Experiences with non-O157 STEC and implications on Public Health Programs

FLEMMING SCHEUTZ

STATENS SERUM INSTITUT

The International *Escherichia* and *Klebsiella* Centre (WHO)
Copenhagen

- O157 references
- Non-O157 references
- VTEC
- STEC
- Shiga-Like Toxin
Non-O157 STEC studies

K. E. Johnson et al. CID 2006;43

16 countries, 1988-2006 (1,402/2,892) 48%
Range 19%-100%

USA, Canada, UK, Germany, Spain, Italy, Czech Republic, Belgium, France, Denmark, Finland, Sweden, Australia, Chile, Argentina & Japan

Netherlands, 2006 80%
Australia, 2004 64%
Belgium, 2006 81%
Brazil, 2007 100%
Poland, 2004 100%
Germany, 1998 88%
Non-O157 STEC surveillance
27 countries; 18,302 isolates

% Non-O157 in the Enter-net database

Country

CZ
GR
MT
PL
SK
GB
ES
GZ
NZ
IE
NL
EE
SE
JP
BE
FR
HU
AT
FI
NO
CH
IT
LU
DK
DE
SI
ZA

70
3
20
13
6
87
607
158
642
457
27
510
314
322
48
312
115
130
18
164
42
872
246
4232

5949
1517
1576

Country
Non-O157 STEC surveillance
27 countries; 6,480 isolates

O Groups in the Enter-net database
STEC incidences in Europe

Selected incidences of STEC
Data from "The Enter-net database"
STEC in Denmark 2006

Incidence of STEC (per 100,000)

- > 4
- 2 – 3,99
- 1,5 – 1,99
- 1 - 1,49
- 0,5 - 0,99
- 0 - 0,49
- No cases

Number of diagnosed STEC infections by county, and annual incidence of all STEC infections in 2006
Detection ratio of STEC in counties using molecular methods vs "other methods" in Denmark
Non-O157 STEC outbreaks

| O22:H8       | O117:H4               |
| O26:H11     | O118:H2               |
| O103:H2     | O119                  |
| O103:H25    | O121:H19 / H21        |
| O104:H21    | O128:H2               |
| O111:H- / H2 / H8 | O145:H-            |
| O113:H21    | O?:H19                |

Citrobacter freundii
O103:H25 outbreak in Norway

Date of onset of disease

- HUS cases
- Diarrhea cases

HUS "outbreak" notified

O103 pos / stx2 neg
Source: Sliced, dry fermented lamb’s sausage
# Product and environmental samples

<table>
<thead>
<tr>
<th>Category</th>
<th>O103 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured meat products</td>
<td>2462</td>
</tr>
<tr>
<td>Sheep meat</td>
<td>171</td>
</tr>
<tr>
<td>Environment</td>
<td>296</td>
</tr>
<tr>
<td>Spices, additives, culture</td>
<td>57</td>
</tr>
<tr>
<td>Minced meat</td>
<td>1000</td>
</tr>
</tbody>
</table>

All isolates were *stx2* negative & *eae* pos. BUT clustered with patient isolates by MLVA (DNA fingerprint)
**O103:H25**
outbreak in Norway

*stx2 & eae*

17 cases; 15 children

10 with HUS

1 child died

HUS notification

Massive media attention

**O26:H11**
outbreak in Denmark

*stx1 & eae*

20 cases; all children

median 2 years

Very mild symptoms

Discovered by PFGE

Little media attention
Lessons learned in Norway

- Outbreak discovered due to notification of cases of HUS

- Methods in clinical laboratories were inadequate for detection of non-O157 in 5 out of the six first cases of HUS

- stx2 negative isolates dominated

- MLVA was used to identify cases and the source
Lessons learned in Denmark

- Real-time PFGE of Danish non-O157 detected a "mild" outbreak
- Only possible because isolates were available for typing

Source identified using

- access to purchase records
- cooperation with supermarkets searching their central computers
## HUS and STEC notifications worldwide

<table>
<thead>
<tr>
<th>HUS &amp; STEC</th>
<th>Australia, Cyprus, Denmark, Germany, Hungary, Japan, Poland</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Notification dates from 1998 – 2005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STEC only</th>
<th>Austria, Canada, Estonia, Finland, Greece, Iceland, Ireland, Luxembourg, Malta, New Zealand, Norway, Slovenia, Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Notification dates from 1990 – 2005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Not mandatory</th>
<th>England &amp; Wales, France †, Italy ‡, Romania, Spain, Scotland*, South Africa Pediatric- nephrology network since</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>† 1996 &lt; 15 Yrs ‡ 1988 &lt; 14 Yrs *Laboratory based since mid-eight’ies</td>
</tr>
</tbody>
</table>
# Shiga toxin 2 (stx2) subtype and clinical presentation

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Non-HUS</th>
<th>HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx2</td>
<td>60</td>
<td>11</td>
</tr>
<tr>
<td>stx2c</td>
<td>49</td>
<td>1</td>
</tr>
<tr>
<td>stx2d-activatable</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>stx2d</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>stx2e</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>stx2-variant</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>stx2 + stx2c</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>stx2 + stx2d</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2x stx2-activatable</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>stx2c + stx2-activatable</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>186</strong></td>
<td><strong>19</strong></td>
</tr>
</tbody>
</table>

*stx2 OR* 32.5 > *stx2c OR* 4.7 for HUS

*) OR: odds ratio; multivariant analysis adjusted for age

Ethelberg et al. 2004 EID; 10
Virulence profile and clinical manifestation in 559 Danish STEC patients 1994-2005
Stx1 : 4 subtypes a - d
7-8 variants

Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0%
VT1 translated sequences

Stx1a-S._dysenteriae-3818T
Stx1a-S._sonnei-CB7888
Stx1b-O111-CB168
Stx1b-O157-EDL933
Stx1b-O48-94C
Stx1b-O111-PH
Stx1c-O174-DG131-3
Stx1d-ONT-MHI813

SP A1 A2 SP B
Stx2:
7 subtypes
a - g
35 variants
Subtyping Method for *Escherichia coli* Shiga Toxin (Verocytotoxin) 2 Variants and Correlations to Clinical Manifestations

Søren Persson,1* Katharina E. P. Olsen,1 Steen Ethelberg,1 and Flemming Scheutz1,2

*Department of Bacteriology, Mycology and Parasitology, Unit of Gastrointestinal Infections, Statens Serum Institut, Copenhagen, Denmark,1 and The International Escherichia and Klebsiella Centre (WHO), Unit of Gastrointestinal Infections, Statens Serum Institut, Copenhagen, Denmark2

Received 27 December 2006/Returned for modification 27 January 2007/Accepted 4 April 2007

Shiga toxin 2 (Stx2) from Shiga toxin-producing *Escherichia coli* (STEC) was subtyped by a method involving partial sequencing of the stxAB2 operon. Of 255 strains from the Danish STEC cohort, all 20 cases of hemolytic-uremic syndrome were associated with subtype Stx2 (11 cases), subtype Stx2c (1 case), or the two combined (8 cases).
Only 12 Stx2 variants found in Danish patients

New variants
First time in humans

Stx2a-O48-94C
Stx2a-O157-EDL933
Stx2c-O157-C394-03
Stx2c-O157-FLY16
Stx2d-O73-C165-02
Stx2d-O8-C466-01B
Stx2d-O157-7279
Stx2e-O101-E43
Stx2e-ONT-26725-97
Stx2g-O2-7v
Stx2b-O111-PH
Stx2b-O118-EH250

0.01
# Attack rate of Stx2 variants associated with HUS

<table>
<thead>
<tr>
<th>Variant Description</th>
<th>No. of Cases</th>
<th>Attack Rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stx2a-O157-EDL933 + Stx2c-O157-FLY16</strong></td>
<td>6/23</td>
<td><strong>26%</strong></td>
</tr>
<tr>
<td><strong>Stx2a-O157-EDL933</strong></td>
<td>3/17</td>
<td><strong>18%</strong></td>
</tr>
<tr>
<td><strong>Stx2c-O157-FLY16</strong></td>
<td>1/18</td>
<td><strong>6%</strong></td>
</tr>
<tr>
<td><strong>Stx2a-O157-SF + Stx2c-O157-FLY16</strong></td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td><strong>Stx2a-O157-EDL933 + Stx2c-O157-FLY16</strong></td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td><strong>Stx1b + Stx2a-O157-EDL933</strong></td>
<td>1/3</td>
<td></td>
</tr>
<tr>
<td><strong>Stx2a-O157-EDL933 + Stx2c-O157-FLY16</strong></td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td><strong>Stx1b + Stx2a-O157-EDL933 + Stx2c-O157-FLY16</strong></td>
<td>1/5</td>
<td><strong>20%</strong></td>
</tr>
<tr>
<td><strong>Stx1b + Stx2a-O157-EDL933 + Stx2c-O157-FLY16</strong></td>
<td>1/5</td>
<td><strong>20%</strong></td>
</tr>
</tbody>
</table>

**O157**

**Non-O157**
Conclusions

Two Stx2a variants associated with HUS

Stx2a-O157-EDL933 \( \text{in NSF O157} \)
(& Stx2-O157-FLY16)

Stx2a-O157-SF \{ \}
Stx2a-O48-94C \( \text{in SF O157} \)

Stx2a-O48-94C \( \text{in Non-O157} \)

New Paradigm:

How may STEC be classified?

Are certain virulence ”cocktails” associated with severe disease rather than the serotype?
### Classification of STEC in 5 Sero-pathotypes

Based on the reported occurrence of serotypes in human disease, in outbreaks and/or in hemolytic-uremic syndrome (HUS)


<table>
<thead>
<tr>
<th>Sero-pathotype</th>
<th>Relative incidence</th>
<th>Frequency of involvement in outbreaks</th>
<th>Association with severe disease (HUS or HC)</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>High</td>
<td>Common</td>
<td>Yes</td>
<td>O157:H7, O157:NM</td>
</tr>
<tr>
<td>B</td>
<td>Moderate</td>
<td>Uncommon</td>
<td>Yes</td>
<td>O26:H11, O103:H2, O111:NM, O121:H19, O145:NM</td>
</tr>
<tr>
<td>C</td>
<td>Low</td>
<td>Rare</td>
<td>Yes</td>
<td>O91:H21, O113: H21, O104:H21, others</td>
</tr>
<tr>
<td>D</td>
<td>Low</td>
<td>Rare</td>
<td>No</td>
<td>multiple</td>
</tr>
<tr>
<td>E</td>
<td>Non human only</td>
<td></td>
<td></td>
<td>multiple</td>
</tr>
</tbody>
</table>
Problems with this classification

Association with serotype and not with virulence profile

- More than 120 O:H serotypes have been associated with HUS (Bergey’s Manual of Systematic Bacteriology, 2nd ed.)
- Many O:H serotypes display extensive heterogeneity

Involvement in outbreak may rapidly change

Relative incidence

- is skewed by lack of efficient detection methods
- will vary depending on the epidemiology of specific types
1. HUS inducing STEC and/or an epidemic outbreak potential
   - eae and stx2a
   - eae negative and stx2d (activatable)
   - eae and stx1 Less common but certain O:H serotypes have been associated with HUS

2. Diarrhea inducing in humans!
   - Many different virulence profiles
   - Capacity to produce Stx and association with diarrhea in humans

3. Animal-associated STEC
   - High prevalence in the animal reservoir
   - Seems to be their natural habitat
   - No human cases
   - Candidates for this group are stx2e positives
Should management and treatment of patients be adjusted according to virulence cocktail?
Background

Danish Practice since 2000

ALL patients with STEC are excluded or quarantined if they are

- Children in institutions and day care
- Staff of health care facilities
- Hospital staff or hospitalized patients
- Food handlers

and until they have had two consecutive STEC negative stool samples

Prolonged shedding of STEC has resulted in huge social problems especially for parents
Danish example of consequences

Revision of guidelines for treatment of Danish patients with STEC may include antibiotic treatment of asymptomatic patients with

- eae negative STEC
- eae & stx1 - except some serotypes

Asymptomatic patients are likely to be allowed back in institutions and day care after treatment
Recommendations

• Adequate detection methods should include the isolation of bacteria

• Typing methods should be standardized

• Subtyping methods for Stx2a variants associated with HUS should be implemented

• Urgent need for standardized nomenclature
Questions

1. How much is detection and surveillance screwed?
2. Can case definitions for HUS to be notified within the Public Health system be established?
3. Will management and treatment of STEC patients depend on
   - a case-to-case based assessment?
   - an outbreak-to-outbreak approach?
   - local epidemiology?
Question

Should non-O157:H7 STECs be considered to be adulterants as *E. coli* O157:H7?

YES - some
Acknowledgements
Statens Serum Institut, Copenhagen
Joan Neverman Jensen
Søren Persson
Katharina E. P. Olsen

Co-authors on nomenclature
Lothar Beutin, Federal Institute for Risk Assessment, Berlin
Denis Piérard, Academisch Ziekenhuis Vrije Universiteit, Brussels
Nancy A. Strockbine, National Center for Zoonotic, Vector-borne and Enteric Diseases (CDC), Atlanta