Shiga Toxin Producing *Escherichia coli*

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Dr. Angela M. Shaw
Assistant Professor of Food Science and Human Nutrition
Food Safety Extension and Outreach Specialist
Biofilm on Apple

• SEM image showing attachment and biofilm formation by *E. coli* cells in the calyx area of an inoculated Golden Delicious apple
• Formed with 72 hours

Tarver. 2009. Biofilms: A threat to food safety. Food Technology
ESCHERICHIA COLI
Escherichia coli

- Both pathogenic and non-pathogenic serotypes
- Habitat: GI tract of humans and animals
- Rod shape
- Non-spore forming
Three Types of Antigens

• The somatic lipopolysaccharide antigen (O)
• The flagellar antigens (H)
• The capsular antigens (K)
• ~174 O antigens, 56 H antigens, and 103 K antigens
E. coli serotyping

Lipopolysaccharide (LPS)
- = O antigen
- = H antigen

O1-O181
H1-H56
Flagella

O157:H7
O111:H8
O26:H11

Non-O157 Shiga Toxin-producing E. coli: Status and Relevance to Food Safety
U.S. Meat Animal Research Center, USDA-ARS; Clay Center, Nebraska
Escherichia Coli
5 Categories of enterovirulent

1. Enterotoxigenic
2. Enteroinvasive
3. Enteropathogenic
4. Enteroaggregative
5. Enterohemorrhagic

http://www.filterclean.co.uk/images/infobacterial
controlimages/infoecoliimage.jpg
Enterotoxigenic (ETEC)

Colonization of Intestinal Wall
Enterotoxigenic (ETEC)

- Travelers diarrhea (Gastroenteritis)
  - 11 million travelers each year
    - 1% hospitalized, 20% confined to bed, 40% forced to change plans
- Similar to V. cholerae
- Heat Labile Enterotoxin
- High Infectious Dose ($10^6$)
Enterotoxigenic (ETEC)

- Multi-drug Resistant- 90% resistant to ampicillin
- Source
  - Water Contamination with Human sewage
  - Infected workers
- Implicated foods
  - Ice, Street Vendors
  - Undercooked seafood, Dairy products (semi soft cheese)
Enterotoxigenic (ETEC)

- Non-invasive
- Symptoms
  - Severe watery diarrhea
  - No Inflammation
  - No Fever
  - Abdominal cramps, nausea and malaise
Enteropathogenic (EPEC)

On Human RBC

Human intestinal cell
Enteropathogenic (EPEC)

- Infant diarrhea (1940-1950’s problem)
  - Dehydration, electrolyte imbalance and death
  - Highly infectious
  - Bottle feeding & Malnutrition
- Sporadic food borne outbreaks
- Virulence mechanism is unknown
  - Enterotoxin mediated infection
  - Shiga-like toxin or verotoxin
Enteropathogenic (EPEC)

- Serogroups O127 and O142
- Symptoms
  - Watery diarrhea WITH MUCUS
  - Bloody diarrhea (Shigella)
  - Vomiting, malaise
  - Some inflammation
  - No fever
Enteroinvasive (EIEC)
Enteroinvasive (EIEC)

- Bacillary dysentery
- EXTREMELY DIFFICULT TO DETECT IN FOODS
- Do not produce Shiga-Toxins
- Resembles Shigella and non-pathogenic E.coli
- Onset time: 12-72 hours
- Cruise ships, mental institutions and travelers
Enteroinvasive (EIEC)

- Implicated Foods
  - Hamburger meat
  - Unpasteurized milk
- Invade lining of intestinal tract
  - BLOOD AND MUCUS
  - Severe inflammation
  - Fever and chills
  - Hemolytic uremic syndrome (HUS)
Enteroaggregative (EAEC)

Aggregative pattern of adherence
Enteroaggregative (EAEC)

- Tropical countries
- Infant diarrhea (more than 14 days)
- Intestinal mucosa damage
  - Causes mucous biofilm
- Heat stable Enterotoxin
Enterocolitigenic (EAEC)

- Associated with HIV patients
- Symptoms
  - Watery and mucous diarrhea
  - Low grade fever
  - Grossly bloody stools
Enterohemorrhagic
Enterohemorrhagic

- 11,000 cases a year in US
- 3,200 hospitalizations a year
- Symptoms:
  - Acute bloody diarrhea
  - Cramping
  - Abdominal cramps, nausea
- Moderately invasive
Enterohemorrhagic

- Zoonotic Disease
- Onset time: 1 to 6 days
- Duration: up to 8 days (Self-limiting)**
- Implicated foods
  - Hamburger, Roost beef
  - Seed sprouts, produce
  - Cider apple, Unpasteurized milk
Enterohemorrhagic- 2 diseases

• Hemorrhagic colitis
  – Lining of Large Intestines
  – Severe bloody diarrhea, painful
  – 6,000 cases in Japan

• Hemolytic uremic syndrome – HUS
  – Occurs 8% of the time
  – 61 fatal per year (3-5%)
  – Kidney failure
Escherichia coli O157:H7

- Gram negative bacteria
- Associated with cattle
- Cost US Residents $993 million
  - 66,905 cases per yr
  - $14,838 cost per case
- Produce related
  - 39% of outbreaks and 54% of illness
Leafy Greens

Twenty-six lettuce/leafy green-associated *E. coli* outbreaks 1995-2005

- 751 ill, 94 hospitalized, 2 HUS, 1 death
- Lettuce second most common food vehicle in *E. coli*O157 outbreaks
Vectors of Contamination

- Water
- Manure and Land Use
- Wild animals
- Humans
- Air
- Equipment
Symptoms

- Symptoms appear in 3 to 8 days
- Symptoms last 5-7 days
- Symptoms
  - Severe stomach cramps
  - Bloody diarrhea
  - Vomiting (30-50% of cases)
  - Complications: hemolytic uremic syndrome (2-7% of cases)
Non-O157 STEC

- Shiga Toxin (Stx) producing *Escherichia coli* (STEC)
- First recognized 1975 in France outbreak of O103
- Link between STEC and HUS established in study in Canada in 1980’s
Top Non-O157 Serotypes (CDC)

- O26  22% of non-O157 STEC
- O111 16% of non-O157 STEC
- O103 12% of non-O157 STEC
- O121  9%  of non-O157 STEC
- O45   7%  of non-O157 STEC
- O145  5%  of non-O157 STEC
Non-O157 STEC: Virulence Factors

- Shiga-like toxins 1 and/or 2 (Stx1, Stx2)
- Alpha-hemolysin
- EHEX-hemolysin
- Intimin (eae) genes
- An enzyme-activated subtype of Stx2d has been associated with HUS in STEC strains lacking eae

Source: Draft Risk Profile for Pathogenic Non-O157 STEC
Symptoms

• Stx2 and Stx2c subtypes are associated with severe symptoms (bloody diarrhea and HUS)
• Severe stomach cramps, bloody diarrhea, and vomiting
  – If there is fever, it usually is not very high (less than 101°F/less than 38.5°C).
Infection

• 1-10 cells
• Onset is 1-10 days
• Duration is 5–7 days
• Most infections are mild
• ~10% are severe or even life-threatening
Reservoir of STEC

• Not host specific
• Guts of ruminant animals, including cattle, goats, sheep, deer, and elk
• Mostly found in cattle and beef products
  – Up to 70.1% prevalence in beef cattle (341 serotypes with 36% pathogenic)
  – Up to 74% prevalence in dairy cattle (152 serotypes with 49% pathogenic)
Implicated Foods

• Meat: beef, lamb, pork, chicken
• Produce: Sprouts, peppers, leafy greens
• Raw Milk and cheese products

• Several studies found O157:H7 in 10% of animal feed
Prevalence: Comparison

- *E. coli* O157:H7 causes ~73,000 cases per year in the U.S. (CDC)
- Non-O157 STEC cause ~37,000 cases per year in the U.S. (CDC)
  - More than 200 non-O157 serotypes have been isolated from outbreaks and sporadic cases
## Background of Non-O157

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>non-O157:H7 STEC</th>
<th>E. coli O157:H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal contact</td>
<td>6.2%</td>
<td>9.7%</td>
</tr>
<tr>
<td>Water</td>
<td>10.0%</td>
<td>25.6%</td>
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<tr>
<td>Person-person contact</td>
<td>28.8%</td>
<td>6.8%</td>
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<tr>
<td>Dairy</td>
<td>10.0%</td>
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<tr>
<td>Meat</td>
<td>11.2%</td>
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</tr>
<tr>
<td>Produce</td>
<td>6.2%</td>
<td>9.2%</td>
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<tr>
<td>Other Food</td>
<td>8.8%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Unknown</td>
<td>18.8%</td>
<td>5.8%</td>
</tr>
</tbody>
</table>

Doyle, UW-Madison, Dec. 2009/April 2010
New Statistics

• Dr. Elisabeth Hagen, Under Secretary for Food Safety at the U.S. Department of Agriculture, has reported non-O157 STECs in significant numbers within the food supply and in some cases in much higher numbers than *E.coli* O157:H7

• CDC showed non-O157 STEC at rates twice that of *E.coli* O157:H7 in 2011, which is concurrent with the FoodNet’s 2010 data
Survival

• Temperature range: 4-45°C (can survive refrigeration and freezing)
  – Optimal 37°C
  – Most cannot grow above 45°C

• pH range: 5.5 and 7.5
  – EHEC strains can have a high degree of acid tolerance, surviving virtually unchanged during 7-hr exposures at pH 2.5 and 37°C
Survival

- Water activity: as low as 0.90
- Salt conditions of 3.5%
- Survive at 0.5 to 5.0% salt
- Survive several weeks in dry conditions in the refrigerator
- Able to move in the air (5 km)
Survival on Plant

• Biofilm can form and attachment is possible

• Some studies have shown internalization when forced into plant or through injury
  – Death typically occurs within 72 hours by natural defense
History of outbreaks

- Argentina (1982-1991): 433 cases ground beef
- Italy (1992): 9 cases ground beef
- Canada (1992): 6 cases raw milk
- Montana (1994): 4 cases raw milk
- Australia (1994-1995): 161 cases beef sausage
- Germany (2000): 6 cases beef sausage
ESCHERICHIA COLI OUTBREAKS
Outbreak 1: Jimmy John’s Sprouts

- 29 individuals infected in 11 states
- *E. coli* O26
- Onset time December 25\(^{th}\), 2011 to March 3\(^{rd}\), 2012
- Age 9 to 57 (median 26 yrs.)
- 89% of the ill were female
- 7 were hospitalized (no death or HUS)
History of problems

• Between 2005 and 2011, at least 10,571 people became sick

• Summer of 2011 *E. coli* O104:H4 contaminated raw sprouts killed 31 people and sickened over 3,000 persons in Germany

• In 1996 a very large outbreak of over 6,000 confirmed cases of *E. coli* O157:H7 infections occurred in Japan from radish sprouts
Source: Sprouts Growing Conditions

- Sprouting seeds must be soaked in water for several hours and held at 20-26°C for multiple days for germination.
- During this soaking phase of growth if pathogens are present on or in the seed, then microbial growth is favored.
- Within two days of sprouting microbial populations have increase by ~3 logs.
- No kill step.
Outbreak 2: Strawberry

- Oregon outbreak
- Illness occurred between July 10th and July 29th
- 15 people ill: age 4 to 85 (5% hospitalized)
- Source: deer droppings
Outbreak 3: Spinach

- *Escherichia coli* O157:H7 outbreak in 26 states
- 199 illnesses; 102 hospitalizations (31 with HUS); 3 death
- Five firms have initiated recalls
- Previously used fields with cattle
Outbreak 4: Unpasteurized gouda cheese

• Alberta in December of 2002
• 10 cases of *E. coli* O157:H7
  – 77% bloody diarrhea; 42% hospitalized
  – 2 cases of HUS (22 month old and 4 yr. old)
• Attended same local farmers market
• Consumed cheese form same vendor
Investigation

- 3 local farmers markets, specialty stores and restaurants
- 26 samples from farm, retailers, and homes and 2 positive samples
- No positive environmental swabs, raw milk, or well water swabs
- Survived 104 days after production
ADDITIONAL QUESTIONS

Angela Shaw, PhD
Assistant Professor and Extension and Outreach Specialist in Food Safety
Department of Food Science and Human Nutrition
2577 Food Science Building
515-294-0868
angelaml@iastate.edu
http://blogs.extension.iastate.edu/iowagap