Carriage and control of verocytotoxigenic *E. coli* in cattle

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Overview

• Verocytoxigenic E. coli: an evolving pathogen

• VTEC carriage in cattle and shedding patterns

• Control measures
Pathogenic *E. coli*
- Enteropathogenic *E. coli* (EPEC)
- Enteroaggregative *E. coli* (EAggC)
- Entertoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC) (*IpaA*..)

*Shigella dysenteria* **st** (**vt**)

1990’s VTEC new group combining virulence genes above groups

1990’s ...
*E. coli* O157:H7
**eae** and **vt**

Mid 2000’s ....
New VTEC serogroups (O26, O103, O111....)
**eae** and **vt**

2011
*E. coli* O104
**EAggC** and **vt**
54 dead, > 4000 ill:
What VTEC serogroups are now causing human illness in EU Member States (2009-2010, EFSA)

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>No. of cases</th>
<th>% total</th>
<th>Serogroup</th>
<th>No. of cases</th>
<th>% total</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157</td>
<td>1,501</td>
<td>41.1</td>
<td>O157</td>
<td>1,848</td>
<td>51.7</td>
</tr>
<tr>
<td>NT¹</td>
<td>1,230</td>
<td>33.7</td>
<td>NT1</td>
<td>1,008</td>
<td>28.2</td>
</tr>
<tr>
<td>O26</td>
<td>257</td>
<td>7.0</td>
<td>O26</td>
<td>192</td>
<td>5.4</td>
</tr>
<tr>
<td>O103</td>
<td>90</td>
<td>2.5</td>
<td>O103</td>
<td>82</td>
<td>2.3</td>
</tr>
<tr>
<td>O145</td>
<td>61</td>
<td>1.7</td>
<td>O91</td>
<td>48</td>
<td>1.3</td>
</tr>
<tr>
<td>O91</td>
<td>57</td>
<td>1.6</td>
<td>O145</td>
<td>47</td>
<td>1.3</td>
</tr>
<tr>
<td>O83</td>
<td>42</td>
<td>1.2</td>
<td>O146</td>
<td>31</td>
<td>0.9</td>
</tr>
<tr>
<td>O111</td>
<td>41</td>
<td>1.1</td>
<td>O128</td>
<td>26</td>
<td>0.7</td>
</tr>
<tr>
<td>O128</td>
<td>29</td>
<td>0.8</td>
<td>O111</td>
<td>25</td>
<td>0.7</td>
</tr>
<tr>
<td>O146</td>
<td>28</td>
<td>0.8</td>
<td>O113</td>
<td>22</td>
<td>0.6</td>
</tr>
<tr>
<td>Other²</td>
<td>315</td>
<td>8.6</td>
<td>Other²</td>
<td>244</td>
<td>6.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3,651</strong></td>
<td></td>
<td><strong>Total</strong></td>
<td><strong>3,573</strong></td>
<td></td>
</tr>
</tbody>
</table>
### Recent selected food borne outbreaks non O157 EHEC serogroups

<table>
<thead>
<tr>
<th>Food</th>
<th>Country</th>
<th>Year</th>
<th>Serogroup</th>
<th>No. Cases</th>
<th>No. Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenugreek seeds</td>
<td>Germany</td>
<td>2011</td>
<td>O104</td>
<td>3816</td>
<td>54</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>Belgium</td>
<td>2007</td>
<td>O145:H28 and O26:H11</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Fermented beef sausage</td>
<td>Denmark</td>
<td>2007</td>
<td>O26:H11</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Cured mutton sausage</td>
<td>Norway</td>
<td>2006</td>
<td>O103:H25</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Venison</td>
<td>USA</td>
<td>2012</td>
<td>O103:H2 and O145:NM</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Milk</td>
<td>USA</td>
<td>2010</td>
<td>O111</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Romaine Lettuce</td>
<td>USA</td>
<td>2010</td>
<td>O145</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Ground beef</td>
<td>USA</td>
<td>2010</td>
<td>O26</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>
VTEC carriage in cattle
Ruminant animals (waste) considered to be main vehicle of VTEC transmission to humans direct and indirectly via food/water chain

But many diverse *E. coli* which are verotoxin producers now circulating in animals (60-70%) and only a small portion will cause illness in humans

How do you assess whether a VTEC isolated from an animal or food poses a human health risk?
Monitoring of verotoxigenic *Escherichia coli* (VTEC) in animals and food for human pathogenic VTEC

Look for: Selected VTEC serogroups most often seen in human illness and virulence assessment of the isolate

**Top serogroups**
EU  O157, O26, O111, O103, O45  
USA  O157, O26, O111, O103, O45, O145, O21

**Virulence genes**  vt in combination with eae

**But**
Other serogroups or other virulence gene combination may also cause illness  2011: *E. coli* O104: an EAggC with vt and no eae
### Selected studies: Prevalence *E. coli* O157 in Bovine Faeces

<table>
<thead>
<tr>
<th>Country</th>
<th>Serogroup</th>
<th>Place</th>
<th>N</th>
<th>%</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>O157</td>
<td>Feed-lot</td>
<td>891</td>
<td>9.2</td>
<td>Alam <em>et al.</em> 2006)</td>
</tr>
<tr>
<td>UK</td>
<td>O157</td>
<td>Abattoir</td>
<td>3939</td>
<td>4.7</td>
<td>Paiba <em>et al.</em> (2002)</td>
</tr>
<tr>
<td>USA</td>
<td>O157</td>
<td>Beef Dairy</td>
<td>408</td>
<td>4.7</td>
<td>Doane <em>et al.</em> (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>408</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Ireland</td>
<td>O157</td>
<td>Abattoir</td>
<td>400</td>
<td>2.3</td>
<td>Thomas <em>et al.</em> (2012)</td>
</tr>
<tr>
<td>Ireland</td>
<td>O157</td>
<td>Abattoir</td>
<td>400</td>
<td>2.3</td>
<td>Thomas <em>et al.</em> (2012)</td>
</tr>
<tr>
<td>S. Korea</td>
<td>O157</td>
<td>Beef</td>
<td>864</td>
<td>1.7</td>
<td>Jo <em>et al.</em>, (2004)</td>
</tr>
</tbody>
</table>

Majority of these isolates (> 90%) where tested had *vt* and *eae* genes indicating most bovine *E. coli* O157 have human virulence potential.
### Selected studies: Prevalence other VTEC serogroups in Bovine Faeces

<table>
<thead>
<tr>
<th>Country</th>
<th>No. samples</th>
<th>Serogroup</th>
<th>% + (% vt and eae +)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>6086</td>
<td>O26, O103, O145, O111</td>
<td>4.6 (2.2 %), 2.7 (0.03 %), 0.7 (0.03 %), 0</td>
<td>Pearce et al, 2006</td>
</tr>
<tr>
<td>S Korea</td>
<td>809</td>
<td>O26, O111</td>
<td>6.67 (6 %), 4.57 (3.4 %)</td>
<td>Byung-Woo et al. (2006)</td>
</tr>
<tr>
<td>Ireland</td>
<td>402</td>
<td>O26, O111, O103, O145</td>
<td>2 (1.5 %), 0 - 8.5 (1.5 %), 0.7 (0.7)</td>
<td>Thomas et al, (2011)</td>
</tr>
<tr>
<td>Belgium</td>
<td>399</td>
<td>O26, O103, O145, O111</td>
<td>2.2 (1.5 %), 2.5 (1.7 %), 0.75 (0.25 %), 0.5 (0.5 %)</td>
<td>Joris et al (2011)</td>
</tr>
<tr>
<td>Japan</td>
<td>2436</td>
<td>O26</td>
<td>1.0 (0.4)</td>
<td>Sasaki (2011)</td>
</tr>
</tbody>
</table>

Overall prevalence these VTEC serogroups similar or lower to O157 but much smaller proportion are virulent
Seasonal pattern
General peak in prevalence seen late spring to early Autumn (April to September)

Age of animal
Shedding prevalence generally higher in young cattle than very young calves or older animals

VTEC persistence in herd and farm environment
Some strains can persist in herd and remain detectable in farm environment and housing (pen) for long periods (months / years) (distinct genetic (PFGE) pattern)
VTEC colonisation in cattle

Cattle exposed to VTEC orally (grass, feed, water, grooming hides other animals, licking their environment etc.)

*E. coli* O157 passes though stomachs and colonises the distal colon at a specific site 0 to 3 cm proximal to recto-anal junction (RAJ)
Not known if same case for other VTEC serogroups

When colonised, cattle display no symptoms of illness
VTEC Shedding pattern

• In an animal colonised with *E. coli* O157, shedding continues for up to 6 weeks but is intermittent/random in this time

• Shedding experiment: Teagasc (McGee *et al* 2004) Cattle inoculated with *E. coli* O157:H7 and faeces each animal monitored every day over 6 week

  Each animal shed the pathogen intermittently
  Days of shedding: Days no shedding
  Last positive faecal samples from animals ranged from day 2 to 15
  Counts ranged from $\log_{10}1.4$ to 6.20 CFU g$^{-1}$ faeces.

  Numbers shed in faeces varied considerably between animals
Supershedders

Some cattle “super-shedders” excrete exceptionally high number of *E. coli* O157 (> 10,000 CFU/g) in their faeces

May also occur with other VTEC serogroups *(Menrath et al, 2010)*

Limited studies on frequency at which this phenomenon occurs

- US study: 3.8% heifers supershedders *(Cobbold et al 2007)*

- German study: Dairy herd (10 % super-shedders) *(Menrath et al, 2010)*

- Canadian study: Feed-lot cattle (25% supershedders) *(Cernicchiaro et al 2010)*
• Super-shedders have a significant impact on transmission of VTEC on farm, transport, lairage and slaughter operations.

• Significant impact on hide contamination in co-penned animals. Hide most important source VTEC contamination at slaughter.

• Estimated that super shedding animals contribute up to 80% of all VTEC transmitted and would thus be a key target for risk reduction measures.

• Supershedding identified as a key area for further research: *to understand what causes super-shedding and then how to control it*
Causes supershedding ??

Genetic variation in VTEC super-shedders
Inoculation study with *E. coli* O157 showed strains with particular virulence related genes (intimin, *tir* and PO157) were consistently shed in higher numbers and were more likely to persist and recolonise herds than strains missing even one of these genes  *Sheng et al, 2006*

Differences in micro-flora at RAJ colonisation site ??
Are intermittent modulations (changes) in the composition of the resident micro-flora at the RAJ site allowing VTEC to flourish and dominate in some animals for periods of time ?

*Research needed*
Control of VTEC in cattle
• On farm management practices can help control VTEC transmission
  Good Hygiene
  Clean water / dry bedding…
  Stocking density: reduce hide to hide contact
  But hard to quantify impact

• Petting farms strict hygiene measures critical

• Number treatments developed to reduce colonisation and shedding of VTEC
  Most targeted at *E. coli* O157 and not other serogroups
  : Vaccines and Phage
Vaccines

Cattle’s own immune system is triggered to produce antigens against VTEC

Generally target proteins involved in *E. coli* O157 host attachment/ colonisation

- Type III secreted proteins involved in intestinal colonization of *E. coli* O157

- Siderophore receptor and porin proteins (SRP)

Reported to reduce carriage in herd by up to 50%
Two commercial vaccines available and shown to be effective way to control *E. coli* O157:H7 in cattle

- **Econiche (Bioniche Inc)** targets type III proteins
  - Approved for use Canada 2008.
  - Not yet approved U.S.D.A.
  - Summer 2012 approved in United Kingdom
  Special Treatment Certificate (STC) by DEFRA UK
  *STC issued when no treatment for a given animal disease is approved by EU, but is available in country outside EU*

- **Epitopix** siderophore “receptor and porin” SRP® technology conditional approval by USDA 2009
**Bacteriophage**
Natural viruses that infect and kill bacterial cells by Reproducing within bacteria and disrupting the host metabolic pathways, causing the bacterium to lyse.

**Commercial**
(Finalyse : Elanco food Solutions)
Licenced in US contains a mixture of naturally occurring phages that specifically target *E. coli* O157:H7

**Research** (many)
*Teagasc*: 2 lytic phages (e11/2 and e4/1c) isolated from bovine slurry with activity against *E. coli* O157 (O’Flynn *et al*, 2004)
Teagasc: Application Phage in rumen model (Rivas et al 2010)

- Rumen model set up and inoculated with $10^6$ cfu/ml *E. coli* O157:H7
- Bacteriophage e11/2 or e4/1c added
- Yielded significant ($p<0.05$) reduction within 2 h
Cattle yearling (n=22) orally inoculated $10^{10}$ CFU *E. coli* O157:H7. Dosed daily with (e11/2 and e4/1c) for 3 days post inoculation.

No significant difference (P > 0.05) numbers *E. coli* O157:H7 shed by phage-treated and control animals.
Teagasc: Application of Phage to hide as pre-slaughter treatment *(Coffey et al, 2011)*

Hide inoculated with *E. coli* O157 and then sprayed with phage cocktail (e11/2 and e4/1c) exposure time of 1h

![Graph showing significant reduction in counts](image)

Significant reduction (*p*<0.05) (1.5 log₁₀ cfu/cm²) compared to control
CONCLUSIONS

• O157 still dominant sero-group in cattle but many other emergent serogroups with group of top 5 serogroups EU and top 7 in USA recommended for monitoring in animal and food chain

• Testing VTEC isolate for selected virulence genes VTEC is essential to ascertain public health importance

• Number of options to control O157  *in vivo*

• Research gaps
  : understanding cause of super-shedding
  : carriage and shedding patterns non O157 VTEC
  : Targeted controls against a wider range of clinically significant VTEC serogroups and supershedders
Acknowledgments