From O157 to O26: HUS surveillance and the evolving epidemiology of VTEC/STEC infections

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Aims:

• To analyze the historical data of the HUS Italian surveillance/registry between 1988 and 2015
• To read the Italian data to analyze/understand the evolution of the epidemiology of STEC infections in Italy and beyond.
The Italian HUS Registry

Pediatric Nephrology Units

(Italian Society for Pediatric Nephrology)

- Active since 1988
- Coordinated by ISS since 2012
- Target population: children (< 15 yrs.)
- Standardized HUS case definition (www.iss.it/seu)
- Cases notified to the Registry with clinical and epidemiological information
- Stool and serum samples submitted to the NRL for E. coli for laboratory diagnosis of STEC infection
Cases of HUS reported to the Italian Registry, by year, 1988-2015*

- Total HUS cases recorded: 984
- Cases with prodromal diarrhea: 85% (752/886)
- Mean HUS cases per year: 35.8 (range 12 – 67)
- Age (median): 26 months (range 0 - 15 yrs)
- Mean annual incidence rate:
  - 0.40 cases per 100,000 (0-15 yrs)
  - 1.17 cases per 100,000 (<4 yrs.) - RR=2.9

*Data updated as of 31 August 2015

Source of demographic data: ISTAT (www.istat.it)
HUS and STEC infection in Italy

- Laboratory diagnosis of STEC infection by:
  - Direct examination of feces for Free Stx (Vero cell assay) and Stx genes (Real Time PCR)
  - STEC isolation
  - Detection of serum antibodies against the LPS of E. coli O157, O26, O103, O111, O145

- Clinical specimens obtained from 806 cases
- Evidence of STEC infection in 590 cases (73% of cases examined)
HUS and STEC infection in Italy

STEC serogroups most frequently detected 1988 – 2015*
(520 HUS cases*)

*Data updated as of 31 August 2015

* 127 cases: STEC isolation; 393 cases: serological diagnosis
1983 STEC O157 is a leading cause of HUS
Karmali et al. 1983

1988: Start of HUS surveillance
Caprioli et al. 1992

1987 Cattle are reservoir of STEC O157
Orskov et al. 1987

1993 STEC O111 outbreak
Caprioli et al. 1994

1993 STEC O157 outbreak
Riley et al. 1993; Michino et al. 1999

1994 STEC O157 outbreak
Nicolini et al. 1995

1994 HUS STEC O111 outbreak
MMWR 1995.
1998 – 2007: STEC O26 becomes the most frequent serogroup

2007 First isolation of SF STEC O157 in Italy

Clusters of HUS cases due to STEC O26

Outbreaks due to SF O157
Ammon et al. et al. 1999;
Allerberger et al. 2000

Importance of Sorbitol Fermenting STEC O157

Importance of non-O157 STEC
Bielaszewska et al. 1996; Jenkins et al. 2008
2008 – 2015 STEC O26 remained the most frequent serogroup

- **2009 HUS case by EAgg O104:H4 STEC**
  - *Scavia et al. 2012*

- **2013 Outbreaks with EAgg STEC O127**
  - *Tozzoli et al. in prep.*

- **2013 Community-wide outbreak by STEC O26** (20 HUS cases)
  - *Germinario et al. Eurosurveil. accepted*

- **2015 Day-care school associated outbreak by STEC O26** (11 cases, 1 HUS)

**Importance of non-O157 STEC**

- Enteroaggregative STEC: the O104:H4 outbreak
  - *Frank et al. 2011*
HUS in Italy: the transition from STEC O157 to O26

Three-years rolling average incidence of HUS in <15 years population (cases * 100,000), by STEC serogroup

Source of data: ISTAT (www.istat.it) demographic
## Illness associated with STEC O157 vs STEC O26 infection

<table>
<thead>
<tr>
<th></th>
<th>O157</th>
<th>O26</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HUS cases with STEC infection</strong></td>
<td>n= 166</td>
<td>n= 198</td>
</tr>
<tr>
<td><strong>age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>42,7 months</td>
<td>27,7 months</td>
</tr>
<tr>
<td>median</td>
<td>31,7 months</td>
<td>20,8 months</td>
</tr>
<tr>
<td>range</td>
<td>0 - 176 months</td>
<td>2 - 120 months</td>
</tr>
<tr>
<td><strong>gender</strong></td>
<td>n=166 %</td>
<td>n=198 %</td>
</tr>
<tr>
<td>males</td>
<td>86  53%</td>
<td>89  45%</td>
</tr>
<tr>
<td>females</td>
<td>77  47%</td>
<td>108  55%</td>
</tr>
<tr>
<td><strong>prodromal symptoms reported</strong></td>
<td>n=166 %</td>
<td>n=198 %</td>
</tr>
<tr>
<td>any diarrhea</td>
<td>146  88%</td>
<td>174  88%</td>
</tr>
<tr>
<td>bloody diarrhea</td>
<td>88  60%</td>
<td>90  52%</td>
</tr>
<tr>
<td>neurological symptoms</td>
<td>n=162 %</td>
<td>n=162 %</td>
</tr>
<tr>
<td></td>
<td>37/112  33%</td>
<td>40/127  31%</td>
</tr>
<tr>
<td><strong>acute case fatality rate</strong></td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>death</td>
<td>-</td>
<td>4  2%</td>
</tr>
<tr>
<td><strong>concomitant infection with other STEC</strong></td>
<td>n %</td>
<td>n %</td>
</tr>
<tr>
<td>HUS cases</td>
<td>5  3%</td>
<td>12  6%</td>
</tr>
</tbody>
</table>

*Note: p-values indicate statistical significance.*
Characteristics of STEC O26 strains isolated from HUS cases (1988 – 2015)

Total STEC O26 strains isolated: 48
- stx1+, eae+  2
- stx1+, stx2+, eae+  2
- stx2+, eae+  44

PFGE subtyping:
- strains with information available 41
- highly heterogeneous population
- very good consistency with clustering based on space/time clustering criteria

Stx subtyping:
- strains with information available 37
  - stx1a  2
  - stx2a  34
  - stx2b  1
HUS outbreaks and STEC clusters

1992: 11 cases (Lumbardy)

1997: 3 cases (Naples)

2005: 6 cases (Salerno)

2007: 3 cases (Turin)

2010: 2 cases (Parma)

2013: 2 cases (Treviso)

2013: 4 HUS+2 other cases O127 EAgg STEC

2012: 3 cases (Apulia)

2015: 1 HUS + 10 other cases (Rome)

Are the reservoirs and the transmission routes of STEC O26 infections to humans different or the same as for STEC O157?
Outbreak of HUS linked to STEC O26 infection (1)
Direct contact with sheep flock and/or dairy products thereof (strong evidence)

- Petting Zoo: June 2015, North Italy
  - VTEC O26, vtx2+, eae+
  - A 1 year old girl with severe case of HUS (intensive care unit) + 6-yrs asymptomatic sister
  - Indistinguishable PFGE profile of strains isolated from the HUS case and from sheep sampled in the farm
E.coli O26 vtx2 +: which is the animal reservoir??

E.coli O26 vtx2+ in cattle:
1 out of 26 VTEC strains reported: 3.8%

E.coli O26 vtx2+ in sheep:
7 out of 17 VTEC strains reported: 41.2%

Bonardi et al. Vet Rec Open. 2015 Jan 20;2(1)
Outbreak of HUS linked to STEC O26 infection (2)

• Community-wide prolonged outbreak summer 2013, Italy – South Italy
  ✓ VTEC O26, vtx2a, eae+
  ✓ 20 HUS cases: 15 residents and 5 travel related; median age 17 months
  ✓ Other 2 HUS cases (1 VTEC O80, vtx2f+, eae+; 1 VTEC ONT, vtx2+, eae+)
  ✓ STEC O26 infection detected only among HUS cases. No uncomplicated cases were reported although active case finding was carried out at regional/national level.
  ✓ No cases reported among adults
  ✓ No secondary cases among household contacts (but two cases were brothers)
  ✓ No apparent epidemiological link among cases (cases among brothers)
Outbreak of HUS due to STEC O26 infection (2)
Case-Control Study: foodborne, strong evidence

<table>
<thead>
<tr>
<th>Exposures</th>
<th>Cases (n=15)</th>
<th>Controls (n=52)</th>
<th>Matched Odds Ratio (95% C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>11</td>
<td>33</td>
<td>1.6 (0.4 - 7.7)</td>
<td>0.5</td>
</tr>
<tr>
<td>Frankfurter sausages</td>
<td>4</td>
<td>8</td>
<td>2 (0.4 - 9.2)</td>
<td>0.3</td>
</tr>
<tr>
<td>Hamburger</td>
<td>4</td>
<td>21</td>
<td>0.6 (0.1 - 2.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>Meatballs</td>
<td>4</td>
<td>19</td>
<td>0.5 (0.1 - 2.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>Pork</td>
<td>5</td>
<td>19</td>
<td>0.9 (0.2 - 3.3)</td>
<td>0.8</td>
</tr>
<tr>
<td>Sausages</td>
<td>5</td>
<td>26</td>
<td>0.5 (0.1 - 1.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>Veal</td>
<td>12</td>
<td>28</td>
<td>3.1 (0.7 - 19.2)</td>
<td>0.1</td>
</tr>
<tr>
<td>Fresh fruit</td>
<td>8</td>
<td>32</td>
<td>0.7 (0.2 - 2.7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Green leafy vegetables</td>
<td>3</td>
<td>4</td>
<td>2.9 (0.4 - 19.3)</td>
<td>0.2</td>
</tr>
<tr>
<td>Juice fruit</td>
<td>8</td>
<td>30</td>
<td>0.8 (0.2 - 3.2)</td>
<td>0.8</td>
</tr>
<tr>
<td>Watermelon</td>
<td>12</td>
<td>39</td>
<td>1.3 (0.3 - 8.5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Other vegetables</td>
<td>7</td>
<td>22</td>
<td>1.2 (0.3 - 4.4)</td>
<td>0.8</td>
</tr>
<tr>
<td>Pasteurized milk</td>
<td>5</td>
<td>15</td>
<td>1.2 (0.3 - 4.8)</td>
<td>0.7</td>
</tr>
<tr>
<td>UHT milk</td>
<td>4</td>
<td>24</td>
<td>0.4 (0.1 - 1.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>Yogurt</td>
<td>11</td>
<td>31</td>
<td>1.9 (0.5 - 9.0)</td>
<td>0.3</td>
</tr>
<tr>
<td>Burrata cheese</td>
<td>3</td>
<td>5</td>
<td>2.4 (0.3 - 13.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>Mozzarella cheese</td>
<td>8</td>
<td>22</td>
<td>1.6 (0.5 - 5.9)</td>
<td>0.5</td>
</tr>
<tr>
<td>Ricotta cheese</td>
<td>8</td>
<td>32</td>
<td>0.7 (0.2 - 2.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>Other fresh cheeses</td>
<td>3</td>
<td>28</td>
<td>0.2 (0.0 - 0.9)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Plant A</strong></td>
<td>3</td>
<td>1</td>
<td>10.3 (1.5 - 930.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Plant B</strong></td>
<td>2</td>
<td>5</td>
<td>1.4 (0.1 - 10.1)</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Plant C</strong></td>
<td>7</td>
<td>5</td>
<td>13.9 (2.2 - 43.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Plant E</strong></td>
<td>1</td>
<td>2</td>
<td>1.8 (0.0 - 36.3)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Strong Evidence**
Outbreak of HUS linked to STEC O26 infection (2): PFGE analysis: foodborne, weak evidence
Outbreak of HUS linked to STEC O26 infection (3)

- **Community-wide prolonged outbreak** autumn 2005, North-West France
  - 16 HUS cases; median age 15 months (range 8m. - 6 yrs)
  - VTEC O26:H11, vtx2a, eae+; VTEC O80:H2, vtx2+, eae+;
  - VTEC O26 infection detected only among HUS cases
  - No other cases of bloody or watery diarrhea due to VTEC O26 were reported
  - No secondary cases among household contacts
  - Consumption of camembert of the same brand was reported for 9 cases

<table>
<thead>
<tr>
<th>N° du cas</th>
<th>Consommation de camembert au lait cru</th>
<th>Souche de STEC isolée</th>
<th>Sérologie</th>
<th>Profil PFGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>non</td>
<td>analyse non réalisée</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>oui (marque X)</td>
<td>O26:H11 stx2+ eae+</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>oui (marque X)</td>
<td>analyse non réalisée</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>non</td>
<td>NT* stx2+ eae+</td>
<td>négative</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>oui (marque X)</td>
<td>O80:H2 stx2+ eae+</td>
<td>négative</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>oui (marque X)</td>
<td>O80:H2 stx2+ eae+</td>
<td>O26</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>oui (marque X)</td>
<td>O26:H11 stx2+ eae+</td>
<td>négative</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>oui (marque X)</td>
<td>O26: stx2- eae+</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>9</td>
<td>oui (marque X)</td>
<td>O26: H11 stx2- eae+</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>10</td>
<td>non</td>
<td>O26: H11 stx2- eae+</td>
<td>analyse non réalisée</td>
<td>A</td>
</tr>
<tr>
<td>11</td>
<td>oui</td>
<td>O80:H2 stx2+ eae+</td>
<td>O26</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>oui</td>
<td>O26:H11 stx2+ eae+</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>13</td>
<td>oui (marque X)</td>
<td>O26:H11 stx2+ eae+</td>
<td>O26</td>
<td>A</td>
</tr>
<tr>
<td>14</td>
<td>oui (marque X)</td>
<td>O2:H5 stx2+ eae+</td>
<td>négative</td>
<td>D</td>
</tr>
<tr>
<td>15</td>
<td>oui</td>
<td>O80:H2 stx2+ eae+</td>
<td>O26</td>
<td>B</td>
</tr>
<tr>
<td>16</td>
<td>non</td>
<td>NT* stx2+ eae+</td>
<td>négative</td>
<td>E</td>
</tr>
</tbody>
</table>

Source: InVS, 2007
Outbreak of HUS linked to STEC O26 infection,

- **Community-wide prolonged outbreak** autumn 2005, North-West France. **Foodborne, strong evidence**

<table>
<thead>
<tr>
<th>Profil PFGE B</th>
<th>Marqueur moléculaire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O2:H5 stx2+ eae+</td>
</tr>
<tr>
<td></td>
<td>O80:H2 stx2+ eae+</td>
</tr>
<tr>
<td></td>
<td>O80:H2 stx2+ eae+</td>
</tr>
<tr>
<td></td>
<td>O80:H2 stx2+ eae+</td>
</tr>
<tr>
<td></td>
<td>O80:H2 stx2+ eae+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Profil PFGE A</th>
<th>Marqueur moléculaire</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O80:H2 stx2+ eae+</td>
</tr>
<tr>
<td></td>
<td>O26:H11 stx2+ eae+</td>
</tr>
<tr>
<td></td>
<td>O26:H11 stx2- eae2+</td>
</tr>
<tr>
<td></td>
<td>O26:H11 stx2+ eae- (ensilage)</td>
</tr>
<tr>
<td></td>
<td>O26:H11 stx2+ eae+ (camembert)</td>
</tr>
<tr>
<td></td>
<td>O26:H11 stx2- eae+ (lait)</td>
</tr>
</tbody>
</table>

Source: InVS, 2007
Outbreak of HUS linked to STEC O26 infection (2)

- **Limited epidemic setting:** creche, May 2015, Central Italy
  - VTEC O26, vtx2a+, eae+
  - 1 case of HUS, 6 cases with bloody or watery diarrhea, 4 cases asymptomatic
  - Secondary cases reported among schoolmates, nurses, household contacts (either adults or children)
  - Epidemic curve suggested a person to person transmission
Outbreak of HUS linked to STEC O26 infection (2): person-to-person transmission

- Asymptomatic shedders !!
- Indistinguishable PFGE profiles
- Median duration of STEC O26 shedding 24 days (range 5 – 44)
- Antimicrobial treatment of healthy shedders or recovered patients was decided to limit further spread (10 subjects)
- The treatment was administered safely!
STEC serogroups in humans - trend in Europe:

Distribution frequency of the most frequently reported serogroup, by year (2008 – 2013)

Source of data: European Centre for Disease Prevention and Control - The European Surveillance System (TESSy)
STEC O26 trend in Europe: food and animals

<table>
<thead>
<tr>
<th>Year</th>
<th>food</th>
<th>animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>32767</td>
<td>8392</td>
</tr>
<tr>
<td>2012</td>
<td>25547</td>
<td>10134</td>
</tr>
<tr>
<td>2013</td>
<td>25008</td>
<td>6683</td>
</tr>
</tbody>
</table>
CONCLUSIONS

- Stx2 *E. coli* O26 represents a major and emerging cause of HUS in Italy
- The Italian historical data on HUS indicate that the epidemiology of STEC O26 may be different from that of O157.
- Despite the high number of STEC O26 cases observed, the reservoir and the routes of transmission of these particular STEC strains remain elusive.
- Importance of routinely maintaining an intersectoral collaboration to support analysis of long-term epidemiological trends of STEC infection in population
Acknowledgments: The Italian Registry for HUS

HUS Registry Reference Laboratories

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AOU Padova, Padua
E. Vidal

IRCCS Bambino Gesù, Rome
F. Emma

AOU Regina Margherita, Turin
L Peruzzi

AO Santobono- Pausilipon, Naples
C. Pecoraro

AO G. Di Cristina, Palermo
S. Maringhini

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