437 samples total)
Nanosphere Verigene® Enteric Pathogens Test

- Detects 7 pathogens/targets
- Cary Blair stool: can be cultured
- Variable throughput, random access, simple method, ~2 hour TAT

- No publications to date!
So many methods…

How should a lab test for STEC?
## Strategies for detecting STEC

<table>
<thead>
<tr>
<th>Method</th>
<th>Detect all STEC</th>
<th>Detect non-toxigenic O157</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157 Culture only</td>
<td>NO</td>
<td>YES*</td>
<td>• Must reflex to PHL to confirm toxin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Contributes to biased reporting</td>
</tr>
<tr>
<td>O157 culture, toxin detection based on criteria</td>
<td>NO</td>
<td>YES</td>
<td>• Will detect some additional STEC, but not all</td>
</tr>
<tr>
<td>(e.g. – bloody diarrhea, age &lt;5 or &gt;60, summer months, HUS, ruminant/farm exposure)</td>
<td></td>
<td></td>
<td>• Must reflex to PHL to confirm toxin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Contributes to biased reporting</td>
</tr>
<tr>
<td>O157 culture + Toxin detection</td>
<td>YES</td>
<td>YES</td>
<td>• Provides recognizable name on preliminary report</td>
</tr>
<tr>
<td>Toxin detection reflex positive to O157 culture</td>
<td>YES</td>
<td>NO*</td>
<td>• Delayed ID of O157 if present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Need to educate physicians as to importance of Stx detection</td>
</tr>
<tr>
<td>Toxin detection w/rapid submission to PHL</td>
<td>YES</td>
<td>NO*</td>
<td>• Delayed ID of O157 if present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Need physician education as to importance of Stx detection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• May lead to broad appreciation of Stx vs serotype</td>
</tr>
</tbody>
</table>
1,945 Isolates of Non-O157 STEC Serogrouped by CDC, 1983-2005

CDC, unpublished data
Increased reporting of non-O157 EHEC in Washington

Figure 1. Rate of reported O157 and non-O157 Shiga toxin (Stx)–producing *Escherichia coli* (STEC) infections and number of laboratories performing Stx testing by year, Washington State, USA, 2005–2010.
Stool tested by PCR, submitted for viral enteritis

\[ N = 2725 \ (2006-2008) \]

- 38 Stx+ specimens
- Cx pos. O157 (3) vs non-O157 (12)
- Previous outbreak strains O26:HNM, O26:H11, O103:H25, O121:H19, O145:HNM

### Table 2. Shiga-toxigenic E. coli isolated from patient stool from Alberta and Northern Territories

<table>
<thead>
<tr>
<th>Yr</th>
<th>Serotype</th>
<th>Patient age</th>
<th>stx type(s)</th>
<th>Stool appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>O26:H11</td>
<td>83 yr</td>
<td>stx₁</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>Rough:H11</td>
<td>3 yr</td>
<td>stx₁</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>O11:H30</td>
<td>3 yr</td>
<td>stx₁</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>O157:H16</td>
<td>2 yr</td>
<td>stx₁/stx₂</td>
<td>Solid, brown</td>
</tr>
<tr>
<td></td>
<td>O6:H16/O103:H25</td>
<td>31 yr</td>
<td>stx₁</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td>2008</td>
<td>O145:HNM</td>
<td>8 mo</td>
<td>stx₁</td>
<td>Liquid, tan</td>
</tr>
<tr>
<td></td>
<td>O26:HNM</td>
<td>13 yr</td>
<td>stx₁</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>O69:H11</td>
<td>52 yr</td>
<td>stx₁</td>
<td>Solid, brown</td>
</tr>
<tr>
<td></td>
<td>O26:H11</td>
<td>6 mo</td>
<td>stx₁</td>
<td>Liquid, yellow</td>
</tr>
<tr>
<td></td>
<td>Rough:HNM</td>
<td>16 mo</td>
<td>stx₁</td>
<td>Semisolid, brown</td>
</tr>
<tr>
<td></td>
<td>O121:H19</td>
<td>10 yr</td>
<td>stx₁/stx₂</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>O157:H7</td>
<td>48 yr</td>
<td>stx₁/stx₂</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>O157:H7</td>
<td>3 yr</td>
<td>stx₁/stx₂</td>
<td>Liquid, brown</td>
</tr>
<tr>
<td></td>
<td>O103:H25</td>
<td>16 yr</td>
<td>stx₁</td>
<td>Semisolid, brown</td>
</tr>
</tbody>
</table>

Couturier et al. J Clin Micro 2011
Comprehensive detection

N = 2328 (2009-2010)

- 21 Stx+ identified
- 8, O157:H7 (5 bloody)
- 13, Non-O157 (5 bloody)
- Culture only method (8/21 detected)

Criteria-driven and culture-only methods would underdiagnose

<table>
<thead>
<tr>
<th>E. coli serotype</th>
<th>Age (yr)</th>
<th>Stool sample description</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157:H7</td>
<td>5</td>
<td>Liquid</td>
</tr>
<tr>
<td>O157:H7</td>
<td>23</td>
<td>Liquid, bloody, mucoid</td>
</tr>
<tr>
<td>O157:H7</td>
<td>26</td>
<td>Mucoid, bloody</td>
</tr>
<tr>
<td>O157:H7</td>
<td>37</td>
<td>Liquid</td>
</tr>
<tr>
<td>O157:H7</td>
<td>66</td>
<td>Bloody, liquid</td>
</tr>
<tr>
<td>O157:H7</td>
<td>15</td>
<td>Blood speckled, liquid</td>
</tr>
<tr>
<td>O157:H7</td>
<td>22</td>
<td>Bloody, liquid</td>
</tr>
<tr>
<td>O157:H7</td>
<td>51</td>
<td>Liquid</td>
</tr>
<tr>
<td>O26:H11</td>
<td>3</td>
<td>Liquid</td>
</tr>
<tr>
<td>O6:H2</td>
<td>20</td>
<td>Bloody, liquid</td>
</tr>
<tr>
<td>O111:HNT</td>
<td>21</td>
<td>Bloody, mucoid</td>
</tr>
<tr>
<td>O5:HNM</td>
<td>28</td>
<td>Liquid</td>
</tr>
<tr>
<td>O111:HNM</td>
<td>34</td>
<td>Bloody</td>
</tr>
<tr>
<td>O103:H25</td>
<td>45</td>
<td>Mucoid</td>
</tr>
<tr>
<td>O121:H19</td>
<td>54</td>
<td>Liquid</td>
</tr>
<tr>
<td>O111:HNM</td>
<td>57</td>
<td>Liquid</td>
</tr>
<tr>
<td>O121:H19</td>
<td>17</td>
<td>Semiformed</td>
</tr>
<tr>
<td>O121:H19</td>
<td>17</td>
<td>Liquid</td>
</tr>
<tr>
<td>O5:HNM</td>
<td>21</td>
<td>Liquid, bloody</td>
</tr>
<tr>
<td>O145:HNM</td>
<td>21</td>
<td>Bloody, liquid</td>
</tr>
<tr>
<td>ONT:H25</td>
<td>34</td>
<td>Mucoid</td>
</tr>
</tbody>
</table>
Review

• Testing for shiga-toxins is necessary for comprehensive detection of STEC: non-O157 are a significant proportion of infections

• Sporadic nature of these infections paired with outbreaks demands constant surveillance through incorporation into routine testing

• Number of infections overall is still underappreciated based on current testing practices
## Infamous outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Serotype</th>
<th>Source</th>
<th>Location</th>
<th>Cases</th>
<th>HUS</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>O157:H7</td>
<td>Jack in the Box hamburgers</td>
<td>US Northwest</td>
<td>Several 100</td>
<td>3</td>
<td>4 (children)</td>
</tr>
<tr>
<td>1996</td>
<td>O157:H7</td>
<td>Odwalla apple juice</td>
<td>NW US/Canada</td>
<td>65</td>
<td>13</td>
<td>1 (16 mo)</td>
</tr>
<tr>
<td>2006</td>
<td>O157:H7</td>
<td>Bagged spinach (CA)</td>
<td>26 states</td>
<td>199</td>
<td>31</td>
<td>3</td>
</tr>
<tr>
<td>2008</td>
<td>O111:HNM</td>
<td>Country Cottage Buffet</td>
<td>Oklahoma</td>
<td>341</td>
<td>17</td>
<td>1</td>
</tr>
</tbody>
</table>

## Recent outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Serotype</th>
<th>Source</th>
<th>Location</th>
<th>Cases</th>
<th>Hospitalized</th>
<th>HUS</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>O157:H7</td>
<td>JBS Swift Beef Co/Fairbanks Farms Beef</td>
<td>8 states</td>
<td>26</td>
<td>19</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>2009</td>
<td>O157:H7</td>
<td>Nestle Raw cookie dough (flour)</td>
<td>30 states</td>
<td>72</td>
<td>34</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2010</td>
<td>O145</td>
<td>Shredded romaine lettuce</td>
<td>5 states</td>
<td>30</td>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2011</td>
<td>O157:H7</td>
<td>Romaine lettuce</td>
<td>10 states</td>
<td>60</td>
<td>30</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2012</td>
<td>O126</td>
<td>Jimmy John’s clover sprouts</td>
<td>11 states</td>
<td>29</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

## Recent outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Serotype</th>
<th>Source</th>
<th>Location</th>
<th>Cases</th>
<th>Hospitalized</th>
<th>HUS</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>O157:H7</td>
<td>Organic spinach/spring mix blend</td>
<td>5 states</td>
<td>33</td>
<td>15</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>O121</td>
<td>Frozen food products</td>
<td>19 states</td>
<td>35</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>O157:H7</td>
<td>Ready-to-eat salad (sold @ Trader Joe’s)</td>
<td>4 states</td>
<td>33</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2014</td>
<td>O121</td>
<td>Raw clover sprouts Evergreen Fresh Sprouts (Idaho)</td>
<td>6 states</td>
<td>19</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

http://www.cdc.gov/ecoli/outbreaks.html
## Recent outbreaks

<table>
<thead>
<tr>
<th>Year</th>
<th>Serotype</th>
<th>Source</th>
<th>Location</th>
<th>Cases</th>
<th>Hospitalized</th>
<th>HUS</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>O157:H7</td>
<td>Ground beef Wolverine Packing Co.</td>
<td>4 states</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2015</td>
<td>O26</td>
<td>Chipotle Mexican Grill</td>
<td>14 states</td>
<td>60</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2015</td>
<td>O157:H7</td>
<td>Costco Rotisserie chicken salad</td>
<td>7 states</td>
<td>19</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2016</td>
<td>O157</td>
<td>Alfalfa sprouts Jack &amp; The Green Sprouts</td>
<td>2 states</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Source and cause summary

Major outbreaks since 2009 (advent of broad toxin testing adoption):
  • Produce vs Meat = 9 vs 4
  • O157 vs non-O157 = 8 vs 5

Non-O157 likely still underreported
  • Pre 2009: 5 O157 vs 1 non-O157
The worst case scenario

THE 2011 O104:H4
GERMAN STEC OUTBREAK
Timeline of events - 2011

- May 23rd – Robert Koch Institute reports unusual number of HUS & bloody diarrhea attributable to STEC
  - Samples first detected May 8th
- May 24th – WHO contact Danish *E. coli* network after German patient diag/w STEC
  - Isolate typed as O104:H4 & positive for shiga-toxin production (Stx$_{2a}$)
  - Subsequent German samples all type as O104:H4
- May 26th – Spanish cucumbers implicated in outbreak by PCR
- May 30th – O104:H4 specific PCR developed in Germany
- June 6th – whole genome sequence assembled & released
- June 10th – O104:H4 detected on fenugreek sprouts by PCR

The final numbers *(circa July 22)*

- Total diagnosed infections: 4075
- HUS cases: 908 cases
  - 22.3% (atypical)
  - 34 deaths (3.7 % fatal)
- Non-HUS cases: 3167
  - 16 deaths (0.05% fatal)
- Sporadic cases in Europe, US, & Canada

*Mellmann et al. 2011, PLoS One: 6(7).*
<table>
<thead>
<tr>
<th>Country</th>
<th>HUS Cases</th>
<th>HUS Deaths</th>
<th>STEC Cases</th>
<th>STEC Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Canada</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Denmark</td>
<td>10</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>France</td>
<td>7</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Germany</td>
<td>857</td>
<td>32</td>
<td>3078*</td>
<td>16</td>
</tr>
<tr>
<td>Greece</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Luxembourg</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Netherlands</td>
<td>4</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Norway</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Poland</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sweden</td>
<td>18</td>
<td>1</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Switzerland</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>UK</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>USA</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>908</strong></td>
<td><strong>34</strong></td>
<td><strong>3167</strong></td>
<td><strong>16</strong></td>
</tr>
</tbody>
</table>
Atypical presentation

- Median age of patients with HUS, 42 y.o.
- HUS distribution:
  - 2% < 5 y.o. vs normally 69%
  - 88% of HUS cases were in adults (20X normal)
- Bloody diarrhea (adults 91% vs children 64%)
- Severe neurological symptoms (48%, 104/217)
- Many received Abx (mero, azithro, rifaximin, tigecycline)
  - Outcome data pending; carbapenems and rifampin were recommended in some cases
- Person-to-person??

Magnus et al. 2012. Brain, Apr. 26 (Epub)
Strain characteristics

• The German STEC is not actually STEC!!
• Screening PCR found genes for the aggregative adhesin locus of EAggEC
• All biochemical profiles similar for EAggEC & less for STEC
• Genome sequence suggests EAggEC was phage-transduced w/ Shiga-toxin bacteriophage

A strange duck

• Intimin-negative – rare for STEC
  – Typically don’t cause HUS in children

• O104:H4 is a rare serotype for Stx production

• Encodes antibiotic determinants
  – TEM-1 broad spectrum β-lactamase
  – CTX-M-15 ESβL also confers cefepime resistance

• Because shiga-toxin is major factor, strain has been termed an STEC

Not as unique as we thought

- Review of unpublished data from PulseNet & EU Surveillance Network
- Stx2+ O104:H4 in databases
  - 2001 – 2 patients w/HUS, Germany (EAggEC/STEC)
  - 2004 – 1 patient (no clinical Hx), France
  - 2005 – 1 HUS, Korea
  - 2009 – 2 HUS, Republic of Georgia (EAggEC/STEC)
  - 2010 – 1 severe diarrhea, Finland (EAggEC/STEC)

Scheutz, F. Euro Surveill 2011, 16(24)
The Forgotten French experience

• 1995, small outbreak of HUS linked to O111 STEC/EAggEC strain (Stx$_{2a+}$)

• 1° children

• Severe disease & ↑ HUS

• Suggests this combination may be more potent for disease

All Quiet on the Western Front

- Outbreak diminished per mid-July 2011
- Largest & deadliest STEC outbreak in history
- Food inspection agencies reviewed processes
  - Egyptian sprout seeds implicated
- Multinational effects - 16 nations w/ cases
How would labs fare in this outbreak now?

<table>
<thead>
<tr>
<th>Method</th>
<th>Detect EAggEC/STEC emerging pathogen??</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157 Culture only</td>
<td>No</td>
<td>• Miss all infected patients!</td>
</tr>
</tbody>
</table>
| O157 culture, toxin detection based on criteria (e.g. – bloody diarrhea, age <5 or >60, summer months, HUS, ruminant/farm exposure) | Maybe | • Symptoms were atypical  
• Most patients were >5 and <60  
• Many children non-bloody  
• Ruminant/farm exposure absent  
• Severe cases without HUS  
• BUT, all were summer, and many adults were bloody… |
| O157 culture + Toxin detection/PCR          | YES                                   | • Toxin is unchanged, will detect  
• No delay                                                                   |
| Toxin/PCR detection reflex positive to O157 culture | YES                                   | • Toxin is unchanged, will detect  
• No delay                                                                   |
| Toxin/PCR detection w/rapid submission to PHL | YES                                   | • Toxin is unchanged, will detect  
• No delay                                                                   |
Key Points

• Not a seasonal illness – occurs year round
• Geographically unpredictable
• Bloody diarrhea – poor universal predictor of STEC
• >60% of sporadic cases and 40% of outbreaks are non-O157
  – Must screen for shiga toxins or genes
• Prevention of HUS only achievable 3-4 days after onset of illness
  – Delays are critical
Questions?