**Escherichia coli**

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*PHR 250*

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**E. coli overview**

- Most common (up to $10^8$/g) facultative bacterium in intestines of mammals & birds — almost all nonpathogenic.
- Until *E. coli* O157:H7 (& other EHEC?), *E. coli* pathogenic for humans were human-specific.

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**Historical**

- First characterized in late 1800s (Theodor Escherich)
- Recognized as common to intestinal tract of humans and animals

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**Characteristics**

- Member of *Enterobacteriaceae*
- Coliform: ability to ferment lactose with acid and gas
- Facultative, gram-negative rod
- Acid and gas from glucose

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**Characteristics**

- Temperature range: 7–8 to 44–46°C, optimum 37°C
- pH range 4.4–9.0, optimum 6–7
- Water activity: minimum 0.95; optimum 99.5

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**Taxonomy**

- Somatic antigen O (outer-membrane protein): >200 O antigens (173 defined?)
- Capsular antigen: 103 K antigens
- Flagellar antigen H: 56 H antigens (note “NM”)
- Fimbriae/pili
- Over 700 serotypes recognized
**Food-associated illness: six virulence groups currently recognized**
- Enteroaggregative (EA_{gg}EC)
- Enteroinvasive (EIEC)
- Enteropathogenic (EPEC)
- Enterotoxigenic (ETEC)
- Enterohemorrhagic (EHEC)
- Diffusely adherent (DAEC)

**Enteroaggregative (EAEC)**
- Unclear whether cause foodborne illness
- Persistent diarrhea in children
- Diarrhea watery and mucoid.
- Up to 30% grossly bloody stools.

**EAEC - 2**
- EAEC bind in clumps (aggregates) to cells of the small intestine and produce toxins.
- Virulence factors include aggregative adherence and heat-stable enterotoxin; plasmid-mediated

**Enteroinvasive (EIEC)**
- Acute dysenteric diarrhea
- EIEC invade cells in the colon and spread laterally, cell to cell.
- Virulence factors include cell invasion and intracellular multiplication; plasmid and chromosomally mediated.

**EIEC - 2**
- Est. infectious dose: $10^8$
- Incubation: 8–24 h, mean 11 h
- Profuse diarrhea or dysentery, chills, fever, headache, muscular pain, abdominal cramps
- Duration: days to weeks

**Entero-aggregative E. coli (EAEC) form a biofilm and secrete cytotoxins**
**EPEC - 2**
- Immunity thought to play a role in low incidence.
- WHO report suggests that 50% of children carry antibodies which may lead to immunity.

**EPEC - 3**
- EPEC attach to intestinal mucosal cells causing cell structure alterations (attaching and effacing). EPEC cells invade the mucosal cells.
- Virulence factors include attaching and effacing lesions, localized adherence mediated by bundle-forming pilus; plasmid- and chromosomally mediated

**EPEC - 4**
- Est. infectious dose: $10^5-10^{10}$
- Incubation: 17–72 h, mean 36 h
- Symptoms described in Table 4
- Duration: 6 h–3 days, mean 24 h

**Entero-pathogenic E. coli (EPEC)**
- Children under age of 1 year
- Traveler’s diarrhea
- Acute and/or persistent profuse watery diarrhea, vomiting, fever
- Uncommon in temperate climates with good hygienic standards
**Enterotoxigenic (ETEC)**
- Diarrhea, vomiting, and fever
- Traveler’s diarrhea
- Profuse watery diarrhea without blood or mucus

**ETEC - 2**
- ETEC adhere to the small intestinal mucosa and produce toxins that act on the mucosal cells.
- Virulence factors include adherence (host-species specific) and heat-stable or heat-labile toxins; plasmid- and chromosomally mediated.

**ETEC - 3**
- Est. infectious dose: $10^8$–$10^{10}$
- Incubation: 8–44 h, mean 26 h
- Symptoms described in Table 4; severe version like cholera
- Duration: 3–19 days

**Diffusely adherent (DAEC)**
- Childhood diarrhea
- Fimbrial and non-fimbrial adhesins identified; plasmid- and chromosomally mediated.
- Cause elongation of microvilli

**Enterotoxigenic E. coli (ETEC) “inject” toxins into enterocytes**

**Diffusely adherent Escherichia coli cause elongation of microvilli**
Enterohemorrhagic (EHEC)

- Shiga-toxin production reported in over 100 Escherichia coli serotypes (STEC).
- Enterohemorrhagic E. coli (EHEC) strains are a subgroup of STEC that are associated with serious disease in humans.

Background

- *Escherichia coli* O157:H7 is the most frequently reported EHEC and an important cause of infectious bloody (enterohemorrhagic) diarrhea and hemolytic uremic syndrome (HUS) in North America.
- An estimated 73,000 human cases and 61 deaths occur in the United States each year (Mead et al., 1999).

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### Escherichia coli O157:H7

**An Evolving Story**

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982</td>
<td><em>E. coli</em> O157:H7 first recognized as a human pathogen</td>
</tr>
<tr>
<td>1993</td>
<td>Large western states outbreak due to undercooked hamburgers served as a fast-food restaurant chain</td>
</tr>
<tr>
<td>1994</td>
<td><em>E. coli</em> O157 added to the list of nationally reportable diseases</td>
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<tr>
<td>1995</td>
<td>First documented lettuce-associated outbreak</td>
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<tr>
<td>1996</td>
<td>Outbreak associated with unpasteurized apple juice affecting the U.S. and Canada</td>
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<tr>
<td>1997</td>
<td>Multi-state outbreak linked to using raw sprouts grown from contaminated seeds</td>
</tr>
</tbody>
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### Escherichia coli O157:H7

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<td>1998</td>
<td>FDA issues guidance document for produce safety</td>
</tr>
<tr>
<td>2000</td>
<td>Outbreaks of <em>E. coli</em> O157:H7 infections among children after direct contact (gutting) with farm animals</td>
</tr>
<tr>
<td>2001</td>
<td><em>E. coli</em> O157:H7 genome sequence completed and published in Nature</td>
</tr>
<tr>
<td>2003</td>
<td>Two separate outbreaks linked to consumption of fresh-cut produce (lettuce and spinach) in California</td>
</tr>
<tr>
<td>2006</td>
<td>Spinach and shredded lettuce outbreaks traced to CA fields: first identification of outbreak strains on farms</td>
</tr>
<tr>
<td>2007</td>
<td>Marketing Agreement/Metrics program initiated in California</td>
</tr>
</tbody>
</table>

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Background

- Virulence factors include
  - Attaching and effacing adherence
  - Shiga toxins
  - Hemolysin
  - Plasmid- and chromosomally mediated.
Background

- Shiga toxins are a family of related phege encoded cytotoxins divided into two major groups:
  - stx1 has 99% homology to shiga toxin of Shigella dysentariae (immunologically identical)
  - stx2 has 55% homology to stx1 (immunologically distinct; multiple variants)
- STEC strains may have stx1, stx2 or both genes.

Background

- Hemolytic uremic syndrome (HUS) in some cases (<5 yr?)
- More rarely, thrombotic thrombocytopenic purpura (TTP)

Background

- EHEC attach to and efface mucosal cells and produce toxin(s) which cause an irreversible inhibition of protein synthesis in eukaryotic cells.

Background

- A subunit shows enzymatic activity
- B subunit binds to receptors on the surface of some tissues (kidney).
- Predominant serotype: O157:H7

Background

- Est. infectious dose: <100 (10?)
- Incubation: 3–9 days, mean 4 days
- Symptoms described in Table 4; hemorrhagic colitis, HUS, TTP
- Duration: 2–9 days, mean 4 days
Entero-hemorrhagic *E. coli* (EHEC) attach intimately to enterocytes and inject toxins

**EHEC reservoir, vehicles**
- Livestock (cattle, sheep, goats)
- Wildlife (deer, rabbits, wild pigs)
- Foods often undercooked beef
- Contaminated RTE foods — meat to other food
- Naturally contaminated produce (unpasteurized juices, sprouts, leafy greens (lettuce, spinach))

**E. coli O157:H7 in meat**

**E. coli O157:H7 Testing**

**E. coli O157 Detection**

**E. coli O157**
DIP STICK EXAMS (Fiction!)
Detection & Isolation of E. coli O157:H7

**SAMPLES**
- Sample collection
- Sample processing
- Enrichment
- RIMS

**IMMUNO-MAGNETIC BEADS**
- Real-time PCR screen
  - Direct plating
  - TC-SMAC/Cهام agar

**TYPICAL COLONIES**
- Red L-EMB
  - Transfer to TSA-VF

**TYPICAL COLONIES**
- Blue SMAC/Cهام agar
  - Transfer to L-EMB

**ISOLATES**
- Motility, specific biochemical tests
  - Transfer to SMAC/sheep blood agar

**ISOLATES**
- Serology for O157 & 57
  - Transfer to BHI broth/TSA

**ISOLATES**
- Complete biochemical tests
  - Real-time PCR confirmation

Rapid detection and identification of foodborne pathogens

- Contaminated food or environmental samples
- 5-hr enrichment
- Microbial capture system (1 hr)
- Overnight enrichment
- Real-time PCR (2 hr)
- Conventional culture method
  - FDA-BAM (3-5 days)
  - Molecular typing
  - 2-7 days

Presumptive positive

Improved Detection & Isolation of E. coli O157:H7

- Direct plating on TC-SMAC
- RIMS (Patharrnx™)

Recirculating Immuno-magnetic separation system (RIMS, Patharrnx™)

- Multiplex real-time PCR method to identify Shiga-toxin genes
  - stx1, stx2, and E. coli O157:H7

- Assay:
  - SRT and SRT
  - ATV:
  - ATV

Recirculating Immuno-magnetic separation (RIMS)

Presumptive Colonies
Serotyping

Biochemical Analysis

Real-time PCR Confirmation

Identification of Shiga-toxin genes and E.coli O157:H7/H

DNA Fingerprinting of Foodborne Pathogens

“Outbreak strains” are tracked through “PulseNet,” the Centers for Disease Control and Prevention’s (CDC) national DNA fingerprinting database for foodborne pathogens

Pulsed-field gel electrophoresis (PFGE)
- Photo of 10 E. coli O157:H7 PFGE patterns posted on PulseNet (matching outbreak strains shown in lane 2 and 9)

Strain A: ggtgcogtataacggtgAACCAAGGGGAGTTGATGCATG
Strain B: ggtgcogtataacggtgAACCAAGGGGAGTTGATGCATG
Strain C: ggtgcogtataacggtgAACCAAGGGGAGTTGATGCATG
Strain D: ggtgcogtataacggtgAACCAAGGGGAGTTGATGCATG

Multi-locus Variable Number Tandem Repeat Analysis (MLVA)
- Compare DNA sequences (4 letter code ATCG) of selected genes in the bacteria
- Includes E. coli O157:H7 (locus 2). Note that the number of “tandem repeats” of “ACC” match for strains B, C, and D (4 repeats), but not strain A (4 repeats)
Outbreak #1
- Hamburgers 1993, US Northwest
- Estimated 1–15 cells/g or 40–600 cells per raw patty
- >700 people affected, 195 hospitalized, 55 HUS or TTP, 4 deaths; approximately 10% cases person-to-person

Outbreak #1 (cont.)
- Contributing causes: undercooking
- Control: adequate cooking, avoiding cross-contamination, sanitation

Regulatory Changes
- 1988 FSIS policy change for precooked patties to 160°F (71°C) from 140°F (60°C)
  - Modified in 1990 to include lower temp/longer time options

Regulatory Changes - 2
- 1993 Food Code changed to 155°F (68°C) for 15 sec
  - Consumer recommendations 160°F (71°C)

Regulatory Changes - 3
- 1996 Meat HACCP regulations
  - E. coli O157:H7 defined as an “illegal adulterant” in raw ground beef
- 1999 — Irradiation approved for red meats

“Illegal adulterant”
- One sample of ground beef tested—if O157 detected, entire lot condemned.
- No retesting permitted.
Recent outbreaks

- Spinach, September 2006
  - 205 cases in 26 states and Canada
  - Traced to processor/farms in the California Central Coast
- Shredded head lettuce (tacos), Nov-Dec 2006
  - 81 cases in 3 states
  - Traced to Central Valley
- Shredded head lettuce (tacos), Nov-Dec 2006
  - Traced to Central Valley

Spinach-Associated Outbreak Timeline

- Sept 8: Wisconsin PHD notifies CDC of a small cluster of HUS cases with matching PFGE. Oregon SHD identifies a cluster of 2 cases of *E. coli* O157 with matching PFGE.
- Sept 13: Clusters in Wisconsin now at 17 cases and 6 cases in Oregon. CDC and Wisconsin both notify FDA.
- Sept 14: Nationwide conference call. Thirteen states report 45 cases, 8 HUS, 1 death. FDA issues consumer advisory for fresh, bagged spinach.

Spinach-Associated Outbreak Timeline

- Sept 16: FDA advises consumers not to eat “fresh spinach”
- Sept 18: Calif. ERT deployed to 9 farms.
- Sept 21: Positive spinach leefower sample in NM matches outbreak strain
- Oct 12: First samples (cattle feces collected 9/27) positive for outbreak strain at a single ranch. Joint FDA-CDHS press release
- Oct 26: Press conference announcing outbreak strain found in wild pig colonic feces and river water at the same ranch

Environmental Sampling during the *E. coli* O157:H7 Spinach Outbreak 2006

Principles of Environmental Investigations Into Food Product Microbial Contamination

- Farm
- Harvest
- Transport
- Processor
- Retail
Potential Risk Factors for Pre-Harvest Microbial Contamination of Fresh Leafy Greens

Manure Compost

Farmworkers Septic Sewage

Contaminated Water, Flooding

“From both sides of the valley little streams slipped out of the tall canyons and fell into the bed of the Salinas River. In the winter of wet years the streams ran jubilantly, and they swirled the river until sometimes it sang and boiled, bank full, and then it was a destroyer. The river tore edges of the farm lands and washed whole acres down…”

Dust Aerosols

Wildlife

Domestic animals

Environmental Sampling during the E. coli O157:H7 Spinach Outbreak 2006
What are the best samples to collect during a farm investigation?

How many?

Wells and Holding ponds
Remote-sensing infrared digital surveillance camera

68 individual wild pigs observed within one mile from the spinach field based on physical sightings, live-capture, and cameras.
Harvesting baby spinach

Samples from the Spinach Outbreak 2006

- Animal feces: bird, cattle, coyote, deer, dog, horse, sheep/goat, wild pig (feces, colonic feces, buccal swabs, rectal-anal swabs, tonsils)
- Compost: chicken pellets
- Environmental swabs
- Field products: spinach, leafy greens
- Moore swabs
- Soil/sediment
- Water: well, river, creeks, ponds, cattle troughs

Positive Samples from Spinach Outbreak 2006

- No E. coli O157:H7 isolated from the processing facility or harvesting equipment
- E. coli O157:H7 isolated from 4 farms/ranches and the San Benito river (20 miles upstream of Ranch A)
- 28 isolates matched the “outbreak strain” by PFGE at a single ranch: cattle feces, wild pig feces/tissue, river water and sediment, pasture dirt

Samples from the Spinach Outbreak 2006

- 879 total samples collected from 3 processing facilities, 2 harvesters, and 9 farms/ranches and surrounding watersheds
- Sampling narrowed to a single processing facility (San Benito County) and 4 farms, Ranches A, B, C, and D, in San Benito and Monterey Counties
Positive Samples from Spinach Outbreak 2006

- Ranch B
  - Cattle feces (9/21, 43%)
  - Water, cattle trough (1/4, 25%)

- Ranch C
  - Soil in cattle pasture (4/25, 16%)

- Ranch D
  - Moore swab, Pajaro river (1/2, 50%)

Spinach Outbreak 2006

Conclusions

- Potential pre-harvest environmental risk factors
  - Large wild pig population in close proximity to cattle and spinach fields
  - Proximity of irrigation wells to surface waterways exposed to feces from cattle and wildlife

Spinach Outbreak 2006

Link to the final report from FDA and CDHS

http://www.dhs.ca.gov

Spinach Outbreak 2006

Acknowledgments

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Central California Coast growers, ranchers, and property owners