Non-O157 Verocytotoxin-producing \textit{E. coli}: role in human disease and detection in foodstuff

Alfredo Caprioli

\textit{EU Reference Laboratory for E. coli}

\textit{Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare}

\textit{Istituto Superiore di Sanità}

Rome, Italy
Outbreak of infection with VTEC O145

The New York Times

In E. Coli Fight, Some Strains Are Largely Ignored

Emily C., 18, had kidney failure from an outbreak of a rarer E. coli strain.

By WILLIAM NEUMAN

Published: May 26, 2010
Outbreak of infection with VTEC O145
Outbreak of infection with VTEC O145, USA 2010

MAY 6, 2010
FOOD SAFETY INFOSHEET
Romaine lettuce linked to E. coli O145 outbreak

CLUSTERS OF ILLNESSES IN THE NORTH EAST AND MIDWEST
60 ILL IN MICHIGAN, NEW YORK AND OHIO
LEAFY GREENS HAVE A HISTORY
LETTUCE, SPINACH, GREENS LINKED TO AT LEAST 30 OUTBREAKS OF PATHOGENIC E. COLI SINCE 1993

<table>
<thead>
<tr>
<th>No. of cases detected</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of HUS cases</td>
<td>3</td>
</tr>
<tr>
<td>Source: contaminated lettuce</td>
<td></td>
</tr>
</tbody>
</table>
Verocytotoxin (Shiga toxin)-producing *E. coli*

One of the most dangerous foodborne pathogens!

**Life-threatening** clinical manifestation

**Very low** infectious dose (*10-100 CFU* !)

**Large** community outbreaks
VTEC: Clinical manifestation

- Intestinal
  - Asymptomatic
  - Watery Diarrhoea
  - Hemorrhagic Colitis

- Systemic
  - Hemolytic Uremic Syndrome (HUS)
  - Neurological involvement
Hemolytic Uremic Syndrome (HUS)

- Hemolytic Anemia
- Thrombocytopenia
- Acute Renal failure

- Main cause of renal failure in childhood and usually requires dialysis

- Antibiotic treatment is not effective and is not recommended

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EU Reference Laboratory for E.coli
Are all VTEC pathogenic to humans?

VTEC Associated with Severe Disease (BD, HUS)

VTEC
>100 serogroups in the reservoir

Additional virulence properties
Attaching/Effacing adhesion

O157 which others??....
How the VTEC issue is managed in the EU

European Food Safety Authority (EFSA)

CRL VTEC

European Centre for Disease Prevention and Control (ECDC)

European Committee for Standardization (CEN)

Istituto Superiore di Sanità – Veterinary Public Health and Food Safety Dept.
EU Reference Laboratory for E.coli
EFSA monitoring plans for VTEC

Monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-036)

Adopted on 18 October 2007
Monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types

Scientific Opinion of the Panel on Biological Hazards

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**Recommendations**

*Monitoring of animal populations and foodstuffs*

*.... examining food and animal reservoirs for a defined range of VTEC serotypes, which have been selected using human surveillance data.*
Surveillance of VTEC infections in the EU
### VTEC Infections by Serogroup

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157</td>
<td>52.7 %</td>
</tr>
<tr>
<td>O26</td>
<td>6.5 %</td>
</tr>
<tr>
<td>O103</td>
<td>3.8 %</td>
</tr>
<tr>
<td>O91</td>
<td>2.8 %</td>
</tr>
<tr>
<td>O145</td>
<td>2.3 %</td>
</tr>
<tr>
<td>O111</td>
<td>1.3 %</td>
</tr>
<tr>
<td>O146</td>
<td>0.9 %</td>
</tr>
</tbody>
</table>

**2006 - 2008**

**N = 9,421**
Non-O157 serotypes

VTEC non-O157 in Europe

2000-02  2006-08

No. of strains

O26  O103  O91  O145  O111  O128  O146  O113

615  325  264  214  119  78  88  81

342  357  101  74  54  47  38  0

2000-02  2006-08
VTEC non-O157 in the US, 1993 - 2002


% VTEC non-O157

<table>
<thead>
<tr>
<th>O26</th>
<th>O111</th>
<th>O103</th>
<th>O121</th>
<th>O45</th>
<th>O145</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>16</td>
<td>12</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>
Epidemiology of VTEC non-O157 in Europe

Real, or surveillance artefact?
Surveys on HUS – VTEC serogroups

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EU Reference Laboratory for E.coli
VTEC infections in the European Union
2002-2006

<table>
<thead>
<tr>
<th>O157</th>
<th>Total Cases</th>
<th>7,227</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUS Cases</td>
<td>310</td>
<td></td>
</tr>
</tbody>
</table>
Verocytotoxin (Shiga toxin)-producing *E. coli*

One of the most dangerous foodborne pathogens!

Life-threatening clinical manifestation

Very low infectious dose (10-100 CFU !)

Large community outbreaks
VTEC non-O157 outbreaks: O26 and O145 – Belgium, 2007

OUTBREAK OF VEROCYTOTOXIN-PRODUCING E. COLI O145 AND O26 INFECTIONS ASSOCIATED WITH THE CONSUMPTION OF ICE CREAM PRODUCED AT A FARM, BELGIUM, 2007

K De Schrijver (koen.deschrijver@wwg.vlaanderen.be), G Buvens, B Possé, D Van den Branden, O Oosterlynck, L De Zutter, K Eilers, D Piérard, K Dierick, R Van Damme-Lombaerts, C Lauwers, R Jacobs

<table>
<thead>
<tr>
<th>No. of cases detected (children)</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of HUS cases</td>
<td>5</td>
</tr>
</tbody>
</table>

Source: ice cream
Contaminated by food handlers ??

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EU Reference Laboratory for E.coli
An outbreak of Verocytotoxin-producing *Escherichia coli* O26:H11 caused by beef sausage, Denmark 2007

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases detected</td>
<td>20</td>
</tr>
<tr>
<td>No. of HUS cases</td>
<td>0</td>
</tr>
</tbody>
</table>

**Source:** organic fermented beef sausage
<table>
<thead>
<tr>
<th>Year</th>
<th>No. of cases detected</th>
<th>Total No. of HUS cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1997</td>
<td>??</td>
<td>12 (1 death)</td>
</tr>
<tr>
<td>2005</td>
<td>3 + 3 cases</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>3 cases</td>
<td></td>
</tr>
</tbody>
</table>

VTEC O26: HUS clusters in Italy, (1997-2007)

Sources: ??????
VTEC non-O157 Outbreaks: O103 – Norway,

No. of cases detected (children) | 17
---|---
No. of HUS cases | 10 (1 death)
Source: traditional cured sausage with sheep meat
**VTEC O111: HUS outbreak in Italy, 1992**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases detected (children)</td>
<td>??</td>
</tr>
<tr>
<td>No. of HUS cases</td>
<td>9 (1 death)</td>
</tr>
<tr>
<td>Source: ????</td>
<td></td>
</tr>
</tbody>
</table>

Escherichia coli O111:H8 Outbreak Among Teenage Campers --- Texas, 1999

In June 1999, the Tarrant County Health Department reported that a group of teenagers attending a cheerleading camp during June 9--11 became ill with severe vomiting and diarrhea. Of the 12 persons who were hospitalized with hemolytic uremic syndrome (HUS), eight persons were identified as campers. Eighty-two other persons who attended the camp were examined, and no further cases were identified. The Tarrant County Health Department, the Texas Department of Health, and the CDC were informed by the state health department of the outbreak. The health departments of the states of Texas and Tennessee and the CDC were informed by the state health department of the outbreak. The health department of the state of Tennessee was informed by the state health department of the outbreak.

| No. of cases detected | 58 |
| No. of HUS cases | 2 |

Source: contaminated food (salad?, ice ?)
## VTEC non-O157 outbreaks: O111 – USA, 2008

<table>
<thead>
<tr>
<th>No. of cases detected</th>
<th>341</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of HUS cases</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>(1 death)</td>
</tr>
</tbody>
</table>

Source: restaurant contamination

**International Food Safety Network Infosheet Sept 12/08**

[www.foodsafety.ksu.edu](http://www.foodsafety.ksu.edu)  
[foodsafetyinfosheets.ksu.edu](http://foodsafetyinfosheets.ksu.edu)
VTEC non-O157 outbreaks: O121 – USA, 1999

<table>
<thead>
<tr>
<th>No. of cases detected</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of HUS cases</td>
<td>3</td>
</tr>
<tr>
<td>Source: swimming in a lake</td>
<td></td>
</tr>
</tbody>
</table>

Source: swimming in a lake
VTEC non-O157 outbreaks: O121 – USA, 2006

About E. Coli

<table>
<thead>
<tr>
<th>Source: contaminated lettuce at restaurant</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases detected</td>
</tr>
<tr>
<td>73</td>
</tr>
<tr>
<td>No. of HUS cases</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

Wendy’s E. Coli Outbreak (Utah)

Lettuce served in dishes prepared by a Wendy’s restaurant in Ogden, Utah, was the source of an E. coli outbreak in June of 2006. Following an investigation into an E. coli outbreak among attendees of a CORE Academy luncheon held at Orion Junior High School in Harrisville, Utah, on June 30, the Weber–Morgan Health Department (WMHD) announced that four people had become ill with E. coli infections after eating iceberg lettuce prepared at the Wendy’s restaurant located at 2500 N 400 E in North Ogden, Utah. WMHD announced that three of the four people who were confirmed ill with E. coli infections after eating the food prepared by Wendy’s had developed hemolytic uremic syndrome (HUS).
Scientific Opinion on monitoring of VTEC

Recommendations
Monitoring of animal populations and foodstuffs

Initially monitoring should concentrate on VTEC O157 since this serotype is most frequently associated with severe human infections (including HUS) in the EU. Monitoring should then be extended to other seropathotypes (e.g. those of O26, O103, O145 and O111) that are identified as pathogenic for humans, based on the periodical analysis of human disease and epidemiological data.
Detection of pathogenic non-O157 VTEC in food
Detection of VTEC O157 in food

Serogroup specific method
ISO 16654:2001

25 gr of Sample in Pre-enrichment broth

Magnetic beads coated with O157 antibodies

- VTEC O157
- Other VTEC

Food Sample
Detection of VTEC O157 in food

The method exploits peculiar features of the bug

- Resistant to 20 mg/Lt novobiocin
- Does not ferment sorbitol
- MUG-
- Resistant to Cefixime-Tellurite

Pre-enrichment

Isolation
Detection of pathogenic non-O157 VTEC in food

No peculiar features to discriminate non-O157 VTEC from other *E. coli*
Detection of pathogenic non-O157 VTEC in food

How to test food with the same efficacy as for O157?

Food Sample

- O26
- O103
- O111
- O145
- Other VTEC

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Real-Time PCR for the detection of the “Top 5” VTEC serogroups

Resolution n°: 243

Ad hoc group "Shiga toxin producing E.Coli other than E.coli O157"

WG 6 asked the Secretariat to launch the final vote before publication based on the draft presented \(\text{(N 502)}\), Microbiology of food and animal feeding stuffs Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) belonging to O157, O111, O26, O103 and O145 serogroups – Qualitative Method.

This resolution should be approved:
- at the next CEN/TC275 meeting
- by correspondence by TC275
- at the next ISO/TC34 SC9 meeting

Buenos Aires: 01 June 2010

Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of Shiga
toxin-producing \textit{Escherichia coli} (STEC) belonging to O157, O111, O26, O103 and O145
serogroups - Qualitative Real-time polymerase chain reaction (PCR)-based Method

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EU Reference Laboratory for \textit{E.coli}
Thanks for your attention!