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, the Animal Health Authority is in the process of making findings of VTEC O157 in samples from live animals notifiable.

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 genes for VT production and the eaeA genes are identified. 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.

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.

In Norway, generally no official restrictions are imposed to cattle holdings where VTEC O157 was detected. But in 2002, more stringent restrictions were imposed upon a farm when a widespread occurrence of VTEC O157 was revealed. Advise regarding human health aspects is provided to the farmers.

As regards foods, the measures taken are stricter. In Norway, a carcass that tests positive for VTEC O157 will be condemned. Similarly, in Sweden, actions are taken to ensure that food contaminated with VTEC O157 is not reaching the consumer.

Table EC 1. Studies and monitoring activities on verotoxigenic *E. coli* in animals, 2002

	Method / Agent	Time and place of investigation	Specimen collected	Sample size / frequency
B	B / *E. coli* O157	Screening on cattle in slaughterhouses	Faecal sample	100 carcasses / month, 20 /slaughterhouse, 1600 cm² swabs of carcasses
	B / verotoxigenic *E. coli*	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 / *E. coli* O157	Survey at slaughterhouse	Faecal sample	One animal per herd, monthly sampling (DANMAP)
	B / VT *E. coli*	Survey at slaughterhouse	Faecal sample	Study on the presence of the serotypes O157, O26, O103; O111, O145
NL		KvW-RIVM surveillance project	Cattle, veal calves	Pooled samples of about 160 herds (cattle) and 150 herds (veal)
S	B / *E. coli* O157	At slaughterhouse	Faecal sample	2000 / annually (since 1997)
		At large scaled-slaughterhouse	Carcasses of cattle	500-900 / annually (since 1998) voluntary
		Voluntary at slaughterhouse	Cattle and sheep	Animals from infected herds
		Follow-up of human cases		
N	B	Survey in beef cattle herds	Faecal samples	6 adult cattle and 3 animals <2 years from each farm. Samples already collected and tested for VTEC O157 were in 2002 tested for other potentially human pathogenic VTEC (serogroup O26, O103, O130, O145)
N	B / PCR (stx)	Research projects in cattle		

B / *E. coli* O157 Bacteriological testing specific for *E. coli* O157
B Bacteriological testing
PCR (stx) PCR to detect the stx-gen

Table EC 2. Verotoxigenic E. coli monitoring programmes in food, 2002

	Method	Time and place of investigation	Specimen collected	Sample size / frequency
B	B / *E. coli* O157	Screening at slaughterhouse, cutting plants, retail trades	Beef and veal carcasses	Swabs (1600 cm^2) for beef and veal carcasses 25 g of cutting meat and minced meat of beef
DK	B / VT *E. coli*	Survey at slaughterhouse	Fresh meat cuts	Study on the presence of the serotypes O157, O26, O103; O111, O145
N	B / *E. coli* O157	Official surveillance programme	Cattle carcasses Sheep and goat carcasses Abdominal muscle and skin from a area close to the abdominal incision	Systematic sampling: - every 150 cattle carcass every 150 goat carcass every 1000 sheep/lamb carcass
NL	B / *E. coli* O157	Food survey KvW	Beef and pork	About 450 (beef) and 89 (pork) samples / year respectively

B Bacteriological testing
B / *E. coli* O157 Bacteriological testing specific for *E. coli* 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 cattle herds were monitored for VT *E. coli* O157 and 5,5% of the samples were found positive in 2002. In addition, a study screening for *E. coli* serotypes O26, O103 and O111 in faecal samples and fresh meat cuts, found one VT negative strain of *E. coli* O26 in a faecal sample. VT *E. coli* O157 was not detected in cuts of fresh beef and minced beef sampled at the cutting plants and retail outlets.

Within the follow-up of a VTEC positive finishing unit for fattening cattle in Finland, *E. coli* O157:H7 was isolated from 8 out of 88 faecal samples. In the investigation of a herd suspected to have a connection to human cases, VT *E. coli* O157 was isolated from 21 out of 79 faecal samples. Swab samples were taken from carcasses that originated from one farm previously positive for *E. coli* O157. The agent was detected in nine out of 195 samples.

In Belgium, a total of 16 herds were sampled following identification of *E. coli* O157 on carcasses in the slaughterhouse. On three herds *E. coli* O157 VT2 eae and on eight herds *E. coli* O157 without eae or without verotoxin was isolated. In the screening programme at slaughterhouses, verotoxigenic *E. coli* O157 was detected in 13 beef carcasses out of 1215 sampled.

Four farms associated with outbreaks of VT *E. coli* O157 outbreaks in humans were investigated in 2002 in Great Britain. On one farm, samples from cattle and wild rabbits were negative, but the organism was isolated from faeces of wild birds. On two other farms samples were taken, all with negative results.

In Sweden, as seen in previous years the prevalence is higher in young animals compared to adult animals. In -beef calves (7-9 months at slaughter) 7 out of 91 (7,7 %) were positive, in young bulls (12-18 months at slaughter) 17 out of 1343 (1,3 %) and in adult cattle 5 of 540 (0,9 %) were positive. During 2002, three VT *E. coli* O157 positive cattle herds were found in investigations to trace the source of infection after EHEC disease in humans in Sweden. These strains were identical to the ones that had been isolated from humans. In follow-up investigations of a foodborne outbreak caused by fermented cold-smoked sausages contaminated with VTEC O157, the meat was found to originate from at least 15 farms in the same region. In 5 of these 15 farms, VTEC O157 was isolated, however these strains were different from the one found in the human EHEC cases.

In The Netherlands, within the surveillance program of the KvW/RIVM, 14,1 % of the investigated dairy cattle herds and 23,9 % of the veal calve herds were positive for *E. coli* O157 in 2002. Compared to the previous years, the prevalence of *E. coli* O157 in cattle is gradually increasing. Most of the isolates contained one or both of the stx genes. Thus, the majority of isolates were potentially pathogenic to humans. None of the food samples tested positive for VTEC O157.

Several surveys have been conducted in Norway in 2002. In none of 50 dairy cattle herds, in which 15 individual faecal samples were tested, VTEC O157 was detected. In another survey, in which faecal samples from 453 beef cattle representing 155 farms were tested for the presence of VTEC O26, O103, O139, O145 and O157, 1,1% animals were positive for VTEC O103. In a study on a research farm regarding the safety of organically produced fresh produce, VTEC O157 was detected in several samples of soil and cattle manure. Extended

testing revealed that the pathogen was widespread on the farm. VTEC O157 was isolated from faecal samples from cattle, sheep and poultry. Molecular analyses indicated that all isolates belonged to the same clone. Out of 2364 cattle, 1229 sheep, and 99 goat carcasses tested in the surveillance programme, five cattle carcasses (0,2% of cattle carcasses) were positive for VTEC O157:H7 (stx2). Four of the five positive carcasses originated from the same herd and was sampled the same day (which is not according to the scheme).

In Germany, 12,2 % of the cattle investigated were harbouring VT *E. coli*, which is higher compared to the previous year. Among the isolates a few VTEC O157 were detected. In beef, VT *E.* coli was isolated from 3 out of 183 samples tested, and two of the isolates were VTEC O157. In processed meat samples, VT *E.* coli was isolated at considerable rates.

Other animals

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

In Norway, no VTEC O26, O103, O139, O145 or O157 was detected in a survey on faecal samples from 45 red deer, 69 roe-deer and 43 elk.

In Germany, 76% of the sheep tested were positive for VT *E. coli,* but none of the isolates were reported to be VTEC O157. Similarly, 39% of the in farmed deer tested were positive for VT *E. coli.*

Other foodstuffs

Besides beef, several other types of foodstuff were tested for Verotoxigenic *E. coli.* Most of the samples were negative. A few VT *E. coli* O157 were isolated from pork, mutton, poultry meat, prepared meat meals, milk products and once from a fish product.

11.2. Verotoxigenic *E. coli* in humans

Source of information

The surveillance systems in the individual countries are still 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

In the 13 countries (12 MS and Norway) where information is provided, 2664 laboratory confirmed cases of verotoxigenic *E. coli* infections or HUS were reported. Out of the total number of reported cases, 1253 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 238 laboratory confirmed HUS cases were reported in 12 countries where notification provides for that information (Table EC 3). Out of these, 91 cases were caused by VT *E.coli* O157, and 33

cases by other serotypes which is more frequent than expected. For the remaining cases no information on the serotype was available.

Compared to the previous years, the number of reported cases has increased again. This might be attributable, at least to some extent, to an increased awareness and to changes or improvements of the diagnostic methods used.

Mainly clinical cases caused by *E. coli* O157 were reported as 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 81 clusters of infection with enterohaemorrhagic *Escherichia coli* (EHEC) involving 200 cases were reported corresponding to 16 % of cases complying with the case definition (2001: 13 %). In 20 of the total number of 81 clusters of EHEC cases reported in 2002, cases with HUS symptoms were involved. Thus, the observed increase in number of reported cases might be due to some outbreaks. Two outbreaks caused by a sorbitol-fermenting variant of *E. coli* O157:H- had been identified where cases were spread over several regions.

In Sweden, a total of 129 cases were reported either by physicians or by the laboratory. 108 (87 %) of the cases reported by physicians were of domestic origin. Altogether, 19 cases of HUS were reported, of which 12 were reported in children younger than 14 years of age. One reason for the unusual high number of HUS is because of one outbreak with nine recorded HUS cases. The increase in the total number of cases compared to the last years can be explained by two outbreaks that occurred during 2002. In an outbreak involving 11 persons the beach and sea-water were suspected sources of infection, but the agent could not be isolated from the environmental samples. In the other outbreak, involving 28 persons, the source of infection was a cold smoked sausage from a local producer. In this outbreak, nine persons developed HUS.

In Denmark, four cases of HUS were reported. VTEC O26:H- was isolated from the case which tested positive by microbiological examination. No large outbreaks were reported in Denmark, but in eight instances two members of the same family were infected with the same serotype and in one instance two children from the same day–care facility.

In Belgium, 39 typical EHEC isolates and 7 atypical EHEC isolates (without intimin or enterohemolysin genes) were characterised. The majority of the EHEC isolates belonged to serogroup O157. Seven typical EHEC isolates belonging to serotype O157:H7 were from children between 1 and 4 years old suffering from HUS.

In Finland, as in 2001, 17 (0,3/100 000 inhabitants) clinical cases were reported. Three of them were HUS. 8 cases were caused by *E.coli* O157:H7 and 7 by non-O157 VTEC strains. One of the strains could not be serotyped.

In Norway, altogether 16 cases of VTEC infection in humans were reported in 2002, which is comparable to the 15 cases in 2001. Eleven cases were due to VT *E.coli* O157 and six of the 11 cases were indigenous. For two of the NON-O157 cases, HUS was reported.

In The Netherlands, the number of cases of VT *E.coli* O157 reported in 2002 is higher compared to previous years. In 2002, 49 cases of VT *E.coli* O157 were found and 53% of these patients were hospitalised. 27% of the patients developed HUS. In 2002, the second sorbitol-fermenting STEC O157 was found in the Netherlands. No outbreaks were observed, however, on the basis of PFGE typing a common source of infection was suggested for patients in 5 different clusters. In the Netherlands, all human cases are traced back when anamnesis reveals animal contact (especially in HUS cases). From 2000 to 2002, three isolates from children could not be distinguished from animal isolates. KvW has therefore published a Hygienecode for children's farms with measurements focussed on risk reduction.

In England and Wales, there was a further reduction in the number of recorded cases. In 2002, 595 cases were recorded. Similarly, there were 28 reports of *E. coli* O 157 in Northern Ireland in 2002, compared with 52 reports in 2001. There was no outbreak reported in 2002.

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

In Austria, the number of reported HUS cases and clinical cases was slightly higher compared to 2001. 5 out of 12 HUS cases were caused by serotypes other than *E. coli* O 157. In France, 75 clinical cases of HUS were notified and 43 HUS cases were laboratory confirmed. About 60% of the confirmed cases were caused by *E. coli* O 157. In Ireland, predominantly cases caused by *E. coli* O 157 were notified. Interestingly, in Italy all HUS cases and most of the other VTEC infections were caused by other serotypes than *E. coli* O 157. In Spain, altogether 9 clinical cases of VT *E. coli* infection were reported in the microbiological information system. No information at all on this disease is available from Greece, Luxembourg and Portugal.

Table EC 3. Human cases of verotoxigenic *E. coli* 2002

	HUS Cases				Clinical cases (non HUS)				All cases			
Country	Clin	Conf.	O157	Non-O157	Clin	Conf.	O157	Non-O157	Clin	Conf.	O157	Non-O157
Austria	17	12	7	5	30	30	21	9	47	42	28	14
Belgium	-	7	7	0	-	32	19	13	-	39	26	13
Denmark	2	1	0	1	136	136	23	113	138	137	23	114
Finland	3	2	2	0	-	14	6	7	3	16	8	7
France	75	43	27	16	-	-	-	-	75	43	27	16
Germany [2]	114	114	-	-	1139	1139	-	-	1253	1253	-	-
Ireland	9	5	5	0	66	66	65	1	75	71	70	1
Italy	22	9	0	9	18	18	7	11	40	27	7	20
Portugal	-	-	-	-	-	-	-	-	-	-	-	-
Spain (SIM)	-	-	-	-	-	9	-	-	-	9	-	-
Sweden	19	14	14	0	105	96	96	0	124	110	110	0
The Netherlands	12	12	12	0	37	37	37	0	49	49	49	0
Scotland	-	-	-	-	-	229	-	-	-	229	-	-
Northern Ireland	-	-	-	-	-	27	-	-	-	27	-	-
England and Wales	-	17	17	0	8	579	578	1	8	596	595	1
Norway	2	2	0	2	12	14	11	3	14	16	11	5

[1] Not notifiable
- No information available