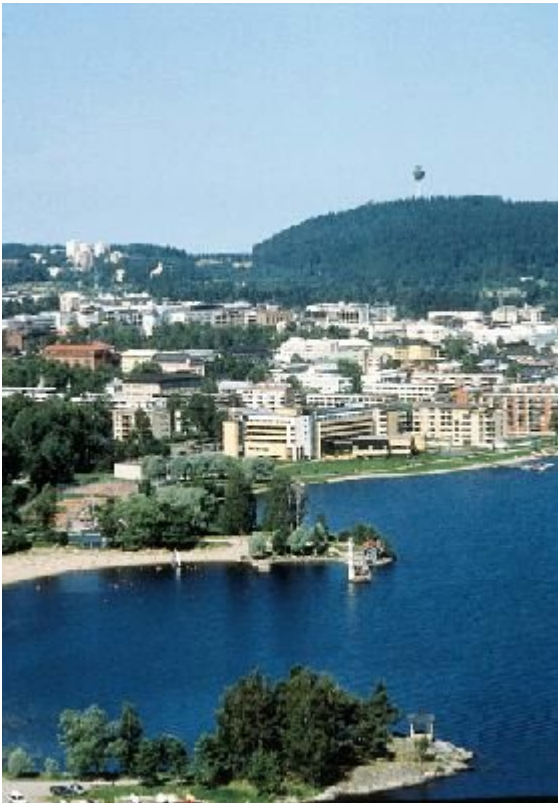


Surveillance of VTEC O157 in slaughter houses

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Compulsory control program for VTEC O157 in bovine slaughter houses

- started in 2004
- sampling based on expectancy rate of 1 %, accuracy 5 % and CI 95 %
- sampling is divided between bovine slaughterhouses depending on their capacity
- approximately 1500 animals sampled each year
- faecal samples, samples are collected by industry and analyzed in approved laboratories



Results from faecal sampling

Year	Samples	Positive	Prevalence	Farms
2004	1599	20	1,25 %	15
2005	1560	8	0,51 %	5
2006	1586	10	0,63 %	9
	4745	38	0,80 %	

Actions at a VTEC O157 positive farm

- Owner has to inform dairy and slaughter house
- Sampling at farm obligatory (faecal and environmental samples)
 - municipal veterinary officer + veterinarian from Association for Animal Disease Prevention takes samples
 - costs covered by state
- Risk management plan
 - Good hygiene: prevent feed contamination with faeces, disinfection of drinking cups and feed alleys
 - Only clean animals for slaughter (approved by the municipal veterinarian)
 - Animals from positive farm are slaughtered at the end of day
- Re-sampling and follow-up of the risk management plan 2-3 months after the first sampling



Verocytotoxigenic *Escherichia coli* in ground beef in Finland

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Background: VTEC in bovines in Finland



Slaughter house survey 2003:

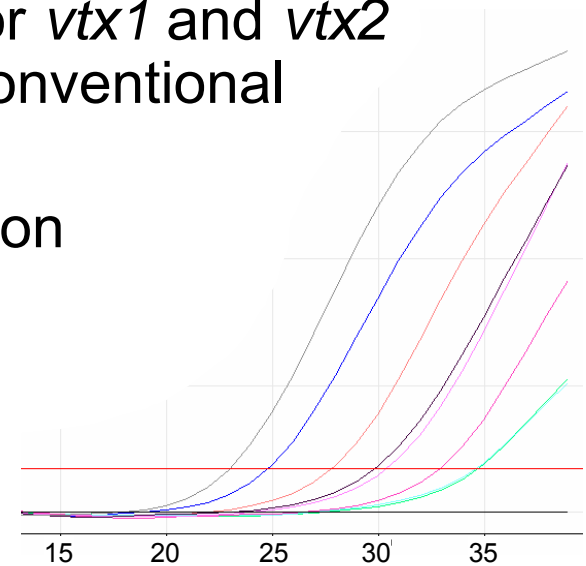
- 533 faecal and carcass swab samples collected in January, May, August and October
- non-O157 VTEC prevalence 30,8 % in faeces (seasonal variation 16,1 – 45,2 %) and 10,7 % (seasonal variation 5,6 – 15,4 %) in carcass swabs (method PCR)
- 129 non-O157 isolates obtained (11,6 % *stx1*, 72,1 % *stx2* and 16,3 % *stx1/2*)
 - 7 (5,4 %) *eae* positive, all *stx1*

VTEC in ground beef

- 402 ground beef samples bought from supermarkets in Kuopio August-October 2006
- samples from five different slaughter house companies, one organic meat producing company and 41 samples of meat ground in the supermarkets
- 90 % of samples were packed in protective gas
- 30 % of the samples had fat content ≤ 10 % and 70 % had fat ≥ 15 %
- the analysis was started as close to the last recommended day of usage as possible

VTEC in ground beef, methods

- 25 g of ground beef was enriched in 225 ml modified tryptone soy broth with 20 mg/l novobiocin overnight at $41,5 \pm 0,5$ °C
- DNA was isolated from 1 ml of the enrichment broth with QIAamp DNA Mini Kit (Qiagen)
- VTEC was detected by real-time PCR for *vtx1* and *vtx2* (Perelle et al. 2004) and confirmed by conventional PCR (Paton and Paton 2002)
- VTEC was isolated by colony hybridization
- Isolates were characterized for their serotype, virulence genes, *vtx* subtypes, VT production and phylogeny group



Results

- real-time PCR: 37/402 (9,2 %) of samples *vtx* positive (conventional PCR: 7,2 %)
- 34 (8,5 %) *vtx2* positive, 2 (0,5 %) *vtx1* and one sample (0,25 %) *vtx1/2* positive
- low fat content: 5/120 samples positive (4,2 %)
- high fat content: 32/278 samples positive (11,5 %)
- no significant difference ($p=0.123$)
- there were significant ($p < 0,001$) differences in *vtx* positivity between different slaughter house companies

Company	Number of samples	Number of positive samples (%)
A	153	26 (17)
B	80	1 (1,3)
C	42	2 (4,8)
D	35	0 (0)
E	25	4 (16)
Organic	26	1 (3,8)
Markets	41	3 (7,3)

Results cont.

- colony hybridization yielded *vtx* + isolates from 14/37 samples
- 12 isolates *vtx2*, two isolates *vtx2*, *ehxA*, *saa*
- all *eae* negative
- serogroups: O8:H8, O8:H9, O22:H8 (two isolates), O113:HNT (4 isolates), O113:H21, ONT:H8, ONT:H9 (two isolates), ONT:H21
- *vtx2* subtypes:
 - *vtx2*-NV206 5 isolates (all O113)
 - *vtx2c* 3 isolates
 - *vtx2d2* 2 isolates (O22)
 - *vtx2*, *vtx2d1*, *vtx2c* + ? and *vtx2e* each one isolate

Conclusions

- during August – October cattle has the highest excretion rate of VTEC; of the fresh retail ground beef analyzed at the same time 9,2 % was *vtx* positive (Hussein et al. 2007: 2,4 – 30 % of ground beef *vtx* positive in different countries)
- in most positive samples the contamination level is probably low
- *vtx2/vtx2c*, *eae* genotype is strongly associated with HUS and HC in many countries (Eklund et al. 2002, Persson et al. 2007) – strains of this type were not detected
- 6,5 % of VTEC strains isolated from German patients had the *vtx2d* activatable, *eae* negative genotype (Bielaszewska et al. 2006) – 8 isolates from ground beef had this genotype
- on the other hand, 5 of these strains are phylogeny group A (and some of them are low toxin-producers), which may indicate that they belong to non-virulent animal associated VTEC population (Girardeau et al. 2005)

Thank you for your attention !

