Pre-harvest Control of STEC’s in Cattle:
Late-breaking news & challenges to food safety

Tom Besser
WSU College of Veterinary Medicine
STEC and Foodborne Illnesses in the USA

- Diarrheal disease, all causes: 47.8 million / yr
  - STEC = 3.6%

- Hospitalizations, all causes: 128 thousand / yr
  - STEC = 1.85%

- Deaths, all causes: 3,000 / yr
  - STEC = 0.7% (~21)

Nearly 4,180 Sickened in E. coli O104:H4 Outbreak

BY MARY ROTHSCILD | JUL 05, 2011

The European Centre for Disease Prevention and Control (ECDC) reported Monday that the European toll in the outbreak of E. coli O104:H4 in Germany and France linked to sprouts had risen to 4,173 illnesses and 49 deaths.

Those numbers include 892 hemolytic uremic syndrome (HUS) cases, according to ECDC.

A single lot of fenugreek seeds -- lot number 48088 -- from an Egyptian exporter appears to be the common link between the German and French outbreaks, the European Food Safety Authority (EFSA) reported Tuesday, even though microbiological tests carried out on the seeds have thus far been negative.
European *E. coli* O104:H4 outbreaks

- **Severity**
  - 2011 O104:H4 >4,000 cases 50 deaths
  - 1993 ‘Jack in the Box’ 700 cases 4 deaths

- **Previously unrecognized *E. coli* pathotype**
  - Enteroaggregative *E. coli* + Stx2a encoding phage
  - (Antibiotic resistant)

- **Epidemiology**
  - Known important vehicle (sprouts); amplification
  - Rarely isolated from human disease prior to outbreak
  - Not isolated from environmental or animal sources
FSIS will begin testing trimmings for six additional STEC on June 4. As of that day, any raw, non-intact beef products or components contaminated with STECs O26, O45, O103, O111, O121 and O145 will be legally considered adulterated -- just as the agency has long treated E. coli O157:H7.
Non-O157 STEC as adulterants

- Big Six: O26, O111, O45, O145, O121, O103
  >300 STEC serotypes in cattle (Hussein JAS 2006)
- Severity: more frequent, less severe
  - But consider *E. coli* O104:H4!
- Diversity: STEC clades w/i serotypes (Norman 2012)
- Surveillance of ground beef components (9/2/12)
  - 6/610; 0.98%
- Diagnostics: under development
- Control measures
  - No specific control measures known
STEC and Foodborne Illnesses

• Diarrheal disease: 47.8 million / yr
  – 3.6% STEC: 110,000 non-O157; 63,000 O157

• Hospitalizations: 128 thousand / yr
  – 1.85% STEC: 2,100 O157; 270 non-O157

• Deaths: 3,000 / yr due to foodborne illness
  – 0.7% STEC: 20 O157; 1 non-O157

Testing and Safe Food

P(detection) proportional to contamination level
  – Industry vs FSIS

• Total *E. coli* O157:H7, 2009
  – 16 recalls; 1,326,982 lbs products

• FSIS routine microbiologic or records monitoring
  – 13 recalls; median 218 lbs.; 602,922 lbs total

• Illness trace-back
  – 3 recalls; median 248,000 lbs.; 723,898 lbs total

http://www.fsis.usda.gov/recalls/
Testing and Safe Food

• Useful for monitoring contamination frequency
• Likely misses most contaminated batches
• Unlikely to measurably impact public health
FSIS is proposing to launch trace-back investigations earlier and identify additional potentially contaminated product when the Agency finds *E. coli* O157:H7 through its routine sampling program. When FSIS receives an indication of contamination through presumptive positive test results for *E. coli*, the Agency will move quickly …
Traceback on Preliminary Positive Results

• May result in improved effectiveness of recalls
  – 2009: 1.36% of recalled product due to FSIS routine monitoring was reported recovered

http://www.fsis.usda.gov/recalls/
• Aug 23: First contaminated product produced
• Sept 3: FSIS test at US border detects contamination
• Eight (+?) people ill in Alberta
• Sept 16: First recall issued, subsequently expanded
  – Includes 890,000 lbs beef trim / boxed beef in US
• Needling steaks?
“There is another, more sinister pattern of bacteriophage infection termed a ‘lysogenic’ cycle. Once inside the host cell, lysogenic viral DNA can integrate itself within the chromosome of the host and stay there, dividing whenever the bacterial chromosome divides.”

- Shiga toxins are produced by lysogenic bacteriophage
- Different Shiga toxin encoding bacteriophages known to infect different *E. coli* O157:H7 lineages
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cattle</th>
<th>Human</th>
<th>Total</th>
<th>Genogroup</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WY12</td>
<td>40.5%</td>
<td>50.5%</td>
<td>189 (45.1)</td>
<td>Clinical</td>
<td>0.048</td>
</tr>
<tr>
<td>AY2</td>
<td>4.8%</td>
<td>24.5%</td>
<td>58 (13.8)</td>
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<td>&lt;0.0001</td>
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<tr>
<td>SY2c</td>
<td>19.8%</td>
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<td>Bovine biased</td>
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# Host-association of SBI genotypes

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### Association of Clinical Genotypes with Stx2a

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<tr>
<th>Stx associated bacteriophage</th>
<th>Insertion</th>
<th>SBI genotypes</th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clinical</td>
<td>Bovine-biased</td>
<td>Unclassified</td>
<td>Total</td>
</tr>
<tr>
<td>Stx2a</td>
<td>wrbA</td>
<td>Bovine</td>
<td>92 (92)</td>
<td>0 (0)</td>
<td>11 (9)</td>
<td>103 (101)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>97 (97)</td>
<td>0 (0)</td>
<td>9 (9)</td>
<td>106 (106)</td>
</tr>
<tr>
<td></td>
<td>argW</td>
<td>Bovine</td>
<td>17 (17)</td>
<td>38 (0)</td>
<td>7 (2)</td>
<td>62 (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human</td>
<td>74 (74)</td>
<td>8 (0)</td>
<td>1 (1)</td>
<td>83 (75)</td>
</tr>
</tbody>
</table>
Neonatal Piglet Challenge Model

• Bacterial Strains
  – Ten strains of bovine-biased genotypes
  – Ten strains of clinical genotypes
  – Oral challenge of newborn piglets

• Disease and pathology scored by personnel blind to challenge strain identity
Clinical genotypes are more virulent in a conventional neonatal piglet model

Sham inoculated
Bovine biased
Clinical
Strain Sakai PC

Shringi et al., 2012
Correlation of genogroup prevalence in cattle with human HUS incidence?

![Graph showing correlation between AY2 in cattle and HUS incidence with data points for Scotland, USA, Japan, and Australia.](image-url)
Correlation of *E. coli* O157:H7 genotypes with cattle production systems

- Single nucleotide polymorphism genotyping

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<tr>
<th>Genotype</th>
<th>Dairy / Cow-calf</th>
<th>Beef feedlot</th>
</tr>
</thead>
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<tr>
<td>I-a Bovine-biased</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>I-b Clinical</td>
<td>89</td>
<td>221</td>
</tr>
<tr>
<td>II-b Clinical</td>
<td>25</td>
<td>4</td>
</tr>
<tr>
<td>IV-b Unclassified</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>IV-c Bovine-biased</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td>V-a Unclassified</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>V-c Bovine-biased</td>
<td>36</td>
<td>16</td>
</tr>
</tbody>
</table>

Likelihood of a Clinical Genotype

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<th>Effect</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td>Feedlot vs Dairy</td>
<td>8.38</td>
<td>4.8 – 14.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Bono et al., 2012 Mol Biol Evol
“The probability of illness, given that a serving is contaminated during production, is roughly constant throughout the year. This suggests that the seasonal change in the probability of exposure to a contaminated serving is the primary driver of the season pattern in illnesses, rather than any seasonal changes in consumer storage and handling.”

Williams et al., FPD 7(10):1247, 2010
Seasonal Variation in *E. coli* O157:H7

Data from CDC, Stanford et al, 2005
Seasonal Variation in *E. coli* O157:H7 Shedding by Cattle

What causes it?

– Seasonal effects on cattle physiology?
– Seasonally variable cattle rumenal microflora?
– Seasonally variable exposure of cattle?
– Other factors?
Seasonal variation: Driven by exposure

![Graph showing seasonal variation in percent positive cattle. The graph compares summer and winter conditions, with summer having a higher percent positive cattle compared to winter. The x-axis represents days post-challenge, ranging from 0 to 56, and the y-axis represents the percent positive cattle, ranging from 0 to 100. The graph includes error bars for each data point. The red line indicates summer (n=20), and the green dashed line indicates winter (n=20).]
Seasonal Variation in *E. coli* O157:H7 Shedding by Cattle

What causes it?

– Seasonal effects on cattle physiology?
  No; same dose gives same colonization

– Seasonally variable cattle rumenal microflora?
  No; oral vs rectal give same colonization

– Seasonally variable exposure of cattle?
  Probably!
Supershedders!

- 10% of cattle = 90% of fecal shedding
- Is a supershedder a different kind of cow or just a different phase of infection?
Study design

- Natural STEC O157 shedding
- Research pens in commercial feedlot operation
- Random assignment, 20 pens x 8 per pen.
- No direct contact between pens or sharing feed / water sources
- Fed, managed, and slaughtered as in commercial feedlot

Cobbold et al., AEM 73:1563, 2007
Sample collection

- Individually sampled twice per week over 14-weeks
- Four cattle withdrawn
- Personnel sampling cattle and processing samples were blinded to results throughout the study.
Supershedders: High Summer in a Research Feedlot

Each rectangle represents a pen from entry (left) to full feed (right). Colors represent STEC O157 shedding: yellow <1000 cfu/g, red >1000 cfu/g.
Within pen reproduction number vs shedding quantity

- To sustain infection within the North pens, shedding must be at least \(10^4\) cfu/g.
- To sustain infection in the South pens shedding must be over \(10^{5.2}\) cfu/g.
- High shedding individuals cause more infections.

Taking into account expected duration of colonization and shedding

NP French, S Spencer, R Cobbold
Summary

• News!
  – STEC O104:H4 – Something new under the sun!
  – (Other) non-O157 STEC – ‘Big Six’
    • Little public health impact to be expected

• WSU research
  – Clinical genotypes associated with feedlot cattle
  – Seasonal variation – environmental exposure?
  – STEC O157 - every animal can be a supershedder