Escherichia coli and the Hemolytic Uremic Syndrome (HUS):
The Critical Initial Encounter

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Disclosure

Federal: NIDDK, NIAID, CDC, USDA
Industry: Johnson and Johnson
Wiley-Blackwell
Bill and Melinda Gates Foundation
Perspectives

• Rare infection (0.9/100,000 annum)*
• Children most frequently affected
  (~ 2/3rds of cases, ~ 90% of HUS)
• In North America and most of the world, O157:H7 is the near-exclusive cause of post-diarrheal HUS
• HUS: 500-750 cases per annum in US
• Most cases sporadic, rural, not food borne
• Rare infections need good protocols, vigilance, and discipline

*MMWR Morb Mortal Wkly Rep 2011;60:749
Diarrhea  Bloody diarrhea

Resolve without HUS
Diarrhea
Lesson 1: Time is not on your side!

Diarrhea

Bloody diarrhea
Lesson 2: Teamwork and collaboration are critical
Three partners:

- Health Department
- Diagnostic microbiologist
- Physician

**Diarrhea**

**Bloody diarrhea**

Emergency physician, internist, pediatrician, GI, ID, nephrologist, surgeon, hematologist, intensivist, neurologist
First Contact: Frequently an ER

Profile *E. coli* O157:H7 infections:

- Nonbloody diarrhea, becomes bloody in 1-3 days
- No fever at presentation
- Tender Abdomen
- > 5 stools in past 24 h
- Pain worse on defecation
- Not many fecal leukocytes
- No relative bandemia

This is a Medical Emergency!
Fortunately, primary care offices cannot cope with such ill children ➔ Emergency Facilities

Lesson 3: Stay Focused

- Misleading and irrelevant in acute bloody diarrhea: urinalysis, ear and rectal examinations, viral and parasite studies, occult blood testing, CT scans, family history of IBD

- No exposure interviews - good interviews obligate 45 minutes (often need to be repeated). Health Departments are trained: call a professional
Lesson 4: Admit, Isolate

Inpatient (contact) precautions:
- dedicated equipment, gowns, gloves

Outpatient advice:
- “Wash your hands well!”


0 hospital – acquired infections St. Louis and Seattle, 1983-2012 vs. 5-10% secondary attack rate in community.
O157:H7: Yes or No?

- Stool to agar ASAP!
- CBC, electrolytes, BUN, Creatinine

Stool ↓ Broth ↓ Incubate O/N ↓ Shiga Toxin EIA
Why focus on O157:H7?

USA, Canada, Japan, UK, South America: *E. coli* O157:H7 is the near exclusive (> 95%) cause of post-diarrheal HUS.

Pediatrics. 1987;80:37  
J Infect Dis. 1990;162:553  
J Pediatr. 1998;132:777  
Jpn J Infect Dis. 1999;52:33  
J Infect Dis. 2001;183:1063  
J Pediatr. 2002;141:172  
Foodborne Pathog Dis. 2006;3:88  
Epidemiol Infect. 2007 Mar 5 (epub)1-7  
Arch Pediatr Adolesc Med.2011;165:884  
Clin Infect Dis 2011;53:269
Recommendations for Diagnosis of Shiga Toxin--Producing Escherichia coli Infections by Clinical Laboratories

“All stools submitted for testing from patients with acute community-acquired diarrhea … should be cultured for O157 STEC on selective and differential agar.”

Best practice: Agar and toxin assay

Lesson 5: SMAC agar best for O157:H7

Three pediatrics series (Seattle, St. Louis)
SMAC plus EIA testing on all stools
- O157 (68)
- non-O157* (26)

<table>
<thead>
<tr>
<th></th>
<th>O157</th>
<th>non-O157</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUS</td>
<td>18%</td>
<td>0%</td>
</tr>
<tr>
<td>Bloody</td>
<td>92%</td>
<td>50%</td>
</tr>
<tr>
<td>Laboratory blood</td>
<td>70%</td>
<td>22%</td>
</tr>
</tbody>
</table>

EIA missed 4 of 39 *E. coli* O157:H7 easily recovered from SMAC agar

Klein, E, et al, J Peds 2002; 172
Unpublished data

* O26, O103, O111, O118 (O121, O165, O174, O177, O165, O174, Orough
<table>
<thead>
<tr>
<th>Setting</th>
<th>O157:H7</th>
<th>Non-O157:H7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HUS</strong></td>
<td>95-99%</td>
<td>1-5%</td>
</tr>
<tr>
<td><strong>ER</strong></td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Seattle, 1991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroenterology. 1993;105:1724</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seattle, 1998-2001</strong></td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>J Pediatr. 2002;141:172</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seattle, 2003-2005</strong></td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Denno, et al, submitted</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>State</strong></td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td><strong>MT, 1998-2000</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J Infect Dis.2003;188:719;</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CT, 2000-2009</strong></td>
<td>163</td>
<td>229</td>
</tr>
<tr>
<td>Clin Infect Dis 2011;53:269</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Lesson 6: Accelerate diagnosis

Plate 24/7
Call with presumptive + (don’t wait for *E. coli* ID, H7 antigen)
Receipt to +: 23 hours, 53 min (14-56h)
Diarrhea

Bloody diarrhea

Resolve without HUS
**Preliminary Communication**

**SPORADIC CASES OF HAEMOLYTIC-URAEMIC SYNDROME ASSOCIATED WITH Fecal CYTOTOXIN AND CYTOTOXIN-PRODUCING ESCHERICHIA COLI IN STOOLS**

**MOHAMMED A. KARMALI**  
**BRIAN T. STEELE**  
**MARTIN PETRIC**  
**CORAZON LIM**

*Departments of Bacteriology and Virology, Research Institute, and Division of Nephrology, Department of Pediatrics, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8 and Department of Microbiology, University of Toronto*

**Summary**  
A cytotoxin active on Vero cells, less active on hela cells, and inactive on WI38 cells (Vero toxin [VT]) was detected in stool isolates of *Escherichia coli* from 8 of the 15 sporadic cases of haemolytic-uraemic syndrome (HUS). Stools from 5 of these 8 patients were...

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>VT * E. coli (serotype) in stools</th>
<th>Faecal VT titre</th>
<th>VT antibody titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17 mo</td>
<td>M</td>
<td>Yes (O26:K60)</td>
<td>NA</td>
<td>NT</td>
</tr>
<tr>
<td>B</td>
<td>18 mo</td>
<td>M</td>
<td>Yes (O113:K75:H21)</td>
<td>NA</td>
<td>NT</td>
</tr>
<tr>
<td>C</td>
<td>12 yr</td>
<td>M</td>
<td>Yes (O111:K89:H88)</td>
<td>NA</td>
<td>NT</td>
</tr>
<tr>
<td>D</td>
<td>114 mo</td>
<td>F</td>
<td>Yes (O111:K58:NM)</td>
<td>1:5120</td>
<td>NT</td>
</tr>
<tr>
<td>E</td>
<td>11 mo</td>
<td>M</td>
<td>Yes (O157:H7)</td>
<td>1:320</td>
<td>1:80 (day 9)</td>
</tr>
<tr>
<td>F</td>
<td>16 mo</td>
<td>F</td>
<td>Yes (O157:H7)</td>
<td>1:320</td>
<td>1:1280 (day 43)</td>
</tr>
<tr>
<td>G</td>
<td>45 mo</td>
<td>F</td>
<td>Yes (UT)</td>
<td>1:320</td>
<td>NT</td>
</tr>
<tr>
<td>H</td>
<td>44 mo</td>
<td>F</td>
<td>Yes (O111:K57:H21)</td>
<td>1:40</td>
<td>NT</td>
</tr>
<tr>
<td>I</td>
<td>26 mo</td>
<td>F</td>
<td>α0111:5α</td>
<td>1:80</td>
<td>NT</td>
</tr>
<tr>
<td>J</td>
<td>13 mo</td>
<td>F</td>
<td>No</td>
<td>1:80</td>
<td>NT</td>
</tr>
<tr>
<td>K</td>
<td>38 mo</td>
<td>F</td>
<td>No</td>
<td>-ve</td>
<td>1:80 (day 17)</td>
</tr>
<tr>
<td>L</td>
<td>32 mo</td>
<td>F</td>
<td>No</td>
<td>-ve</td>
<td>1:320 (day 28)</td>
</tr>
<tr>
<td>M</td>
<td>18 mo</td>
<td>F</td>
<td>No</td>
<td>-ve</td>
<td>NT</td>
</tr>
<tr>
<td>N</td>
<td>32 mo</td>
<td>F</td>
<td>No</td>
<td>-ve</td>
<td>NT</td>
</tr>
<tr>
<td>O</td>
<td>1 mo</td>
<td>F</td>
<td>No</td>
<td>-ve</td>
<td>NT</td>
</tr>
</tbody>
</table>
HEMORRHAGIC COLITIS ASSOCIATED WITH A RARE ESCHERICHIA COLI SEROTYPE

Lee W. Riley, M.D., Robert S. Remis, M.D., M.P.H., Steven D. Helgerson, M.D., M.P.H.,
Harry B. McGee, M.P.H., Joy G. Wells, M.S., Betty R. Davis, M.S., Richard J. Hebert, M.D.,
Ellen S. Olcott, R.N., Linda M. Johnson, R.N., M.S., Nancy T. Hargrett, Ph.D.,
Paul A. Blake, M.D., M.P.H., and Mitchell L. Cohen, M.D.

Stool cultures did not yield previously recognized pathogens. However, a rare Escherichia coli serotype, 0157:H7, that was not invasive or toxigenic by standard tests was isolated from 9 of 12 stools collected within four days of onset of illness in both outbreaks combined, and from a beef patty from a suspected lot of meat in Michigan. The only known previous isolation of this serotype was from a sporadic case of hemorrhagic colitis in 1975. This report describes a clinically distinctive gastrointestinal illness associated with E. coli 0157:H7, apparently transmitted by undercooked meat. (N Engl J Med. 1983; 308:681-5.)
$E. \text{ coli O55:H7} \rightarrow E. \text{ coli O157:H7}$
THE KILLER GERM

It's turning up everywhere: in your water, your food, the pool. How to protect yourself from E.Coli
Shiga toxins: Cardinal Virulence Traits

- Shiga toxin (Stx) 1 and 2
  (Verotoxins, verocytotoxins, Shiga-like toxins)

Found in Shiga toxin-producing E. coli (STEC), AKA enterohemorrhagic E. coli

Shiga toxin 1: 99% identical to Shiga toxin, produced by *Shigella dysenteriae* serotype 1

Stx 2: ~2/3 homologous to Stx 1
A-B$_5$ Toxin

B subunit and its receptor

- Binds to GB3 (globotriaosylceramide)
- Cells that don’t express GB3 resist effects of Stx
- Expression is probably age dependent
- Not expressed on human intestinal cells (paracellular transport from gut)
- GB3 upregulated by cytokines
A subunit

- N-glycosidase (like ricin)
- Disrupts large ribosomal subunit, halting protein synthesis
- Sublethal toxin injury: upregulates injury response in eukaryotic cells (JCI, 2012;122:759)
Stx 1 and 2

- About 12 allelic variants of Stx1 and Stx2
- Stx2 more virulent than Stx1
- Almost all E. coli O157:H7 produce Stx2, and about 2/3 also produce Stx1
- Stx1/Stx2+ E. coli O157:H7 less virulent than those containing Stx2 alone.
Animal Diseases

- Edema Disease of Pigs (Stx2e)
- Swollen head syndrome (avian disease)
Animal Carriage

- Cattle, Deer (O157:H7 and non-O157:H7)
- GB3 is not found on vascular cells in cattle (Proc Natl Acad Sci U S A. 2000;97:10325-9)
- Stx1 inhibits bovine leukemia virus cell proliferation (Infect Immun. 2000;68:4462)
100 patients < age 10
75 no HUS

4 partial HUS (PLTS<150K, HCT<30%)
3 severe partial HUS (plts < 20K, requires RBC transfusion)

18 HUS (PLTS<150K, HCT<30%, Creatinine > ULN (age)
12 have oligoanuric HUS (much worse prognosis)
Lesson 7: Prothrombotic lesions begin early

\[ \text{THROMBIN} + \text{F}1+2 \]

\[ \text{FXa} \]

\[ \text{PROTHROMBIN} \]

\[ \uparrow \text{D-dimer Before HUS} \]

\[ \text{NEJM 2002; 346:23} \]

\[ P<0.01 \]

\[ p<0.001 \]
<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Uncomp</th>
<th>Pre-HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT (%)</td>
<td>36 ± 3</td>
<td>37 ± 3</td>
<td>38 ± 5</td>
</tr>
<tr>
<td>Plts (k/mm(^3))</td>
<td>321 ± 70</td>
<td>317 ± 74</td>
<td>322 ± 97</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.4 ± .1</td>
<td>0.4 ± .1</td>
<td>0.4 ± .2</td>
</tr>
</tbody>
</table>
Lesson 8: not much toxin in Stool

<table>
<thead>
<tr>
<th></th>
<th>Stx Frequency</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-HUS:</td>
<td>40%</td>
<td>320 (160-1280)</td>
</tr>
<tr>
<td>Uncomplicated:</td>
<td>48%</td>
<td>1689 (160-40,000)</td>
</tr>
<tr>
<td>At HUS:</td>
<td>16%</td>
<td></td>
</tr>
</tbody>
</table>

Cornick, N., J Infect Dis. 2002;186:57
Lesson 9: Antibiotics are not beneficial

Recent data:
Adjusted (age, WBC, vomiting) OR: 3.5 (1.2-10.3)
P=0.02

Antibiotics are commonly prescribed:
36% in 11 centers (US, Scotland) (Arch Pediatr Adolesc Med. 2011; 165:884)
23% in Minnesota (Pediatr Infect Dis J. 2011 Sep 1)
44% in multi-state CDC analysis (Clin Infect Dis. 2011;52:1130)
10% in multi-state childhood study (Clin Infect Dis, in press)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>HUS rate</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>12 %</td>
<td>27/234</td>
</tr>
<tr>
<td>Yes</td>
<td>36 %</td>
<td>9/25</td>
</tr>
</tbody>
</table>

Specific antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>HUS rate</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimethoprim-sulfamethoxazole (n=9)</td>
<td>44 %</td>
<td>4/9</td>
</tr>
<tr>
<td>β-lactams (n=9)</td>
<td>22 %</td>
<td>2/9</td>
</tr>
<tr>
<td>Metronidazole (n=3)</td>
<td>67 %</td>
<td>2/3</td>
</tr>
<tr>
<td>Azithromycin (n=4)</td>
<td>25 %</td>
<td>1/4</td>
</tr>
</tbody>
</table>
Tennessee Medicare, 1995-2004

Episodes of diarrhea: 315,828

Cultures performed: 15,820 episodes (5.0%)

Antimicrobials: 32,949 episodes (10.4%), 89.4% not accompanied by stool culture.

100 patients < age 10
75 no HUS

4 partial HUS (PLTS<150K, HCT<30%)
3 severe partial HUS (plts < 20K, requires RBC transfusion)

18 HUS (PLTS<150K, HCT<30%, Creatinine > ULN (age)
12 have oligoanuric HUS
Oligoanuric HUS is categorically worse than non-oligoanuric HUS


Lesson 10: IV fluids (rapid microbiology) associated with maintained urine output in children who subsequently develop HUS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Good outcome</th>
<th>Poor outcome</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>Day 2</td>
<td>Day 3</td>
<td>0.066</td>
</tr>
<tr>
<td>First IV started</td>
<td>Day 3 (0-4)</td>
<td>Day 4.5 (2-9)</td>
<td>0.01</td>
</tr>
<tr>
<td>First Culture obtained</td>
<td>Day 2 (0-4)</td>
<td>Day 3 (2-9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Culture +</td>
<td>Day 4 (2-4)</td>
<td>Day 7 (3-9)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

11 Center Study of children with HUS (CA, WA, NM, MO, IN, AR, WI, OH (2), Glasgow)

### Table 4. Logistic Models

<table>
<thead>
<tr>
<th>Logistic Model</th>
<th>Variable</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Age</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td></td>
<td>Antibiotics</td>
<td>2.9 (0.7-11.4)</td>
</tr>
<tr>
<td></td>
<td>Total intravenous fluid given during the first 4 days of illness</td>
<td>6.1 (0.8-46.8)</td>
</tr>
<tr>
<td></td>
<td>Total intravenous sodium given during the first 4 days of illness</td>
<td>1.0 (0.97-1.0)</td>
</tr>
<tr>
<td>Second&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Antibiotics</td>
<td>3.1 (0.8-11.9)</td>
</tr>
<tr>
<td></td>
<td>Total intravenous fluid given during the first 4 days of illness</td>
<td>1.4 (1.0-2.0)</td>
</tr>
<tr>
<td>Final&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Total intravenous sodium given during the first 4 days of illness</td>
<td>1.4 (1.0-1.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Variables with *P* values of greater than .20 were eliminated from the first model. Also, the collinear variable of sodium given in the first 4 days of illness was taken out because it had less significance than volume given.

<sup>b</sup>Variables with *P* values of greater than .05 were eliminated from the model. The final model has only volume given during the first 4 days.
Lesson 10

• If oligoanuria does not ensue by day 10 of illness, it probably won’t happen

HUS develops
HCT <30%, hemolytic smear
Platelets <150K/mm3
Creatinine > ULN for age
Lesson 12: No evidence for a lesion that will respond to therapeutic plasma exchange

“The vWF antigen concentration increased from 137 ± 37% of the control value in 37 samples at colitis to 230 ± 74% (p < 0.001) in 16 samples at HUS. In the eight patients investigated at both stages, the vWF antigen level increased from 184 ± 65% to 234 ± 62% (p = 0.04).”


“We did not detect anti-ADAMTS13 antibodies … ADAMTS13 activity was normal in all patients, thereby excluding the classic pathogenesis of TTP. However, we observed a two-to three-fold increase from normal concentrations of vWF antigen … suggesting vWF is affected in these patients.”

Lessons

1. Time is not on your side
2. Teamwork and collaboration are critical
3. Profile, and Stay Focused
4. Admit, isolate
5. SMAC agar is best for O157:H7
6. Accelerate laboratory diagnosis
7. Prothrombotic lesions begin early
8. Not much toxin in stool
9. Antibiotics not beneficial
10. Early volume expansion mitigates severity of HUS
11. Anuria ensues by day 10 of illness
12. No evidence for plasma exchange-responsive lesion
But, you don’t have to:

- Admit a patient with a guiac + stool, unless red blood is visible
- Admit infants (HUS very rare < 9 mos)
- Admit chronic bloody diarrhea
Additional Practical Points

- “Transfer with plates” (“if it isn’t negative in a good lab, it isn’t negative”)
- Swab on admission (don’t wait for next stool) – start the culture replicating
- Fibrinogen normal or high; PT only slightly prolonged
- Call Health Department
- Adults have similar illnesses, with perhaps more early and late in illness mortality
Thanks

• Patients, families, RNs, laboratorians, house officers

• Sandy Watkins, Craig Wong, Han-Mou Tsai, Wayne Chandler, Nancy Cornick, Peggie Neill, Christina Hickey, Julie Ake, Eileen Klein, Lori Holtz

• Helge Karch and colleagues

• NIDDK52081, CDC, USDA, Doris Duke Clinical Scholars Program
Lessons

1. Time is not on your side
2. Teamwork and collaboration are critical
3. Profile, and Stay Focused
4. Admit, isolate
5. SMAC agar is best for O157:H7
6. Accelerate laboratory diagnosis
7. Prothrombotic lesions begin early
8. Not much toxin in stool
9. Antibiotics not beneficial
10. Early volume expansion mitigates severity of HUS
11. Anuria ensues by day 12 of illness
12. No evidence for plasma exchange-responsive lesion