

Comparison of *E. coli* O145 isolates from animal, food and humans with special attention to the O145 real-time PCR

9th Annual Workshop of the NRLs for *E. coli*
Rome, 21. October 2014

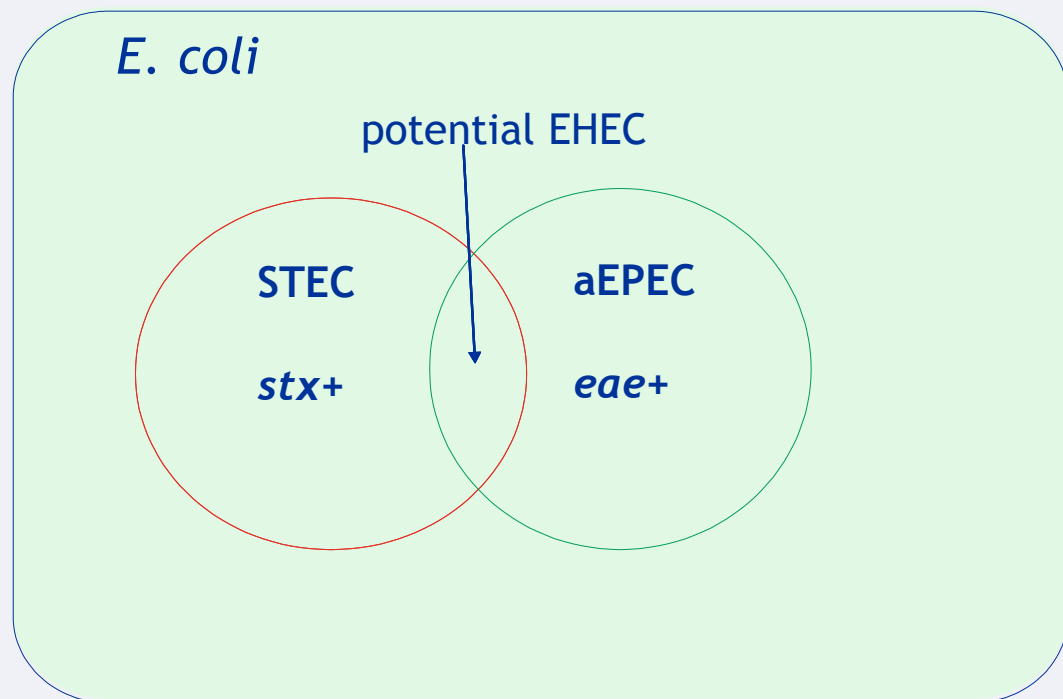
Camilla Sekse
Gro Johannessen
Anne Margrete Urdahl



Veterinærinstituttet
Norwegian Veterinary Institute

STEC and aEPEC

- STEC - Shiga toxin-producing *E. coli*
- **STEC = *stx+* *eae-/+ E. coli***
- aEPEC = atypical Enteropathogenic *E. coli*
- **aEPEC = *stx-* *eae+* *E. coli***



Introduction

- O145 one of the big-5 human pathogenic STEC serogroups
 - Many strains are characterized as O145 without known H-type
- Several outbreaks
 - STEC O145:H-, Japan 1984, 100 cases, elementary school
 - STEC O145:H28, Norway 2009, 9 cases, child day-care centre
 - STEC O145, USA 2010, 26 cases (3 HUS), romaine lettuce



Survey of *E. coli* in sheep



Photo: Anne Mette Kirkemo

- Samples submitted from 94 flocks in 2006 and from 498 flocks in 2007
- ~50 single samples per flock
- Analysed as pooled samples (10 per flock)
- Good representativity

- From this, 149 flocks analysed for *E. coli* O145
 - random sampling among the 2007-flocks
- Pre-enrichment with detection and isolation using AIMS-ELISA -specific for O145
- Conventional serotyping
- *stx* and *eae* real-time PCR



Results O145 survey in sheep

- STEC O145 not detected
- aEPEC O145 in 43/149 flocks
- Surprisingly high prevalence
 - aEPEC O145 (28.9%)

In 2013, we traced a sporadic HUS-case back to a farm (*E. coli* O145, *eae*, *stx2*, unknown H-type) - not detected by O145-ISO-primers



There was a need for identify H-type of *E. coli* O145 other than O145:H28 and to check the specificity of the O145-ISO-primers

And also to compare human and animal O145 isolates



Material for characterization and comparison

- Samples checked for O145 with two different primer sets
- Isolates
 - 78 ovine O145 *E. coli* isolates
 - Sampled 2007
 - 1 ovine O145 *E. coli* associated to a sporadic case
 - 33 human O145 *E. coli* isolates
 - obtained from domestically infected human patients 2007-2013



Analysis of *E. coli* O145

- Real-time PCR for O145
 - Both samples and isolates
- H-typing (PCR, sequencing)
- Virulence characterization
 - 21 virulence-associated genes
- Molecular typing
 - PFGE
 - MLVA - General *E. coli* - 10 loci protocol



Real-time PCR for O145

- Two O145 real-time PCR assays were tested on a selection of samples and isolates
 - O145 primers, *ihp1* (Perelle *et al.* 2004)
 - O-island 29 homologue
 - O145 primers, *wzy* (Fratamico *et al.* 2009)
 - O-antigen polymerase, part of the O-antigen cluster



Results -real time PCR on samples

- Looking for *E. coli* O145 in NRL Norway
- Samples from food and faecal samples from animals
 - Varying results
 - Majority of samples positive with Fratamico-primers



Two cases of disease from O145

Water 2011 (one case)

- Screening of enrichment broth: P+/F-
- AIMS and isolation of colonies: all colonies P-/F+
- Isolated aEPEC O145, eae+, stx-, same MLVA as patient, but still not identical, common MLVA profile

Fecal samples 2013 (one case, O145, stx2+, eae+)

- Screening of broth: 1P+/F-, 3 P+/F+, 4 P-/F+
- AIMS and isolation of colonies: all colonies (>60) P-/F+
- Isolated EHEC O145:H?, stx2+/eae+, MLVA profile same as patient



Results -real time PCR on isolates

- All isolates that were O145 by conventional serotyping were also positive using “Fratamico-primers”
 - detecting the *wzy* gene (O-gene cluster)
- 75/112 isolates were positive using “Perelle-primers”
 - detecting the *ihp1*-gene
 - 14% of the selected ovine isolates
 - 45% of the selected human isolates
 - Specific to O145:H28, regardless presence of *stx*



Results *E. coli* O145

Serotype	Human	Sheep	Total
O145:H25	8	67	75
O145:H28	15	11	26
O145:H34	5		5
O145:H40	5		5
Total	33	78	111



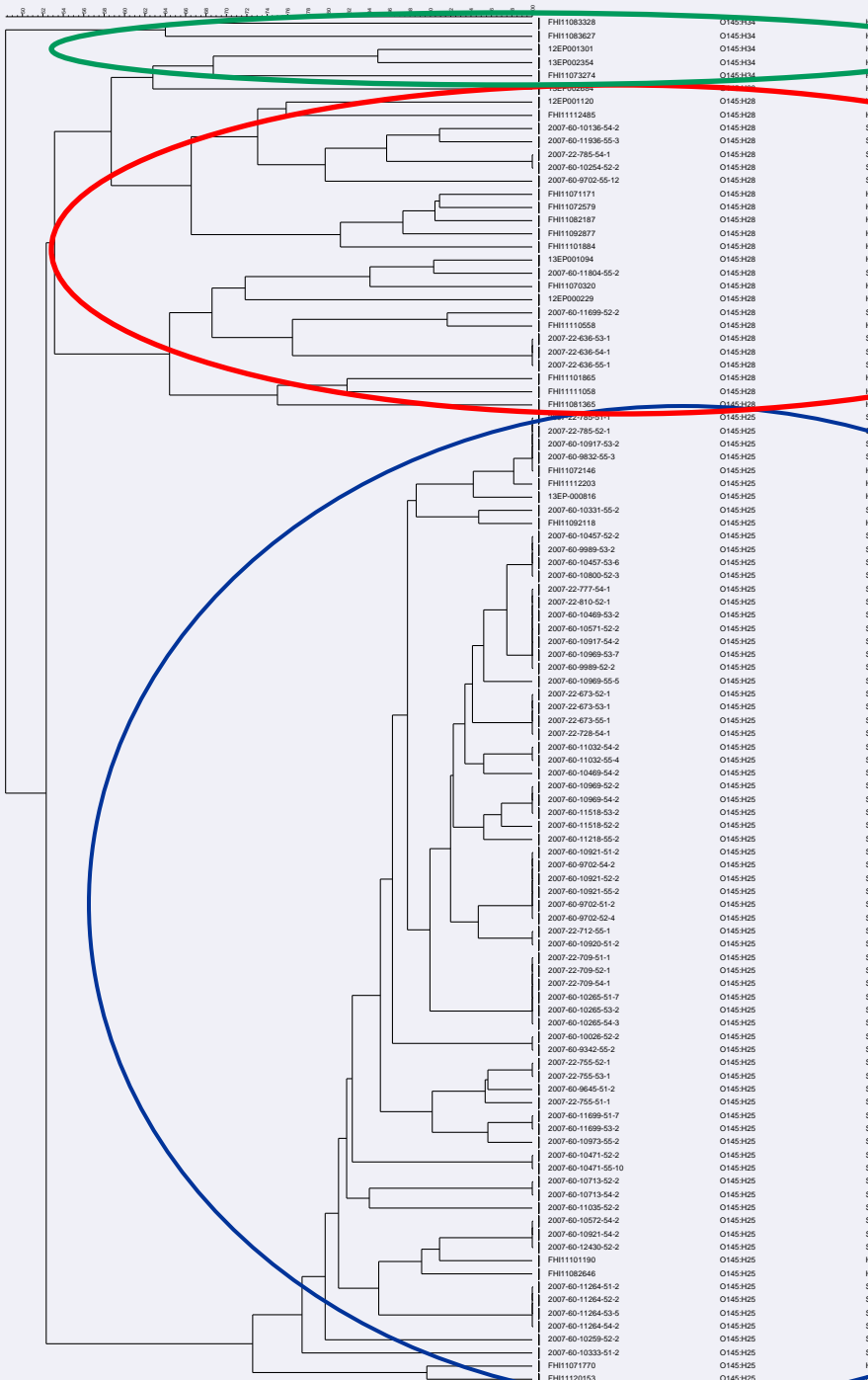
Virulence-associated genes

- *stx, eae*
 - *stx* subtyping (phage-associated) - *stx1a/stx2a* - only some human isolates
 - *eae* (LEE) - all isolates positive
- EAF-plasmid
 - *bfpA* - all isolates negative
- Virulence genes associated with other pathogenic *E. coli*
 - EAaggEC (*aggR, aaiC*)
 - ETEC (*lt, st(p), st(h)*)
 - EIEC (*ipaH*)
 - All negative





Serotype	Source	stx	Virulence profile*	MLVA profiles**
O145:H25 (75)	Sheep (67), Human (8)	stx2a (3, H)	eae ehxA nleA# nleF# nleE ent-espL2 nleB Z2098 Z2099 terB#	5_2_-2_11_4_2_5_0_3_3 (3,SS) 5_2_-2_11_4_2_5_0_3_5 (6, 1 STEC) 5_2_-2_11_4_2_5_0_3_6 (18) 5_2_-2_11_4_2_5_0_3_7 (15, S) 5_2_-2_11_4_2_5_0_3_8 (2) 5_2_-2_11_4_2_5_0_3_10 (3) 5_2_-2_11_4_2_5_0_3_11 (2, 1 STEC) 5_2_-2_11_4_2_5_0_3_12 (6) 5_2_-2_11_4_2_5_0_4_6 (2, SS) 5_2_-2_11_4_2_5_0_4_7 (1, H) 5_2_-2_11_4_2_5_0_4_9 (1, H, STEC) 5_2_-2_11_4_2_5_0_4_10 (3, S) 5_2_-2_11_4_2_5_0_5_4 (1, S) 5_2_-2_11_4_2_5_-2_3_5 (1, S) 5_2_-2_11_4_2_5_-2_3_6 (2, SS) 5_2_-2_11_4_2_5_-2_3_9 (2, S) 5_2_-2_11_4_2_5_0_3_6 (1, S) 5_2_-2_11_4_2_5_0_3_7 (2, SS) 5_2_-2_11_4_2_5_0_3_9 (1, S) 5_2_-2_-2_4_2_5_0_3_8 (2, SS)
O145:H28 (26)	Sheep (11) Human (15)	stx1a (6, H) stx2a (4, H)	eae ehxA ^α katP ^α nleA ^α nleE ent-espL2 nleB Z2098 ^α Z2099 terB	1_2_-2_11_3_5_5_1_-2_-2 (3) 1_2_-2_11_3_6_5_1_-2_-2 (5) 1_2_-2_11_3_7_5_1_-2_-2 (2, H) 1_2_-2_11_3_8_5_1_-2_-2 (1, H) 1_2_-2_11_3_9_5_1_-2_-2 (1, H) 1_-2_-2_11_3_8_5_1_-2_-2 (1, H) 7_2_-2_11_3_3_5_0_-2_-2 (1, H) 7_2_-2_11_3_3_5_1_-2_4 (1, H) 7_2_-2_11_3_3_5_1_-2_8 (4, SS) 7_2_-2_11_3_3_5_1_-2_-2 (5) 7_2_-2_11_3_3_5_2_-2_6 (1, H) 7_-2_-2_11_3_3_5_1_-2_-2 (1, H)
O145:H34 (5)	Human (5)	-	eae katP [§]	4_-2_-2_15_3_9_5_1_-2_-2 (1, H) 4_-2_-2_15_3_10_5_1_-2_-2 (1, H) 4_-2_-2_16_3_6_5_1_-2_-2 (1, H) 6_-2_-2_16_3_6_5_1_-2_-2 (1, H) 6_-2_-2_16_3_6_5_2_-2_-2 (1, H)
O145:H40 (5)	Human (5)	-	eae nleA terB	5_-2_6_11_3_5_5_0_-2_-2 (5, H)



O145:H34
 PFGE results for
 all *E. coli* O145
 O145:H28

O145:H25

5 human O145:H40
 isolates - all
 degraded



Conclusions

- Ovine *E. coli* O145 were of same serotype as most of the human O145 isolates : O145:H25 and O145:H28
- Human and ovine isolates were similar with respect to serotype, virulence gene profile and molecular genotype
- All ovine O145 isolates were lacking the *stx* genes, however, they may be considered potentially human pathogenic
 - either as a cause of diarrhoea by itself or as precursors for STEC



Conclusions

At the NRL, Norway we will use the Fratamico-primers instead of the ISO-primers for the future

