

Non-O157 STEC in Scotland

Lesley Allison

on behalf of

the Scottish *E. coli*
O157/STEC Reference
Laboratory (SERL)

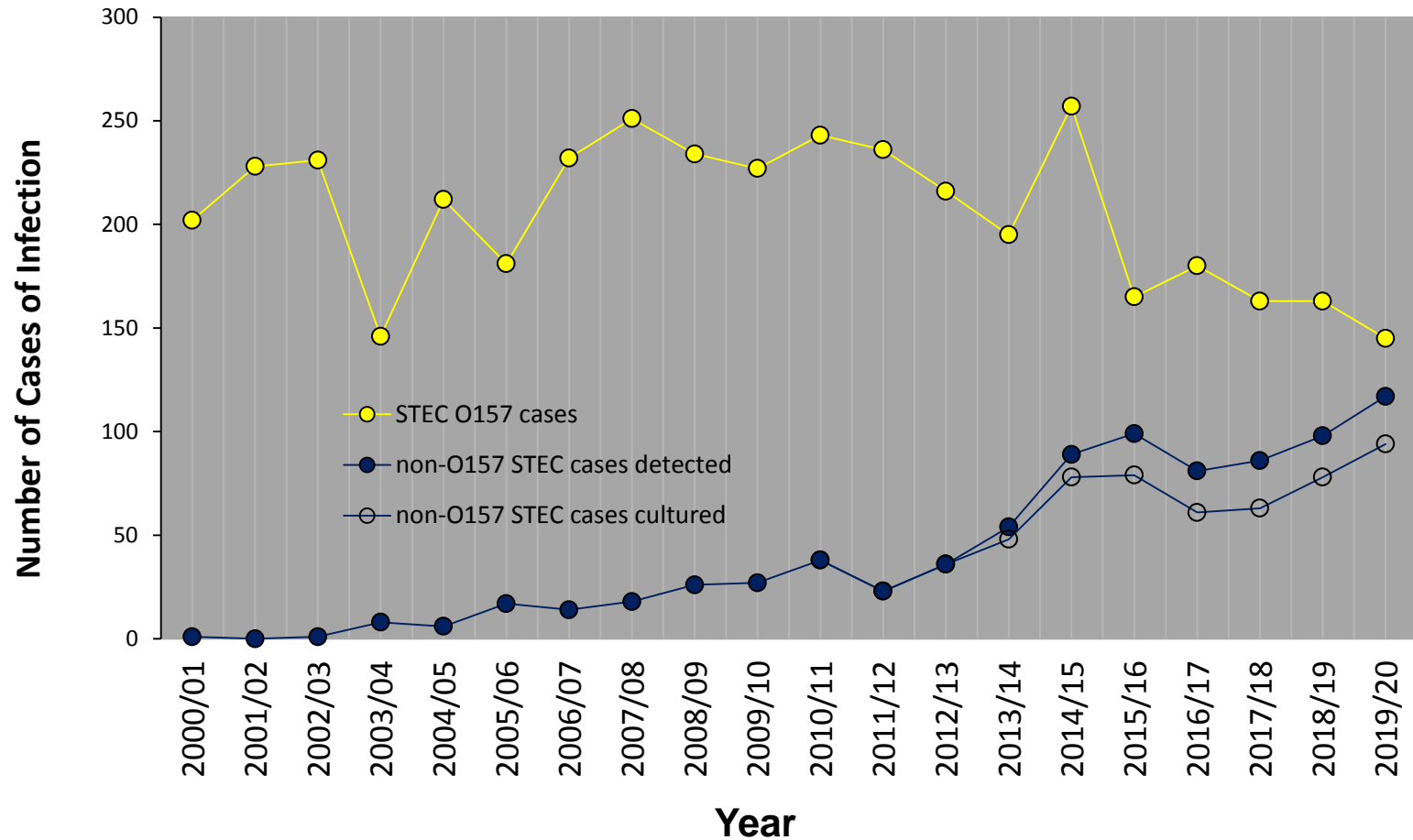
Presentation Summary

- STEC in Scotland
- Faecal screening
- Non-O157 WGS Project
– FSS/2017/027



<https://www.foodstandards.gov.scot/publications-and-research/publications/whole-genome-sequence-typing-and-analysis-of-non-o157-stec>

Prevalence of STEC in Scotland



Scottish Cases of Infection April 2000 to March 2020

Screening Faeces at SERL

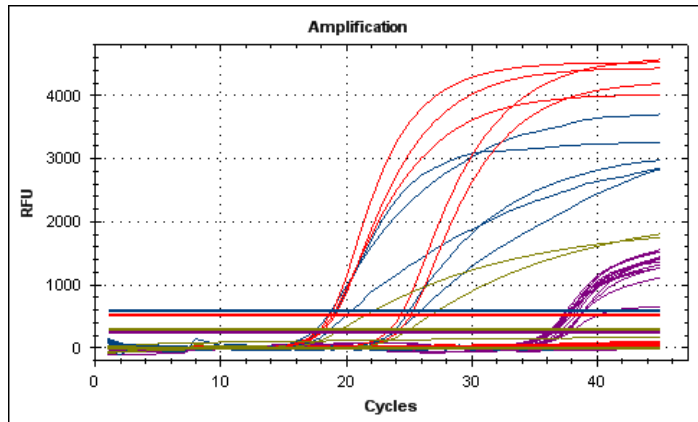
Detection

Faeces (BD/HUS)

Overnight enrichment (TSB)

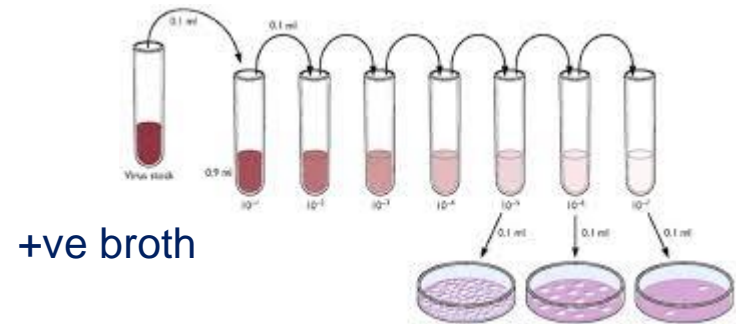
Instagene extraction

In-house PCR

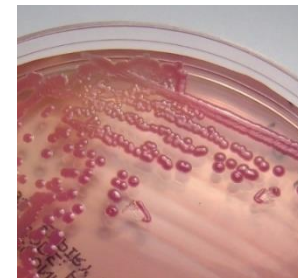


***stx*₁, *stx*₂, *stx*_{2f}, *rfb*₀₁₅₇, IAC**

Isolation



Spread plate 10⁻³ to 10⁻⁵



Individual & pooled colony picks & sweep of growth for PCR

Background to project

- Outbreak associated with raw cheese
- Non-O157 STEC isolated but NOT O157 outbreak strain
- Unable to provide context



NHS
National Services Scotland

Incident Management Team report:
Outbreak of *E. coli* O157 PT21/28
July–September 2016

Published March 2017

 Health Protection Scotland

www.hps.scot.nhs.uk

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Study Details

Aims

- Provide a highly detailed description of the non-O157 STEC causing clinical infection in Scotland over a 16 year period.
- Provide a phylogenomic analysis of the strains
- Assess the potential of each strain to cause clinical disease
- Provide a unique and comprehensive genomic database of Scottish non-O157 STEC for future interrogation.

Methods

The Study Group

525 strains from clinical cases of infection, collected over a 16y period 26/02/02 to 05/02/18

DNA Extraction, Library Preparation and Sequencing

Qiagen extraction

Nextera XT library prep

Miseq v2 500bp kits

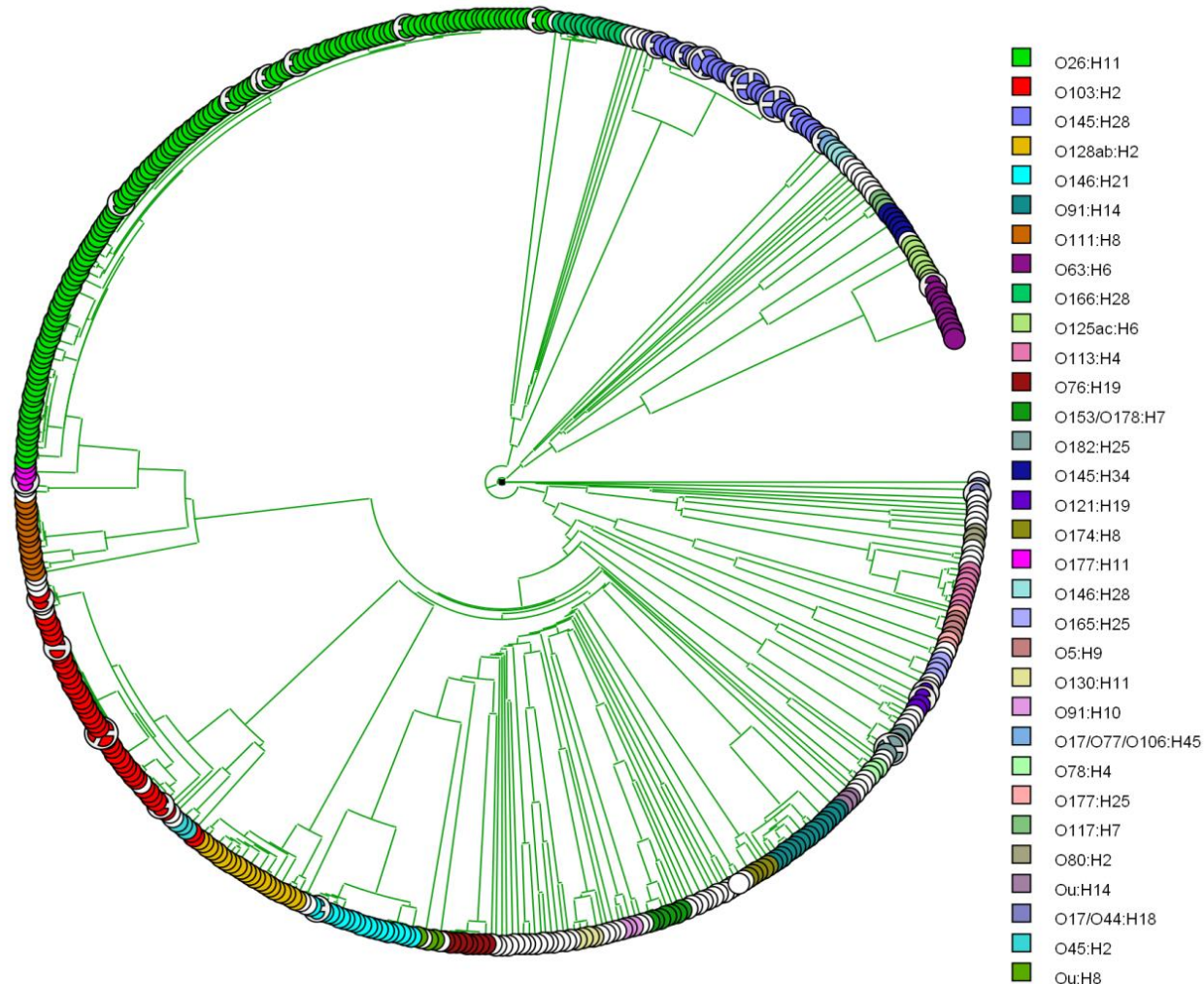
Data Analysis

PHE Bioinformatics protocol

BioNumerics v7.6 (Applied Maths)

Non-O157 Serotypes

- A total of 522 (99.4%) clinical isolates were identified as *E. coli*, while three (0.6%) were *Escherichia albertii*.
- *In silico* serotyping detected 89 different serotypes; 47 were observed on one occasion only.



Serotype & stx subtype

Top Serotypes

Serotype	No. Isolates
<i>E. coli</i> O26:H11	141
<i>E. coli</i> O103:H2	49
<i>E. coli</i> O145:H28	45
<i>E. coli</i> O128:H2	21
<i>E. coli</i> O146:H21	16

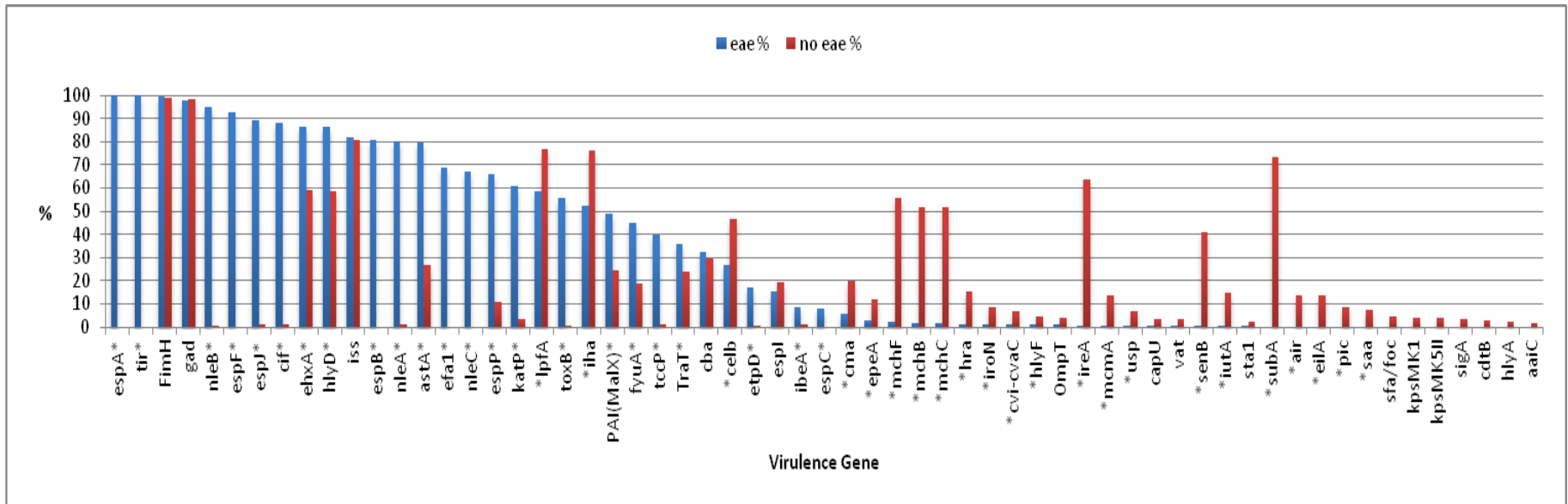
- 53% of all non-O157 STEC
- O26:H11 = 27%

Top stx subtype

stx subtype profile	No. isolates
<i>stx1a</i>	176
<i>stx2a</i>	78
<i>stx2a stx1a</i>	60
<i>stx2b stx1c</i>	46
<i>stx2f</i>	40 (8%)
<i>stx2b</i>	36
<i>stx1c</i>	30
<i>stx2b stx1a</i>	14
<i>stx2d</i>	13
<i>stx1d</i>	3

Virulence Genes

- *eae* was carried by 64.5% of strains.
- 85 other virulence genes were identified



Comparison of virulence genes in *eae*-positive and *eae*-negative STEC

- Top 3 *eae* –ve serotypes: O128:H2, O146:H21, O91:H14
- Predominant genes in *eae* –ve strains: *subA*, *ireA*, *mchF*, *B*, *C*

Potential to cause disease

Expert Meetings on Microbiological Risk Assessment (JEMRA) report, 2018.

JEMRA Level ¹	Subtype combination ²	No. of non-O157 isolates	No. of O157:H7 isolates	% non-O157 in each JEMRA level	% O157:H7 in each JEMRA level
1 (D/BD/HUS)	<i>stx2a, eae</i>	66	53	24.8	58.2
	<i>stx2a, eae</i> (with <i>stx1a</i>)	59	4		
	<i>stx2a, eae</i> (with <i>stx1a, stx2c</i>)	1	13		
	<i>stx2a, eae</i> (with <i>stx2c</i>)	0	107		
	<i>stx2a, aggR</i>	2	0		
2 (D/BD/HUS)	<i>stx2d</i>	13	0	2.7	0.0
	<i>stx2d</i> (with <i>stx2b</i>)	1	0		
3 (D/BD)	<i>stx2c, eae</i>	3	30	1.0	40.1
	<i>stx2c, eae</i> (with <i>stx1a</i>)	2	92		
4 (D/BD)	<i>stx1a, eae</i>	161	5	31.1	1.6
5 (D)	other <i>stx</i> subtypes	209	0	40.4	0.0

¹JEMRA level assignment of non-O157 STEC compared with *E. coli* O157:H7, based on virulence gene combination and potential to cause diarrhoea (D), bloody diarrhoea (BD) and haemolytic uraemic syndrome (HUS).

Conclusions



- **A highly diverse group** of non-O157 STEC are associated with human infections in Scotland, although a small number of serotypes accounted for the majority of cases of infection.
- **We did not identify some of the pathogenic strains** (such as O55:H7 and certain pathogenic O26:H11 clones) currently emerging in certain countries, although we have identified some strains that we will continue to closely monitor, including *E. coli* O145:H28.
- This comprehensive collection of non-O157 STEC sequences from a single country is **a valuable and unique resource**, and will be further enhanced by linking the data to the clinical and epidemiological information for each patient.

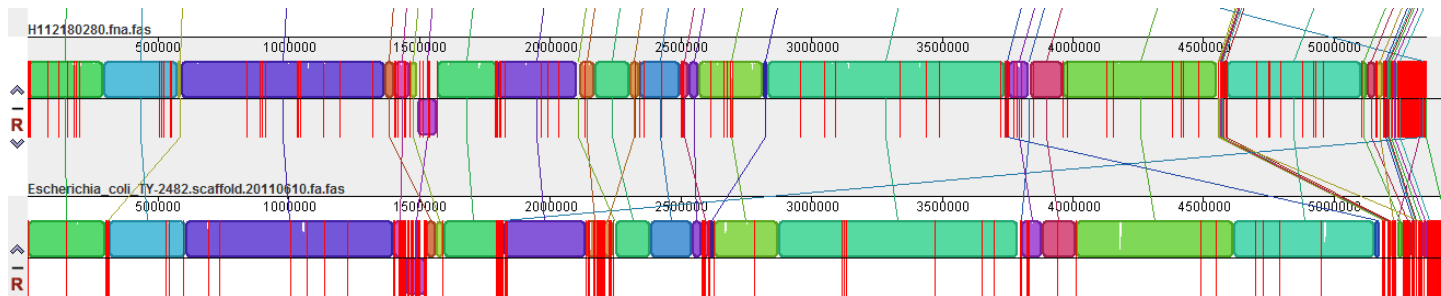
Next Steps



- The second phase of this project will compare the predicted disease potential (the JEMRA Level assigned, based on virulence gene combination) with the actual symptoms experienced by each patient, to determine whether this molecular risk assessment approach might support decisions on public health interventions for STEC infection in the future.
- Sequence data from this project is held in the SERL BioNumerics database and routinely compared with all new strains.
- Ongoing projects/collaborations:
 - Prevalence and diversity of non-O157 STEC carriage in Scottish **Cattle**
 - The Risk of STEC (Shiga Toxin-Producing *E. coli*) contamination in **Wild Venison**
 - Microbiological Survey of **Minced Beef** on Retail Sale in Scotland

Acknowledgments

- All SERL Staff
- NHS Diagnostic Laboratories
- Health Protection Scotland
- Food Standards Scotland (Marianne James)



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Standards
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healthy eating

WHOLE GENOME SEQUENCE TYPING AND
ANALYSIS OF NON-O157 STEC

May 2019



www.foodstandards.gov.scot/publications-and-research/publications/