

## 11. Verotoxigenic *Escherichia coli*

### 11.1. Verotoxigenic *Escherichia coli* in animals and products thereof

#### Monitoring strategies

Usually, findings of verotoxigenic (VT) *Escherichia* (*E.*) or VT *E. coli* O157 are not notifiable. However, in Sweden any case of VT *E. coli* O157 in animals with connection to a human case of enterohaemorrhagic disease is notifiable. Furthermore, if livestock contacts are reported in a human case of *E. coli* O157 infection, the animals are investigated by bacteriological sampling. In Norway, findings of *E. coli* O157 in an official survey or in the official surveillance carcass programme are notifiable. Restrictions may be posed on holdings where VTEC O157 is detected.

As in previous years, several Member States were searching for VT *E. coli* in cattle and beef. The purpose of most studies was to estimate the prevalence of *E. coli* O157 (Table EC 1). Samples were either taken at the farm or at slaughterhouse. Some surveillance programmes in food, covering verotoxigenic *E. coli* or *E. coli* O157 as well, are implemented at national, regional or local level (Table EC 2). In addition, results of diagnostic investigations were reported.

Targeted sampling was done as follow-up to infections in man with *E. coli* O157 or to previous findings in animals or meat/food in Belgium, Finland, Sweden, Norway, and Great Britain.

#### Methods applied

The detection of *E. coli* O157 is usually made by a selective culturing method using enrichment and immunomagnetic separation and plating on selective media. The isolates are confirmed by latex agglutination and biochemistry. In several countries, the presence of virulence genes including shiga-toxin genes and *eae* A genes is tested by PCR. In Belgium and Germany, all clinical isolates sent to the reference laboratory are examined routinely for the toxin and virulence genes. In Finland, the ISO 16654:2001 method is applied with some modifications. In Sweden, NMKL 164 is used. In addition, certain isolates are subtyped by use of Pulse Field Gel Electrophoresis (PFGE).

#### Control measures

In Belgium, in case of a positive finding in a dairy herd, selling of milk or milk products that were not heat-treated is forbidden.

In Sweden, there are guidelines and recommendations established of how to handle VTEC O157 in cattle when associations have been made with human disease. For example, these measures may include that animals should be tested negative prior to transport and slaughter and that hygiene recommendations should be instituted at the farm.

In Norway, in 2002, more stringent restrictions were imposed upon a farm when a widespread occurrence of VTEC O157 was revealed. Advice regarding human health aspects is provided to the farmers. Herds found positive for O157 are tested four times the following year or until two testings are negative.

As regards foods, the measures taken are stricter. In Norway, a carcass that tests positive for VTEC O157 will be condemned. Similarly, in Sweden, carcasses positive for VTEC O157 will be destined for heat-treated products. The abattoirs should be thoroughly cleaned and disinfected after such slaughter.

**Table EC 1. Studies and monitoring activities on verotoxigenic *E. coli* in animals, 2003**

Method / Agent	Time and place of investigation	Specimen collected	Sample size / frequency
B / VT <i>E. coli</i>	Tracing back from - positive cases in slaughterhouse sampling - human outbreaks that can be traced to a farm	Faecal samples	20 % of animals between 6 month and 2 years
DK B / <i>E. coli</i> O157 B / VT <i>E. coli</i>	Survey at slaughterhouse Survey at slaughterhouse	Faecal sample Faecal sample	One animal (calf) per herd, monthly sampling (DANMAP) Study primarily in calves and dairy cows on the presence of the serotypes, O26, O103, O111, O145, O157
FIN B / stx 1 and stx 2 pos <i>E. coli</i> O157	Survey at slaughterhouse	Faecal samples	1490 animals sampled evenly all the year round; 1 sample per animal
NL	KvW-RIVM surveillance project - first quarter of 2003	Cattle, veal calves	Pooled samples of about 160 herds (cattle) and 150 herds (veal)
S B / <i>E. coli</i> O157	At large scaled-slaughterhouse Voluntary follow-up investigations of human cases at slaughterhouses	Cattle carcass swabs Cattle	500-900 / annually (since 1998) – voluntary Animals from infected herds
N B	Survey in dairy cattle herds	Faecal	1233 animals representing 137 dairy farms were tested for the presence of VTEC (serogroup O26, O103, O139, O145 and O157)
N B	Follow-up testing in herds with positive carcasses in the surveillance programme		
UK B / <i>E. coli</i> O157	Follow up investigations of human cases Survey at slaughterhouse	Faecal samples from cattle, pigs, sheep	Random sampling proportional to abattoir throughput put. Maximum of four samples collected on any one occasion
B / <i>E. coli</i> O157	Bacteriological testing specific for <i>E. coli</i> O157		
B / VT <i>E. coli</i>	Bacteriological testing covering all verotoxigenic <i>E. coli</i>		
B	Bacteriological testing		

**Table EC 2. Verotoxigenic *E. coli* monitoring programmes in food, 2003**

Method	Time and place of investigation	Specimen collected	Sample size / frequency
B B / <i>E. coli</i> O157	Screening at slaughterhouse, cutting plants, retail trades	Beef and veal carcasses	Swabs (1600 cm <sup>2</sup> ) for beef and veal carcasses 25 g of cutting meat and minced meat of beef
DK B / VT <i>E. coli</i>	Survey at slaughterhouse	Fresh meat cuts	Study on the presence of the serotypes O157, O26, O103; O111, O145
FIN B / stx 1 and stx 2 pos <i>E. coli</i> O157	Survey at cutting plants Survey at slaughterhouse	Crushed meat from the cutting tables or the conveyor belts Carcasses of cattle	315 samples were taken in 9 cutting plants located in different areas of Finland 2 sponge samples from the surface of randomly chosen carcasses
N B / <i>E. coli</i> O157	Official surveillance programme	Cattle carcasses Sheep and goat carcasses Abdominal muscle and skin from an area close to the abdominal incision	Systematic sampling: – every 150 cattle carcass – every 150 goat carcass – every 1000 sheep/lamb carcass
NL B / <i>E. coli</i> O157	Food survey KvW at retail	Beef and pork	About 450 (beef) and 89 (pork) samples / year respectively
B	Bacteriological testing		
B / <i>E. coli</i> O157	Bacteriological testing specific for <i>E. coli</i> O157		

## Recent situation

### Cattle and beef

Verotoxigenic *E. coli* O157 has been detected in cattle in several countries at varying rates. Data available on animals are summarised in Tables AN – 11.1.1 to AN - 11.1.4 in the Annex. Information on examinations performed in food is given in Tables AN - 11.2.1 to AN - 11.2.7.

As surveillance activities are not harmonised figures given below are not directly comparable between the countries.

In Denmark, faecal samples from slaughtered calves were monitored for VT *E. coli* O157 and 7,1 % of the samples were found positive in 2003. In addition, in a study of the occurrence of five serogroups of *E. coli*, a total of 58 isolates were verified as belonging to serogroups O26 (14), O103 (4), O111 (0), O145 (5) and O157 (35). Three (0,4 %) O26 isolates and 23 (3,1 %) O157 isolates were identified as verocytotoxin producers.

In a survey, run in Finland, 0,4 % of the 1490 faecal samples were positive for VT *E.coli* O157. All the isolates carried the stx 2 gene. The occurrence of *E.coli* O157 in cattle in Finland was in 2003 lower than in 1997. This may indicate variation between different years. However, the same decreasing trend can be seen in human cases. In Finland VTEC O157 was isolated from one cattle carcass. Both, stx 1 and stx 2 were detected in the isolate.

In the British abattoir survey, the prevalence of VTEC O157 carriage in animals at slaughter was 4,7 % for cattle. Within investigations on the origin of outbreaks of VTEC O157 in people, the same types of VTEC O157 were isolated from a dairy farm and an open farm and VTEC O157 was confirmed in a beef farm. No VTEC O157 was isolated from the nature reserve, which had been associated with an outbreak involving 18 people.

In Sweden, three out of six cattle farms were found to be the source of human infection. Some patients had fallen ill after having consumed un-pasteurized milk from two of the three farms.

In The Netherlands, the prevalence of *E. coli* O157 in cattle was gradually increasing up to 2002. In investigations within the first quarter of 2003 the prevalence was already high for the time of the year, rating at 15,4 % positive calves. Most of the isolates contained one or both of the stx genes. Thus, the majority of isolates were potentially pathogenic to humans. For the first time since 1989, VTEC O157 was isolated from beef at retail within the surveillance programme.

In a survey in Norway for the presence of VTEC O26, O103, O139, O145 and O157 in dairy farms, in 126 (92 %) herds VTEC O103 was detected, while VTEC O157 was detected in one herd only (0,7 %). The overall animal prevalence of VTEC O157 was 0,2 % in this survey. In follow-up investigations of 8 farms, none of the samples was found positive in Norway. In the surveillance programme, one (0,04 %) of 2275 carcass swabs were found positive for VTEC O157:H7 (stx 1 and stx 2).

In Germany, in 17,3 % of the cattle VTEC was reported in 2003 compared to 12,2 % in 2002. In 2002, one isolate of VT *E. coli* O157 was reported. In beef, VT *E. coli* was isolated from 6 out of 197 samples tested, but none of the isolates were VTEC O157.

In Belgium, within the national random survey, VTEC O157 was isolated from 0,7 % of beef carcasses at slaughterhouse and cutting meat at processing plants. At retail, 2,4 % of the minced beef samples were positive for VT *E. coli* O157.

In Ireland, 600 samples were taken as part of a once-off localized survey to determine the prevalence of *E. coli* O26 and *E. coli* O111 in minced beef. One sample positive for *E.coli* O126 was identified.

### Other animals

VT *E. coli* have been detected in various animal species at considerable levels. In the British abattoir survey, the prevalence of VT *E. coli* O157 carriage in animals at slaughter was 0,7 % for sheep and 0,3 % for pigs. In Italy, VT *E. coli* O157 has been detected in 29 (19 %) of 150 buffaloes tested.

In the Norwegian surveillance programme, one (0,08 %) sheep carcass and no goat carcass were positive for VT *E. coli* O157.

Verotoxigenic *E. coli* O157 had been detected in pigs in the Netherlands and Portugal in 2002. In 2001, findings in sheep and goats had been described.

### Other foodstuffs

Besides beef, several other types of foodstuff were tested for Verotoxigenic *E. coli*. Most of the samples were negative. As in previous years, a few VT *E. coli* O157 were isolated from pork, mutton, prepared meat meals and milk products. In 2002, in addition findings in poultry meat and once from a fish product were reported.

### New Member States

Cyprus and Lithuania reported on investigations for VTEC in animals and foods. In the New Member States, no isolates of VT *E. coli* O157 were reported.

## 11.2. Verotoxigenic *E. coli* in humans

### Source of information

The surveillance systems in the individual countries are different. Usually reporting systems concentrate on cases caused by *E. coli* O157. Hemolytic uremic syndrome (HUS) is not always separately notifiable.

In Sweden, only cases caused by O157 are notifiable. Similarly, the voluntary laboratory reporting in Great Britain involves only *E. coli* O157. In Denmark and Norway, all VT *E.coli* cases are registered.

### Recent trends

Reports on infections caused by VTEC in humans were submitted by 14 countries (13 MS and Norway). No information is available from Luxembourg and Spain. Altogether, 2607 laboratory confirmed cases of verotoxigenic *E. coli* infections or HUS were reported in 2003 compared with 2664 cases in 2002. Out of the total number of reported cases, 1216 were reported in Germany. This might reflect large differences in surveillance in the individual countries. In Germany, all cases confirmed by clinical laboratory diagnosis or on clinical-epidemiological grounds, or with a clinical picture of an enteropathic haemolytic-uraemic syndrome are notifiable. A total of 203 laboratory confirmed HUS cases were reported in 13 countries (Table EC 3) compared with 238 cases in 2002. Out of these, 156 cases were caused by VT *E.coli* O157, and 45 cases by other serotypes. For the remaining cases no information on the serotype was available.

Compared to the previous years, the number of reported cases has slightly decreased for the first time.

Within the clinical cases of VT *E.coli* infection without HUS, about two third were caused by *E. coli* O157. Usually only this agent is looked for and has to be reported. Thus, there might be significant underreporting of non-O157 strains. Nevertheless figures show the relevance of NON-O157 *E. coli* strains as they have caused bloody diarrhoea or HUS at remarkable frequencies.

In Germany, altogether 78 clusters of infection with enterohaemorrhagic *Escherichia coli* (EHEC) involving 204 cases were reported. 18 cases with HUS symptoms were involved. During summer month, clinical infections including HUS were more frequently detected. Altogether, 3 death were registered, two in small children and one in an elderly.

In Sweden, a total of 73 cases were reported either by physicians or by the laboratory. 108 (87 %) of the cases reported by physicians were of domestic origin. This is a great reduction of the number (about half) in comparison to the year before, which can be explained by the absence of outbreaks. 16 (23 %) persons were infected abroad. There were seven cases of HUS reported, of which all except one were reported in children below 15 years of age. Of the HUS cases, two were infected abroad. VTEC O157 caused six of the HUS cases.

In Denmark, in 2003, 128 episodes of VTEC infections were. Of these, 27 cases (22 %) were O157. This represents an overall decrease in the number of diagnosed episodes of 11 % compared to 2002 and it is the first time since the registration of cases began in 1997 that the number has not increased. In 2003, three cases of HUS were reported, none of them fatal. From these cases VT *E. coli* strains of serotype O157:H7 and O157:H+ were isolated. All strains were vtx1 negative and vtx2 positive.

In Belgium, 41 typical EHEC isolates were characterised. The majority of the EHEC isolates belonged to serogroup O157. Eight typical EHEC isolates belonging to serotype O157:H7 were from children between 10 month and 11 years old suffering from HUS.

In Finland, 15 clinical cases were reported, one of them was HUS, 6 cases were caused by *E.coli* O157:H7 and 9 by non-O157 VTEC strains.

In Norway, a total of 17 cases of VT *E. coli* infection in humans were reported in 2003, which is comparable to the 16 cases in 2002. Thirteen cases were due to VT *E. coli* O157 and seven of the 13 cases were indigenous. HUS was reported for one imported O157 case and the O103 case.

In The Netherlands, again the number of cases of VT *E.coli* O157 reported in 2003 is higher compared to previous years. In 2003, 58 cases of VT *E.coli* O157 were diagnosed and 52 % of these patients were hospitalised. 13 % of the patients developed HUS.

For one patient PFGE-fingerprinting showed the same pattern as that found in cattle in the neighbourhood. In general cluster-analysis of PFGE DNA-finger printings showed quite a few clusters suggesting that the higher incidence in 2003 is related to a number of small epidemics from divers sources. This could be proved only once. This epidemic was also the first time that contaminated beef could be proved to have been responsible for a series of patients.

In England and Wales, the total number of recorded cases was 663. This is a small increase compared to the 595 cases recorded in 2002, but less than the 751 cases recorded in 2001. In 2003, there were 12 laboratory confirmed cases of HUS.

There were 53 reports of *E. coli* O 157 in Northern Ireland in 2003, compared with 28 reports in 2002 and 52 reports in 2001. There was one O157 outbreak reported in 2003.

In Scotland, there were 148 culture positive cases recorded in 2003 compared with 229 cases in 2002 and 236 cases in 2001. This is a reduction from 2002. Scotland continues to have a rate of infection much higher that other parts of the United Kingdom. The reasons for this remained unclear.

The age distribution for the VT *E.coli* infections and HUS cases is depicted in Figure EC1.

**Table EC 3. Human cases of verotoxigenic *E. coli* 2003**

Country	HUS Cases				Clinical cases (non HUS)				All cases			
	Clin	Conf.	O157	Non-O157	Clin	Conf.	O157	Non-O157	Clin	Conf.	O157	Non-O157
Austria	16	11	10	1	28	28	18	10	44	39	28	11
Belgium	-	8	7	1	-	39	14	25	-	47	21	26
Denmark	3	3	3	0	-	128	27	101	3	131	30	96
Finland	-	1	0	1	-	14	6	8	-	15	6	9
France	96	51	42	18	-	-	-	-	96	51	42	18
Germany <sup>3</sup>	81	61	44	6	1135	1100	119	430	1216	1161	163	436
Ireland <sup>1</sup>	7	5	4	1	-	90	84	6	-	95	88	7
Italy	26	13	1	12	5	5	2	3	31	18	3	15
Luxembourg	-	-	-	-	-	-	-	-	-	-	-	-
Portugal	-	3	0	3	-	9	0	9	-	12	0	12
Spain (SIM)	-	-	-	-	-	-	-	-	-	-	-	-
Sweden <sup>2</sup>	7	6	6	-	63	52	-	-	70	58	6 <sup>2</sup>	-
The Netherlands	7	7	7	-	51	51	50	-	58	58	57	-
Scotland	21	20	19	1	170	154	146	8	191	174	165	9
Northern Ireland	-	-	-	-	-	51	-	-	-	51	-	-
England and Wales	-	12	12	-	-	665	663	2	-	677	675	2
Norway	2	2	1	1	11	15	12	3	13	17	13	4

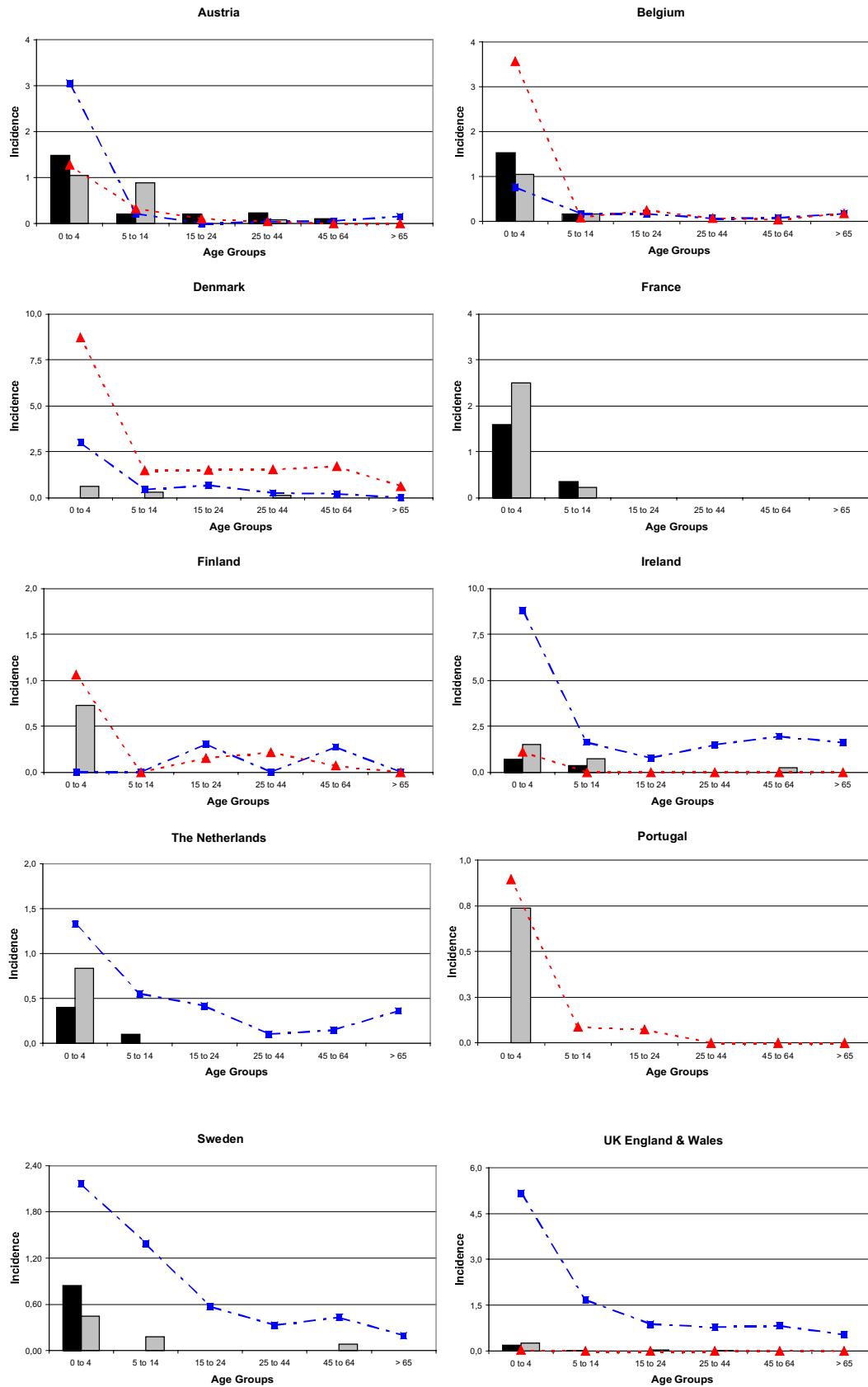
- No information available

<sup>1</sup> Combined data from NDSC VTEC enhanced surveillance and from SWAHB PHL Ireland

<sup>2</sup> *E. coli* serovar known only for HUS cases

<sup>3</sup> Source: RKI 2004: Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2003

Figure EC 1. Incidence rate per 100 000 inhabitants of verotoxigenic *E. coli* infection in different age groups



**Figure EC 1. Incidence rate per 100 000 inhabitants of verotoxigenic *E. coli* infection in different age groups - continued**

