What are verotoxigenic *E. coli*?

- A diverse group of *E. coli*
  - All produce exotoxins, called verotoxins (VT)
    - A. k. a. Shiga toxins (STX), Shiga-like toxins (SLT)
  - Hence they are known VTEC, STEC or SLTEC

- VTEC vary in their ability to cause disease
  - Virulent VTEC such as *E. coli* O157:H7 cause:
    - diarrhea
    - bloody diarrhea (hemorrhagic colitis, HC)
    - the hemolytic uremic syndrome (HUS).
  - Others have not been linked to human disease
Enterohemorrhagic *E. coli* (EHEC)

- Virulent sub-group of VTEC
- Prototype: *E. coli* O157:H7
  - Frequently associated with severe disease
    - HC, HUS/TTP
    - Possess large virulence plasmid
  - Originally only *E. coli* O157:H7, O26:H11, O111:NM
  - Now over 50 serotypes.

Human infections

- Over 400 VTEC serotypes isolated from humans
  - >90% of known infections are caused by fewer than 10 serogroups
  - O157, O26, O45, O103, O111, O121, O145
  - Children and the elderly are most susceptible
  - Most infections (80%) are sporadic
Key virulence attributes of EHEC

- Intimate adherence to intestinal epithelium
  - Attaching and effacing lesions
  - Mediated by eae-encoded intimin, Tir (translocated intimin receptor) and other secreted proteins of the Locus of Enterocyte Effacement (LEE)

- VT production
  - Types and variants of VT
  - Level of production of VT
  - Local action of VT (and other factors) causes HC
  - Translocation and dissemination of VT causes HUS

Intestinal pathology of virulent VTEC

Colon showing lesions of hemorrhagic colitis (HC)

TEM of colon showing attached E. coli and “attaching and effacing” lesion

SEM of colon with E. coli intimately attached to the intestinal epithelium

Molecular basis of intimate adherence via intimin and tir delivered by a Type III secretion system
Incidence: O157 vs non-O157 Human Infections

- Given that most infections are sporadic, what is the true incidence of VTEC infections.

<table>
<thead>
<tr>
<th>Country or Region</th>
<th>IR /100,000</th>
<th>O157 (%)</th>
<th>Non-O157 (%)</th>
<th>Major Non-O157 serogroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>0.2-0.4</td>
<td>~58</td>
<td>~42</td>
<td>O111, O26</td>
</tr>
<tr>
<td>Continental Europe</td>
<td>0.1-0.7</td>
<td>~50</td>
<td>~50</td>
<td>O26, O111, O103, O121, O145</td>
</tr>
<tr>
<td>Italy</td>
<td>&lt;0.2</td>
<td>~50</td>
<td>~50</td>
<td>O26, O111</td>
</tr>
<tr>
<td>France</td>
<td>0.5-0.8</td>
<td>~58</td>
<td>~42</td>
<td>O103, O26, O111</td>
</tr>
<tr>
<td>Germany</td>
<td>0.9-2.0</td>
<td>~70</td>
<td>~30</td>
<td>O26, O103, O145</td>
</tr>
<tr>
<td>UK, Ireland</td>
<td>1.0-2.0</td>
<td>&gt;90</td>
<td>&lt;10</td>
<td>O26, O111</td>
</tr>
<tr>
<td>Scotland</td>
<td>2.0-10</td>
<td>&gt;90</td>
<td>&lt;10</td>
<td>O26, O111</td>
</tr>
<tr>
<td>Canada</td>
<td>3.0-6.0</td>
<td>&gt;85</td>
<td>&lt;15</td>
<td>O26, O111, O121, O91, O103</td>
</tr>
<tr>
<td>Japan</td>
<td>2.0-3.0</td>
<td>~60</td>
<td>~40</td>
<td>O26, O111, O121, O103, ONT</td>
</tr>
<tr>
<td>Argentina (HUS)</td>
<td>10.4-12.2</td>
<td>~60</td>
<td>~40</td>
<td>O145, O26, O113, O174, O8</td>
</tr>
<tr>
<td>USA</td>
<td>1.04-1.2</td>
<td>~70</td>
<td>~30</td>
<td>O26, O111, O103, O121, O45, O145</td>
</tr>
</tbody>
</table>

Non-O157 VTEC in Canada

- National reported incidence of VTEC infection: 4-6/100,000
  - Nationally, VTEC O157 predominates (>80%).
  - Data from two sites indicate non-O157 VTEC are more prevalent.

Alberta Provincial Health Laboratory PROVLAB

Prospective study of 916 pediatric diarrheic stools:
- 47/916 positive for vt genes by PCR
- 24/47 (51%) yielded VTEC O157,
- 23/47 (49%) yielded non-O157 VTEC
- O26:H11 (10) and O121:H19 (3) most common non-O157 VTEC

Chui, Louie, Church, Galbraith and Ng, unpublished.
Non-O157 VTEC in Canada

- British Columbia Centre for Disease Control
- Characterization of VTEC isolates referred from clinical labs
- Detection and isolation of VTEC in stool samples
  - 2000-2004: 509 isolates from clinical labs, 233 from stools after tests for fecal VT by Vero cell assay
  - Paccagnella, Laberge and Isaac-Renton, unpublished

Isolates from direct testing of stool samples

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157:H7/H-/HUT</td>
<td>110</td>
<td>50.9</td>
</tr>
<tr>
<td>O26:H11/NM/HUT</td>
<td>39</td>
<td>18.1</td>
</tr>
<tr>
<td>0111:NM/HUT</td>
<td>20</td>
<td>9.3</td>
</tr>
<tr>
<td>0121:H19</td>
<td>8</td>
<td>3.7</td>
</tr>
<tr>
<td>0103:H2/H10/H25</td>
<td>8</td>
<td>3.7</td>
</tr>
<tr>
<td>O145:NM</td>
<td>5</td>
<td>2.3</td>
</tr>
<tr>
<td>Total of 233</td>
<td>190</td>
<td>88.0</td>
</tr>
</tbody>
</table>

Diagnostic Challenges for VTEC in Foods (1)

- Over 300 serotypes of VTEC isolated from foods
- Many are not virulent for humans
  - Fewer than 10 serotypes cause most serious infections
  - O157 predominates in many countries
  - Non-O157 VTEC now known to cause 30-60% of VTEC infections (CDC, 2010)
- In the US, O26, O45, O103, O111, O121, O145 VTEC cause approx 80% of non-O157 infections
  - Hence VTEC of these “Top Six” priority serogroups are being targeted for risk management.
  - Recently declared adulterants in raw meats in the US
What is needed?

- The ideal test would be a single screening test that is:
  - Fast and reliable
  - Broad enough to identify samples of concern
  - Specific enough to include priority STEC and exclude low risk STEC
  - A pipe dream? Not necessarily.

- Current approaches
  - Many favor screening tests with rapid molecular methods such as PCR for Stx, intimin (eae) and serogroup marker genes.

- Other potential or promising targets include:
  - Stx subtype, intimin, intimin subtypes,
  - other LEE and non-LEE targets, often linked to “seropathotypes”.
    - e. g. nleB, as shown by Patrick Pach and Brian Coombs
  - As yet unidentified markers identified by whole genome sequencing

Serogroup Markers

- Pros
  - Current 6 or 7 are highest risk STEC in HC, HUS.
  - Methodology already or soon available, some in familiar formats (eg IMS).
  - Others (RT PCR) have flexibility.
  - Can be multiplexed.

- Cons
  - Current list covers only ~70% high risk STEC.
  - List is dynamic, with new targets needing new tools.
  - Some concerns about performance of current formats (eg IMS).
  - Increased multiplexing reduces sensitivity.
  - Not all O-group E. coli are VTEC
Alternate markers

Stx Subtyping

- **Pros**
  - Stx1a,2a, 2c, 2d involved in most cases of HC, HUS.
  - Methodology already available.

- **Cons**
  - Historic and geographic evidence suggests this approach alone may be too narrow for screening.

**Other virulence markers**

- **Pros**
  - Can be highly specific for priority STEC.
  - Can be targeted by multiplexed, multi-tiered rapid methods.

- **Cons**
  - Distribution not yet well enough known among other STEC and non-STE to establish sensitivity and specificity

The Challenge (2)

- **Not all E. coli of a serogroup are VTEC**
  - VT-negative E. coli of O157 and non-O157 serogroups of little concern are present in animals and foods, for example:
    - Non-pathogenic O157, O26 and numerous other serogroups
    - Enteropathogenic E. coli (EPEC) O26, O111 and others

_Hence markers such as O-group, VT and intimin (eae) for screening enrichment cultures may be present in different bacteria._
The Challenge

- Consequently, isolation from mixed cultures is necessary to confirm the target markers are in the same isolate
  - Methods for isolation of O157 VTEC are established and effective
  - Unlike O157 VTEC, non-O157 VTEC resemble generic E. coli:
    - VT/VT genes are only common marker
    - enrichment cultures may contain as few as $10^5$ VTEC among $10^8-10^9$ other E. coli/coliforms (1:1,000-10,000)

*Isolation by testing individual colonies, pools, or sweeps is laborious, time-consuming and often unsuccessful*

---

Our Approach: VT ELISA + Colony Immunoblot

- For foods, clinical and other samples

**Day 0**
- Enrich sample
  - 18 h
  - 42°C

**Time**
- 0 hours

**Day 1**
- Test broth by VT ELISA
- VT+ve broth
- Set up colony immunoblot on TSA
  - 16 h
  - 42°C

**Time**
- 24 hours

- Probe immunoblot membranes
- Pick VT+ve colonies

**Day 2**
- Confirm as VT+ve by ELISA
- +/- O-group agglutination test

**Time**
- 48 hours

---

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Our approach (3)

B. Colony immunoblot with HGMF
Diluted VT-positive broths are filtered onto hydrophobic grid membrane filters (HGMFs) as the top membrane, incubated for 16 h.

Capture Ab: Rabbit anti-VT Ab (LFZ)
Detector Ab: Mix of MAb to VT (LFZ)

We prefer HGMFs. They yield higher numbers of individual colonies

Evaluation: VT ELISA + Colony Immunoblot

- Example: 200 samples of ground beef
- VT ELISA vs Verocell Assay
  - Sensitivity 100%; Specificity 99.5%
  - 43 samples (21.5%) VT+ve by ELISA & VCA
- Colony Immunoblot
  - VTEC isolated from 42/43 samples
  - Within 48 h of beginning enrichment
Isolates recovered (n=66)

Table 1. VTEC serotypes isolated from ground beef

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<td>3</td>
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<td>2</td>
</tr>
<tr>
<td>O6:H10</td>
<td>1</td>
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<td>1</td>
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<td>1</td>
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<td>O?::H2</td>
<td>2</td>
</tr>
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<td>1</td>
<td>O?::H4</td>
<td>1</td>
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<td>9</td>
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<td>7</td>
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</tr>
<tr>
<td>O119:H5</td>
<td>1</td>
<td>O?::H28</td>
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Green = also isolated from humans. Red = associated with HC or HUS

VTEC in meats in Canada

Twenty most common STEC serotypes from cattle and beef, LFZ Guelph

Proportions of “Top 7” and other serotypes of varying virulence
Current prevalence in ground beef

• 2010: 223 samples of retail ground beef
• Tested by same methods
  – 13 samples VT-positive
  – Serotypes

  O8:H19
  O28ac:H25
  O91:H28
  O111:H8
  O168:H8
  O77:H41
  O107:H7
  O139:H19
  O174:H21
  O?:H8

Meat processors are doing very well!!!
Colony immunoblotting for isolation of Verotoxin-producing *Escherichia coli*

Roger Johnson, Kim Ziebell, Bob Holtslander, Shelley Frost, Amanda Mazzocco, Shaun Kernaghan, Jennifer Wheeler and Leslie MacDonald

Public Health Agency of Canada
Laboratory for Foodborne Zoonoses
110 Stone Road West, Guelph, Ontario N1G 3W4, Canada
Roger.Johnson@phac-aspc.gc.ca

LFZ Science Retreat Sep 29-30, 2011, Guelph ON
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- +/- O-group agglutination test

Our approach (2)

A. Colony immunoblot with round membrane
- Diluted VT-positive cultures are spread-plated on the round top membrane

- CA round membrane filter
- Antibody-coated VT capture membrane
- Tryptic Soy Agar

Capture Ab: Rabbit anti-VT Ab (LFZ)
Detector Ab: Mix of MAbs to VT (LFZ)

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</table>

*Green* = also isolated from humans. *Red* = associated with HC or HUS

Comments

- We have tested thousands of food, animal and human samples with similar success.
- The method is designed to detect and isolate VTEC of any serotype.
- For C-Enternet, we are currently testing:
  - 8 to 10 samples of ground beef each week,
  - 5 water samples every two weeks.
  - Samples are received by 2:00 pm on Wednesdays, and typically we have VTEC isolates for serotyping on Fridays.
Our approach: selected O serogroups

Colony immunoblots for VT and LPS O-antigens:
HGMF loaded with mix of non-VTEC, target O157, O26, O111 VTEC and non-target O48 VTEC, incubated for 16 h.

VT capture membrane pre-coated with rabbit anti-VT Ab and probed with mix of MAbs to VT (LFZ).
LPS membrane probed with mix of MAbs to O26, O111, (from Dr Brian Brooks, CFIA) and O157 LPS (LFZ).

By LPS immunoblot, 3 VTEC are target serogroups O26, O111 or O157
Agglutination tests confirm the O type of each VT+ target colony picked from the HGMF.
Acknowledgements

Sincere thanks to our great staff in the Immunology and E. coli Laboratories:

Kim Ziebell,
Shelley Frost,
Shaun Kernaghan,
Bob Holtslander,
Amanda Mazzocco,
Jennifer Wheeler
Leslie MacDonald

Now happily welcoming Uma Silphaduang to the IR Lab

Further Acknowledgements

Sincere thanks also to the staff of the E. coli and Salmonella Typing Labs, and the AMR Lab, who diligently test and type the plentiful Salmonella and E. coli isolates from CIPARS, C-Enternet and elsewhere, as you have heard about earlier today.

Kim Ziebell, Irene Yong, Nina Dougherty, Suzanne Johnson
Linda Cole, Ketna Mistry, Anne Perets, Betty Wilkie
Andrea Desruisseau, Chad Gill, Linda Nedd-Gbedemah

Thank you for your attention!