Epidemiological and evolutionary studies on STEC O157 and O26 in cattle and humans in New Zealand –

A summary of findings from PhD and Postdoc research studies

Presented at STEC workshop
Massey University, Palmerston North
15 Dec 2015
PhD research studies (12–13 min)

1) Nationwide prevalence study – Cross-sectional study (2009 – 2011)

2) Transport & lairage study in bobby calves – Cohort study (2010)

3) Source attribution study in humans – Case-control study (2011–2012)

4) Molecular study on bovine and human STEC O157 (2013)

Postdoc research (7 min)

Nationwide prevalence study on STEC O157 and O26

**Research question:** What is prevalence of STEC O157 and O26 in NZ slaughter cattle population? (incl. dairy, beef, adult cattle, bobby calves)

**Sample collection**

- 4x cattle slaughter plants (2x NI, 2x SI)
- Repeated visits over 2 years
  - Fortnightly (calves)
  - Monthly (adult cattle)

**Calves**

\[ n = 695 \]

**Adult cattle**

\[ n = 895 \]
Origin of animals (1,009 different farms)

695 calves (566 farms)  895 adult cattle (536 farms)
### Sample testing

Real-time PCR → Culture isolation → Molecular analysis

#### Results

<table>
<thead>
<tr>
<th></th>
<th>Serogroup</th>
<th>Rt PCR +ve</th>
<th>Culture +ve</th>
<th>STEC</th>
<th>H7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calves</strong></td>
<td>O157</td>
<td>23.5% (163/695)</td>
<td>3.2% (22/695)</td>
<td>2.3% (16/695)</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>O26</td>
<td>33.2% (231/695)</td>
<td>8.3% (58/695)</td>
<td>3.9% (27/695)</td>
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</tr>
<tr>
<td><strong>Adult cattle</strong></td>
<td>O157</td>
<td>7.0% (63/895)</td>
<td>2.5% (22/895)</td>
<td>1.6% (14/895)</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>O26</td>
<td>7.6% (68/895)</td>
<td>3.2% (29/895)</td>
<td>0.4% (4/895)</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions of nationwide prevalence study

Animal-level

• Prevalence of shedding STEC in calves >> adult cattle ($P < 0.001$)
  
  6.0% vs. 1.8%
  
  (95% CI 4.4%–8.1%) (95% CI 1.1%–3.0%)

• Prevalence of shedding STEC O26 > O157 in calves ($P = 0.121$)

• Animals rt PCR +ve for one serogroup more likely to be +ve for other

Farm-level

• STEC+ve: 4.9% all farms (49/1,009; 95% CI 3.6%–6.4%)
  
  2.8% beef farms (10/354; 95% CI 1.4%–5.3%)

  6.0% dairy farms (39/655; 95% CI 4.3%–8.1%)

Jaros et al. *Epidemiol Infect* 2016 (accepted)

Nationwide prevalence and risk factors for faecal carriage of *Escherichia coli* O157 and O26 in very young calves and adult cattle at slaughter in New Zealand
Transport & lairage study in bobby calves

**Research question:** What are the risk factors associated with high prevalence of STEC O157 and O26 in/on bobby calves at slaughter, including the impact of transportation and lairage?

Waikato region, 3 locations
Pre-selected 3 dairy farms/location, pre-tested →
1x ‘high’ risk farm
1x ‘low’ risk farm

<table>
<thead>
<tr>
<th>High risk</th>
<th>Low risk</th>
</tr>
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<tbody>
<tr>
<td>10</td>
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</tbody>
</table>

Transportation: (< 2 h)

Lairage at slaughter plant

Sample collection

Post-slaughter

On-farm

On-plant

Hide

Pre-interv.

Post-interv.
## Results

3x runs, in total 60 calves followed as cohorts from farm to post-slaughter. Samples processed: Real time PCR → Culture isolation → Molecular analysis

<table>
<thead>
<tr>
<th>Samples (n)</th>
<th>Transport and lairage</th>
<th>Post-slaughter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On-farm</td>
<td>On-plant (end of lairage)</td>
</tr>
<tr>
<td>Samples (n)</td>
<td>60</td>
<td>60</td>
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<tr>
<td>Real time PCR +ve</td>
<td>O157</td>
<td>38</td>
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<tr>
<td></td>
<td>O26</td>
<td>37</td>
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<tr>
<td>Culture +ve</td>
<td>O157</td>
<td>15</td>
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<tr>
<td></td>
<td>O26</td>
<td>19</td>
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</tbody>
</table>
### Results – genotype diversity

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Risk type of farm</th>
<th>On-farm</th>
<th>On-plant</th>
<th>Hide</th>
<th>Pre-int.</th>
<th>Post-int.</th>
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</table>

**O157**

**O26**

1. run

2. run

3. run

1. run

2. run

3. run
Conclusions of transport & lairage study in bobby calves

- Little evidence for transmission of infection during transport and lairage
- Increased cross-contamination of hides and carcases from high to low risk calves
- No increased residual contamination on carcases at pre-boning stage
- Calves rt PCR +ve on-plant more likely to be +ve on-farm or originated from a high-risk farm

**Jaros et al. 2016 (in preparation)**

The effect of transportation and lairage on faecal shedding and carcass contamination with *Escherichia coli* O157 and O26 in very young calves in New Zealand
Source attribution study in humans

**Research question:** What are the risk factors associated with sporadic STEC infections in New Zealand?

**Methods**
- Nationwide prospective case-control study
- Expected ~150 cases of STEC
- Random sampling of 506 controls (1:3 case-control ratio)
- Questionnaire (D7, Q42)
Results – Age and spatial distribution of cases and controls

113 STEC cases (52.2% male)  
506 controls (42.9% male)
Results – Identified risk factors

- Other family member contact with animals, Age 0-4
- Other family member contact with animals, Age 5-19
- Other family member contact with animals, Age >19
- Contact with recreational waters
- Travelled to areas in NZ with interrupted water supply
- Contact with animal manure
- Beef livestock present on meshblock
- Eating raw vegetables
- Visiting childcare/kindergarten/school
- Handling raw offal
- Drinking refrigerated fruitjuice from supermarket

* Water supply to home from bore/spring or creek/stream
  * Contact with children wearing nappies
  * Dining outside home
  * Eating seafood
  * Taking antacids

* Confounding variables
Conclusions of source attribution study in humans

- Children 0–4 years-old at higher risk
- Environmental and animal contacts, but not food, as significant exposure pathways
- Strong indications that dairy and beef cattle are most important sources of STEC
- Increased relative risks of STEC infections in Northland, Waikato, Taranaki, Canterbury, and Southland

*Jaros et al. BMC Infect Dis 2013, 13:450*

A prospective case–control and molecular epidemiological study of human cases of Shiga toxin-producing *Escherichia coli* in New Zealand
Molecular study on bovine and human STEC O157

Research question: What are the differences between bovine and human isolates?

Methods

• Isolates
  40 bovine STEC O157 isolates (Prevalence study + other study)

• Molecular subtyping
  Pulsed-Field Gel Electrophoresis (PFGE)
  Stx-encoding Bacteriophage Insertion typing (SBI)
Results – Between-island comparisons

**Dominant SBI types**

- **AY2a**
- **WY12a**
- **ASY2c/SY2c**
- **Other**

**Bovine NI**
(n = 32)

- AY2a: 49 (18%)
- WY12a: 41 (15%)
- ASY2c/SY2c: 9 (11%)
- Other: 175 (63%)

**Bovine SI**
(n = 8)

- P < 0.001 (Fisher’s exact)

**Human NI**
(n = 278)

- Other: 5% (5%)

**Human SI**
(n = 85)

- Other: 7% (7%)

P < 0.001 (Chi-square)
Results – International comparison of SBI genotypes

Mellor et al. AEM, 2013
Conclusions of molecular study on STEC O157

- Geographical distinction between NI and SI O157 isolates (bovine and human)

- Strong indication of localised transmission between both populations

- Distinct geographic divergence of genotypes at international level, suggesting historic introduction of STEC O157 genotypes into NZ


Geographic divergence of bovine and human Shiga toxin-producing Escherichia coli O157:H7 genotypes, New Zealand
Evolutionary study on bovine and human STEC O157

Research questions:

• When was O157 introduced into NZ?
• How often?
• How does that relate to the cattle population in NZ?
• What are the implications for biosecurity and public health?
Methodology

- 144 STEC O157 isolates, 2004–2014
  - 67 bovine
  - 77 human
- Selection of isolates
  - SBI typing data or PFGE profiles
  - Island of origin
  - Year of isolation

1x dominant SBI type / host / island / year
1x other SBI types

- Whole genome sequencing
Preliminary results

Core SNPs

- Used for phylogenetic analysis
- Core SNPs
  Single nucleotide polymorphisms shared by all organisms of interest
- Reads of 144 genomes compared with EDL933, 3,981 core SNPs identified
- NeighborNet tree based on concatenated core SNPs
Preliminary results

**BEAST analysis**
(Bayesian Evolutionary Analysis Sampling Trees)

- To study evolutionary history
- GMRF model using 3,981 core SNPs
- Phylogenetic tree with predicted years of common ancestors

![Phylogenetic tree with predicted years of common ancestors](image)

- **ASY2c/SY2c**
  - 1969 (95% HPD 1950–1984)
  - 1999 (95% HPD 1997–2001)

- **AY2a**
  - 1978 (95% HPD 1971–1985)

- **WY12a**
  - 1848 (95% HPD 1821–1872)

**FigTree software**
Cattle importations into New Zealand

Quantification of historical livestock importations into New Zealand, 1860-1979

Ancestor strains of STEC O157

1969: ASY2c/SY2c
1978: AY2a
Bacterial population sizes of dominant SBI lineages

- WY12a
- AY2a
- ASY2c/SY2c

Log scale graphs showing the population sizes over time.
Bacterial population sizes of dominant SBI lineages

**Number of dairy cows**

- **STEC O157 case**

**Dairy herds**

- Increase of stocking density by 28%
  - Density associated with enhanced pathogen transmission
  - Increase in bacterial populations as number of host increases

Source: NZ Dairy Statistics 2013/14, LIC & Dairy NZ, Hamilton, NZ
Summarising comments on evolutionary study

- Preliminary results indicate historic introduction of ancestral strains
  - livestock importations
  - people

- Unique island ecosystem in New Zealand
  - population biology
  - evolution of pathogens
  - low diversity and unique genotypes of pathogens

- Importance of biosecurity measures
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Anne Midwinter
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Dairy farmers
Transport companies and staff

MPI
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Funding

Ministry for Primary Industries
Manatū Ahu Matua

OIE Collaborating Centre for Veterinary Epidemiology and Public Health

mEpiLab
Publications

• Jaros et al. 2016 (in preparation)
  Genomic epidemiology of bovine and human *Escherichia coli* O157:H7 in New Zealand

• Jaros et al. 2016 (in preparation)
  The effect of transportation and lairage on faecal shedding and carcass contamination with *Escherichia coli* O157 and O26 in very young calves in New Zealand

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