

# A large outbreak of sorbitol-fermenting VTEC O157 associated with unpasteurized milk and contact with cattle in Finland



**Ruska Rimhanen-Finne ,THL  
Saara Salmenlinna, THL,  
Hanna Lundström, City of Turku  
Anniina Jaakkonen, Evira,  
Sirpa Heinikainen, Evira  
Miia Kauremaa, Evira  
Annika Pihlajasaari, Evira  
Saija Hallanvuo, Evira**

# Start of the outbreak

- First case 19.6.2012 (confirmed 20.6.2012), child (4 years) infected with VTEC O157:H7, sorbitol positive, stx2, hlyA and eae positive, FT 88
- The patient had consumed unpasteurized milk originating from a farm near Turku
  - City's visiting farm offers also theme visits like "birthday in the countryside", "day in grandmother's place " > especially for children
  - Historic travel sight with 1950's methods in keeping of cattle

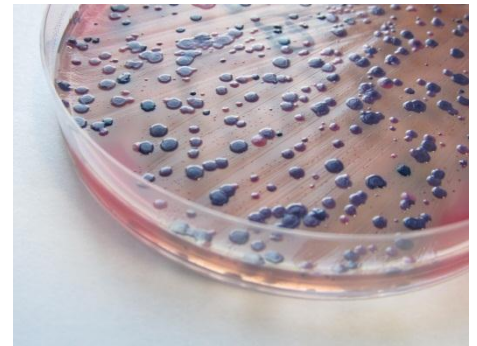
# General view of the outbreak

- Altogether 6 symptomatic children + 2 asymptomatic adults with EHEC
  - All were microbiologically confirmed with identical strain
- Web-based questionnaire was launched for persons attending the farm / regular milk customers during June
- 146 answered, 5 had lab-confirmed EHEC-infection
- All lab-confirmed cases had consumed unpasteurized milk produced by the farm
- Drinking unpasteurized milk from the farm was statistically associated with the infection (RR=6.3, 95%CI 2.1-18.8, p=0.0003)

# Investigations on the farm

Sampling for VTEC conducted 25.6.2012 on farm

- 16 feces samples from cattle > 4 positives (24%)
- 1 feces sample from lambs > negative
- 23 environmental samples > 7 positives (30%)
- 8 milk samples (from different days) > 4 positives
- Positive strains = stx2 and eae positive in real-time PCR
  - Strains were VTEC O157, sorbitol, stx2, and eae positive like patient strains
  - Strains were of subtype stx2a



# Investigations on the farm

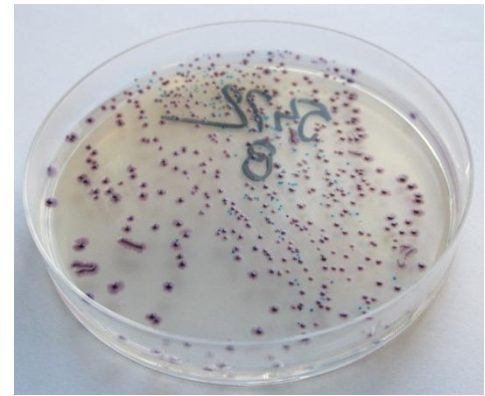
Sampling for *Campylobacter* and *Salmonella* conducted also on 25.6.2012

- Samples were negative for *Salmonella*
  
- Results for *Campylobacter* (NMKL 119: 2007 modified)
  - 15 feces samples from cattle > 4 positives (27%)
  - 1 feces sample from lambs > negative
  - 8 milk samples (from different days) > negative
  
- All positive samples were confirmed to *Campylobacter jejuni*
- 2 of the 4 positive animals had both VTEC and *Campylobacter* isolated

# Laboratory studies

## Faecal and environmental samples >

- Culture method ISO 16654:2001, modified
  - mTSB (20mg/ml novobiocin) at 41,5 °C
  - Enrichment 6h and 24h
  - Plating on Harlequin SMAC-BCIG, CHROMagar STEC (and CT-SMAC)
  - Isolated strains confirmed by stx1, stx2, eae PCR and PFGE



## Milk samples >

- Real-time PCR prCEN ISO/TS 13136
- Enrichment in mTSB (16 mg/l) and BPW at 37 °C
- stx1, stx2, eae
- IMS for O157
- Plating on Harlequin SMAC-BCIG, CHROMagar STEC (and CT-SMAC)
- Isolated strains confirmed by stx1, stx2, eae PCR and PFGE

# Laboratory studies

## Observations >

- Discrepancy between IMS isolation and PCR in ISO/TS 13136
  - Conducted at the same time for milk samples
  - 4 samples were positive in IMS (strains O157, sorbitol, stx2, and eae positive)
  - Only 1 sample was positive in real-time PCR
    - Internal amplification control positive in negative samples (no inhibition of PCR)

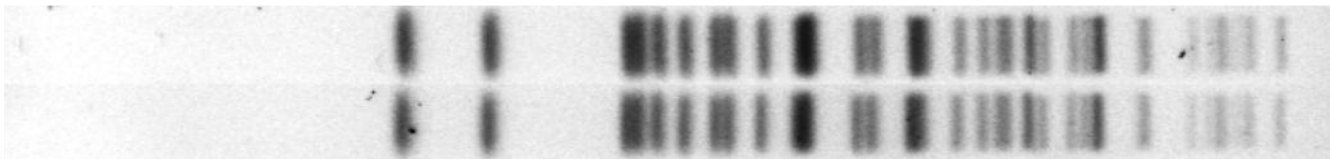
> In this case, following the protocol would have led to 3 false negative results in milk samples

## Further typing by PFGE (pulsed-field gel electrophoresis)

- 34 VTEC O157 strains (originating from 15 samples) assigned to specific types
- Identical patterns for patient strain and strains from environment (feed table) and cattle faeces
- Patient pattern 1.192 found previously in humans (Rovaniemi 2011, Ristiina 2012)
- Altogether 5 new types revealed

### PFGE-XbaI

---



Patient  
Farm, feed table



# Distribution of PFGE types

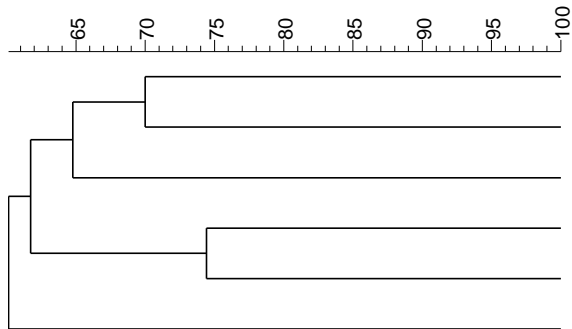
## By sample type

1.192	1.144	1.143	1.72	1.46	1.145
<ul style="list-style-type: none"> <li>• Patient</li> <li>• Cattle faeces</li> <li>• Farm (inside), feed table</li> <li>• <b>Farm (inside), water trough</b></li> <li>• Farm (outside), water trough 1, barn wall</li> <li>• Farm (outside), cattle shed</li> <li>• Milk room, floor</li> </ul>	<ul style="list-style-type: none"> <li>• Unpasteurized milk 6.5.</li> <li>• Unpasteurized milk 20.6.</li> <li>• <b>Unpasteurized milk 23.6.</b></li> <li>• <b>Unpasteurized milk 24.6.</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>Farm (inside), water trough</b></li> <li>• <b>Unpasteurized milk 24.6.</b></li> </ul>	<ul style="list-style-type: none"> <li>• <b>Unpasteurized milk 23.6.</b></li> </ul>	<ul style="list-style-type: none"> <li>• Cattle faeces</li> </ul>	<ul style="list-style-type: none"> <li>• Cattle faeces</li> </ul>

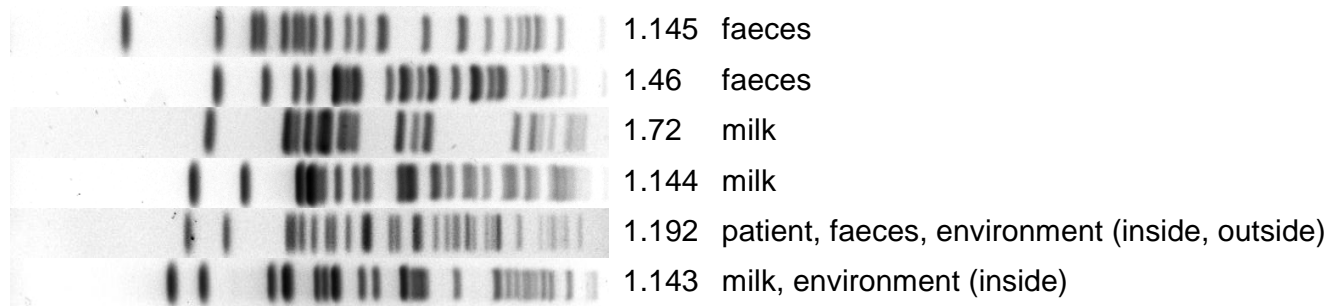
# Distribution of PFGE types

Heterogeneous types on the farm....

PFGE-XbaI



PFGE-XbaI



# Risk management actions

- Selling of unpasteurized milk directly to customers was forbidden immediately 19.6.2012
  - Visitors' contacts to animals was stopped
  - Regular milk customers were informed
  - Hand washing was instructed
- Farm was closed from public 28.6.2012
- Risk management plan and realization of actions in the farm
  - Realization was followed with several inspections
  - VTEC status was followed by resampling (18.9.2012)

# Resampling on the farm

Results for VTEC (ISO 16654:2001, modified and ISO/TS 13136)

- 13 feces samples from cattle > all negative for VTEC
- 6 feces samples from lambs > 2 positives, this time with stx1 and stx2 positive VTEC
- 33 environmental samples > all negative
- 8 milk samples (from different days) > all negative

Results for *Campylobacter* (NMKL 119: 2007 modified)

- All retested *C. jejuni* positive animals remained positive
- 4 feces sample from lambs > 1 positive for *C. jejuni*
- 1 environmental sample > negative
- 8 milk samples (from different days) > negative

Thank you for your attention!

