EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

European Food Safety Authority

TECHNICAL REPORT

Shiga toxin/verotoxin-producing *Escherichia coli* in humans, food and animals in the EU/EEA, with special reference to the German outbreak strain STEC O104

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ECDC / EFSA JOINT TECHNICAL REPORT

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1 Introduction

This report aims to give a short summary of reported Shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC) prevalence and incidence in humans, food and animals. A special reference is given to the strain STEC O104:H4, which has been isolated as the causative agent for the largest outbreak of haemolytic uremic syndrome (HUS) ever reported, which started in Germany in May 2011 and includes several cases from other EU and non-EU countries linked to the outbreak. The report focuses on cases reported in EU/EEA countries through the existing surveillance and monitoring systems in the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) and the comparison of characteristics of strains from earlier reported isolates/cases with the German outbreak strain.

2 Microbiology of Shiga toxin/verotoxin-producing *Escherichia coli*

2.1 *Escherichia coli* as a pathogen

All humans and animals carry *Escherichia coli (E. coli)* bacteria in their intestines – they are part of the normal gut flora and usually harmless. However, there are several types of *E. coli* strains that may cause gastrointestinal illness in humans. These strain types can be divided into six groups or pathotypes:

- Enteropathogenic *E. coli* (EPEC)
- Attaching and effacing *E. coli* (A/EEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterohaemorrhagic *E. coli* (EHEC)
- Enteroaggregative *E.coli* (EAggEC)

E. coli is a bacterium that very easily and frequently exchanges genetic information with related bacteria around it, such as *Salmonella* spp., *Shigella* spp., and other *E. coli* strains, through horizontal gene transfer mechanisms. Therefore, *E. coli* strains may exhibit characteristics that have been acquired from a wide variety of sources. The differences in characteristics of *E. coli* pathogenic groups are not discussed in this report and the focus of the report is on the VTEC group, which includes all Shiga toxin/verotoxin-producing *E. coli* strains.

2.2 STEC, VTEC or EHEC

The Shiga toxin-producing group of *E. coli* strains is capable of producing toxins very similar to the one produced by *Shigella dysenteriae* type 1. Two types of toxins have been described: Shiga toxin 1 (Stx1), which differs from true Shiga toxin by one to seven amino acid differences, and Shiga toxin 2 (Stx2), which shares about 60% homology to Stx1. In spite of these differences from true Shiga toxin (Stx), all Stx1 and Stx2 toxins are considered to belong to the family of Shiga toxins. Therefore, these bacteria are often called Shiga toxin-producing *E. coli* (STEC).

Functionally active Shiga toxins may be detected using Vero cell toxicity test [1]. This is why these bacteria are also called verotoxin or verocytotoxin-producing *E. coli* (VTEC).

The Shiga toxins produced by *E. coli* may cause anything from uncomplicated diarrhoea to haemorrhagic colitis, which can progress into haemolytic uremic syndrome (HUS), composed of a micro-angiopathic haemolytic anaemia, thrombocytopenia and severe acute renal failure requiring intensive care. Thus, the bacterium is often called also enterohaemorrhagic *E. coli* (EHEC).

In this report, the acronyms STEC and VTEC are used, as these are the terms employed in the reporting systems for humans, animals and food at the EU level.

2.3 Characteristics of the outbreak strain STEC O104:H4

On 26 May 2011, Germany reported a nationwide outbreak of haemolytic uremic syndrome (HUS) caused by Shiga toxin-producing *E. coli* (STEC) [2, 3]. Within a week, the number of reported HUS cases had increased to 470 cases [3]. The STEC strain isolated in the outbreak has been very thoroughly characterised at the National Reference Laboratory for *Salmonella* and other enteric bacteria in the Robert Koch Institut[1] (Germany) and it has the following features:

- Sorbitol fermenting
- Serotype: O104:H4
- Shiga toxin 1: - (negative)
- Shiga toxin 2 (subtype 2a): + (positive)
- Intimin (eae): - (negative)
- Enterohaemolysin: - (negative)

1 http://www.rki.de/cln_116/nn_467482/DE/Content/InfAZ/E/EHEC/EHEC__Diagnostik,templateId=raw,property=publicationFile.pdf/EHEC_Diagnostik.pdf

- Enteroaggregative *E. coli* virulence plasmid:
  - *aatA:* + (positive)
  - *aggR:* + (positive)
  - *aap:* + (positive)
  - *aggA:* + (positive)
  - *aggC:* + (positive)
- MLST sequence type: ST678
- ESBL (CTX-M-15)
- Beta-lactamase production:
  - ESBL (CTX-M-15): + (positive)
  - TEM-1: + (positive)
- Resistant against:
  - Ampicillin
  - Amoxicillin/clavulanic acid
  - Piperacillin/sulbactam
  - Piperacillin/tazobactam
  - Cefuroxime
  - Cefuroxime-Axetil
  - Cefoxitin
  - Cefotaxime
  - Cetfazidime
  - Cefpodoxime
  - Streptomycin
  - Nalidixic acid
  - Tetracycline
  - Trimethoprim/Sulfamethoxazole

The outbreak strain possesses an unusual combination of virulence factors of STEC/VTEC and enteroaggregative *E. coli* (EAggEC). This combination is very rare and has been previously described in strains of serotype O111:H2 involved in a small outbreak of HUS in children in France [4]. EAggEC infections are usually associated with prolonged watery diarrhoea, particularly among children and travellers to developing countries [5, 6].

3 Monitoring of STEC/VTEC in the EU/EEA

3.1 Surveillance of STEC/VTEC in humans

Data on cases of STEC/VTEC infections in humans are reported quarterly and annually by EU Member States and EEA countries to the European Surveillance System (TESSy) at ECDC, based on the Decision 2119/98/EC[2]. The data is annually published together with EFSA in the Community and European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks. In 2009, 24 EU Member States plus Iceland and Norway submitted data on STEC/VTEC infections in humans.

When interpreting information on STEC/VTEC cases in humans, it is important to note that the data is not comparable between countries due to underlying differences in the national surveillance systems. In addition, serotyping capacity varies considerably by countries and is mostly targeted to the serogroup STEC/VTEC O157. This serogroup was the first STEC/VTEC to be identified as a source of human disease and has been responsible for a large number of reported outbreaks over the last decades. The concentration of laboratory testing on this serogroup means that the proportion of STEC/VTEC non-O157 strains is largely underreported.

3.2 Monitoring of STEC/VTEC in food and animals

Data on STEC/VTEC detected in food and animals are reported annually on a mandatory basis by EU Member States to the European Commission and EFSA, based on Directive 2003/99/EC[3]. Most Member States have provided data on their VTEC investigations in the past years. In 2009, 23 EU Member States plus Norway reported data on VTEC in food or animals.

EFSA's Biological Hazard Panel recommended in 2007 that monitoring of STEC/VTEC in animals and food should concentrate on VTEC O157, since this serotype is most frequently associated with severe human infections (including HUS) in the EU. Monitoring should then be extended to other serotypes (e.g. those of O26, O103, O91, O145 and O111) that are identified by periodical analysis of European human disease and epidemiological data as most frequently causing for human infections [7]. EFSA issued in 2009 a scientific report on technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food [8]. This report covers primarily the monitoring of VTEC O157 on the hide of young cattle and on sheep fleeces. Member States may extend the monitoring to the serogroups O26, O103, O111 and O145, which are also identified as causes of human infections.

[2] Decision 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the EU. OJ L 268, 3.10.1998, p.1-7.

[3] Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. OJ L 325, 12.12.2003 p. 31-40.

4 Results of surveillance and monitoring of STEC/VTEC in the EU/EEA

4.1 STEC/VTEC in humans

Altogether 16 263 confirmed human VTEC cases have been notified in EU Member States in the period 2005–2009 (Table 1). In 2009, a total of 3 573 confirmed cases were reported from 24 Member States, which is slightly more (an increase of 13%) than in 2008. In 2009, EU notification rate was 0.75 per 100 000 population. Only two to six deaths due to STEC/VTEC infection have been reported annually between 2006 and 2009.

Table 1 Reported VTEC cases in humans, 2005–2009, and notification rate for confirmed cases in 2009

Country	Report type[1]	2009			2008	2007	2006	2005
		Cases	Confirmed cases	Confirmed cases/ 100 000	Confirmed cases			
Austria[2]	C	91	91	1.09	69	82	41	53
Belgium	C	96	96	0.90	103	47	46	47
Bulgaria	U	0	0	0	0	0	-	-
Cyprus	U	0	0	0	2	-	-	-
Czech Republic[3]	-	-	-	-	-	-	-	-
Denmark	C	173	160	2.90	161	156	146	154
Estonia	C	4	4	0.30	3	3	8	19
Finland	C	29	29	0.54	8	12	14	21
France	C	93	93	0.14	85	57	67	-
Germany	C	878	878	1.07	876	870	1 183	1 162
Greece	-	-	-	-	0	1	1	-
Hungary	C	1	1	<0.1	0	1	3	5
Ireland	C	240	237	5.33	213	115	153	125
Italy	C	71	51	0.08	24	27	17	
Latvia	U	0	0	0	0	0	0	0
Lithuania	U	0	0	0	0	0	0	-
Luxembourg	C	5	5	1.01	4	1	2	8
Malta	C	8	8	1.93	8	4	21	23
Netherlands	C	313	313	1.90	92	88	41	64
Poland	U	0	0	0	3	2	4	4
Portugal	-[2]	-	-	-	-	-	-	-
Romania	U	0	0	0	4	-	-	-
Slovakia	C	14	14	0.26	8	6	8	61
Slovenia	C	12	12	0.59	7	4	30	-
Spain	C	14	14	<0.1	21	18	13	16
Sweden	C	228	228	2.46	304	262	265	336
United Kingdom	C	1 339	1 339	2.19	1 164	1 149	1 294	1 171
EU Total		**3 609**	**3 573**	**0.75**	**3 159**	**2 905**	**3 357**	**3 269**
Iceland	C	8	8	2.50	4	13	1	-
Liechtenstein	-	-	-	-	0	-	-	-
Norway	C	108	108	2.25	22	26	50	18
Switzerland	C	42	42	0.54	67	53	47	52

Source: [14]

[1] A: aggregated data report; C: case-based report; -: No report; U: unspecified.

[2] No surveillance system exists.

[3] New electronic reporting system in place since 2009.

The serogroup O157 has been the most commonly detected serogroup in the past two years, representing about 52% of the confirmed cases with known serotypes (Table 2). This reflects the focus of laboratory diagnosis, which is often targeted to detect and confirm STEC/VTEC O157 only.

Table 2 Reported serogroups in confirmed human VTEC cased in 2008–2009

2009			2008		
Serogroup	**N**	**% total**	**Serogroup**	**N**	**% total**
O157	1 848	51.7	O157	1 673	53.0
NT[1]	1 008	28.2	NT	819	25.9
O26	192	5.4	O26	166	5.3
O103	82	2.3	O103	88	2.8
O91	48	1.3	O145	49	1.6
O145	47	1.3	O91	50	1.6
O146	31	0.9	O111	43	1.4
O128	26	0.7	O128	28	0.9
O111	25	0.7	O146	25	0.8
O113	22	0.6	O117	20	0.6
Other[2]	244	6.8	Other	198	6.3
Total	**3 573**		**Total**	**3 159**	

Source: [14]
[1] NT = untyped/untypeable.
[2] 'Other' includes 12 confirmed cases where antigen O was reported as unknown.

The notification rate of STEC/VTEC O157 was highest in children below 5 years (7.2 cases per 100 000 population) in 2009. This group also accounted for almost two thirds (63.2%) of the 242 haemolytic uremic syndrome (HUS) cases with information on age; these cases were mainly associated with STEC/VTEC O157 infections.

Between 2007 and 2009, a total of 548 (6%) HUS cases among 9 637 confirmed STEC/VTEC cases were reported to ECDC by 23 EU/EEA countries (Table 3).

During the period 2007–2009, the HUS cases have been mostly reported in infections with the serogroup O157 (N=282) and serogroup O21 (N=60), covering 86% of cases of the top 10 group (Table 4).

Table 3 Haemolytic uremic syndrome (HUS) cases in reported confirmed STEC/VTEC cases, by EU/EEA countries, 2007–2009

Country	2007	2008	2009
Austria	0	0	13
Belgium	10	11	21[1]
Cyprus	-	0	-
Denmark	1	4	6
Estonia	0	0	0
Finland	-[2]	-	-
France	36	43	62
Germany	31	41	52
Greece	1	-	-
Hungary	1	1[3]	0
Iceland	0	0	0
Ireland	5	11	24
Italy	25	24	35
Luxembourg	0	0	0
Malta	0	0	0
Netherlands	6	6	4
Norway	4	2	14
Poland	0	1	-
Slovakia	0	0	0
Slovenia	1	0	1
Spain	0	0	0
Sweden	0	0	6
United Kingdom	23	4	26
Total	**144**	**148**	**264**

Source: TESSy data as of 4 June 2011 (update from countries 8 June 2011).
[1]Data from Belgium.
[2] Data from Finland.
[3] Data from Hungary.

Table 4 Serogroups and associated HUS cases in 2007–2009, pooled data

O serogroup	N	%
O157	282	71
O26	60	15
O145	19	5
O111	17	4
O103	6	2
O121	6	2
O128	3	1
O55	3	1
O114	2	1
O126	2	1
Total	**400**	**100**

Source: TESSy data as of 8 June 2011.

4.2 STEC/VTEC in food and animals

4.2.1 Meat, milk, cattle and sheep

Most reported data on STEC/VTEC are from animals (mainly ruminants) and meat and milk thereof, since these are considered to be main sources of human infections. However, VTEC findings are also reported from other animal species, such as wild ruminants and game. These data are summarised in the Community and European Union Summary Report on Trend and Sources of Zoonoses and Food-borne Outbreaks in 2004–2009 [9-14].

Similar to the information on human cases, when interpreting the data from food and animals it is important to note that data from different investigations are not directly comparable due to differences in sampling strategies and applied analytical methods. In fact, the most widely used analytical method only aims at detecting VTEC O157, whereas fewer investigations have been conducted with analytical methods aiming at detecting all or selected non-O157 serotypes of VTEC. Therefore, the data between Member States and between reporting years are not necessarily comparable.

During the period 2007–2009, overall 0.3%–2.3% of fresh bovine meat samples were found positive for VTEC in the reporting Member States, and 0.1%–0.7% of these samples were positive for VTEC O157 (Table 5). The proportion of positive samples varied between the Member States, from 0% to 14.9%. Some data were also reported on fresh sheep meat, where 0.7%–3.2% of the samples were positive for VTEC at EU level and no samples were positive for VTEC O157. The proportion of positive sheep meat samples ranged between the Member States from 0% to 10.5%. In addition, VTEC was also reported from some samples of raw cow's milk during the years.

In cattle during the period 2007–2009, STEC was reported in animal samples at levels of 2.2%–6.8% at EU level, and VTEC O157 was found from 0.5%–2.9% of these samples. The prevalence of VTEC in cattle varied widely between the Member States, from 0% to 48.5%. In sheep, overall 0.9%–20% of the animal samples were found VTEC positive in the reporting Member States and 0.3%–3.1% of these samples were VTEC O157 positive in 2007–2009. The prevalence of VTEC in sheep varied also widely between the Member States from 0% to 70.5%. However, the number of reporting Member States was low in case of sheep. The specimens taken from animals varied between the Member States and included faeces, ear, hide and fleece samples.

Table 5 STEC/VTEC in fresh bovine and sheep meat, cattle and sheep in EU, as reported in accordance with Directive 2003/99/EC* in 2007–2009

Animal/food category	N of MS	2007			2008			2009		
		N	VTEC	VTEC O157	N	VTEC	VTEC O157	N	VTEC	VTEC O157
Fresh bovine meat	13–14	14 115	0.3%	0.1%	14 598	0.3%	0.1%	9 285	2.3%	0.7%
Fresh sheep meat	4–5	285	1.8%	0%	1 263	0.7%	0%	248	3.2%	0%
Cattle – animals	9–11	5 154	3.6%	2.9%	5 368	2.2%	0.5%	5 555	6.8%	2.7%
Sheep – animals	4	533	0.9%	0.4%	671	3.1%	1.6%	324	20%	0.3%

**Only investigations with ≥ 25 samples included.*
N=number of samples; N of MS=number of Member States reporting data.

4.2.2 Vegetables and fruits

During the period 2004–2009, 14 Member States tested for and reported data on VTEC in fruits, vegetables and products thereof (Tables 6 and 8). In total 5 910 such samples were examined and out of them only 11 were found positive for VTEC (0.19%) and eight of these samples were identified as VTEC O157 (0.14%). Most of the positive findings were from vegetables, where 0.50% of the samples tested positive for VTEC. In 2008, the Netherlands reported five VTEC O157 positive samples out of 947 samples of vegetables; Portugal found one VTEC non-O157-positive sample of pre-cut ready-to-eat fruits and vegetables from catering; and Spain reported two VTEC-positive findings out of 23 vegetable samples tested. In 2009, Sweden reported three VTEC O157-positive samples out of 57 tested vegetable samples.

Table 6 STEC/VTEC findings in fruits and vegetables and products thereof as reported by the Member States in accordance with Directive 2003/99/EC in 2004–2009

Food category	Number of samples	STEC positive	STEC O157 positive
Fruits and vegetables	691	1* (0.14%)	0
Vegetables	2 019	10 (0.50%)	8 (0.40%)
Fruits	2 774	0	0
Juice	317	0	0
Sprouts	104	0	0
Spices and herbs	3	0	0
Ready-to-eat salads	2	0	0
Total	**5 910**	**11 (0.19%)**	**8 (0.14%)**

**STEC non-O157.*

4.2.3 Food-borne outbreaks

A total of 211 food-borne outbreaks and 13 (drinking) waterborne outbreaks caused by pathogenic *E.coli*, including STEC/VTEC, were reported by the EU Member States in the period 2007–2009 (Table 7). Out of these, detailed data was available from 57 outbreaks and the food vehicle was identified in 40 outbreaks. The implicated food vehicle was meat (mainly bovine meat) in 16 outbreaks, dairy product in nine outbreaks and other or mixed food in 15 outbreaks. None of the outbreaks were reported to be caused by fruits or vegetables.

For the period 2004–2006, when the reporting of food-borne outbreaks was not yet harmonised in EU, the data is less comparable and therefore more difficult to interpret. However, for these years, together 195 food-borne outbreaks caused by *E. coli* were reported by Member States (Table 7). In three of these outbreaks, vegetables and salads were reported as the food vehicle (two outbreaks in Sweden in 2005 and 2006, and one outbreak in Portugal in 2006). The Swedish outbreak in 2005 affected 135 persons, the source was lettuce and the location of exposure was both restaurants and private households. The Swedish outbreak in 2006 affected 10 persons, was caused by vegetables and took place in a kindergarten. The outbreak in Portugal affected 10 persons and occurred at an institutional canteen. In addition, one outbreak in Denmark in 2006 was associated with herbs and spices (pesto sauce and imported basil were implicated) and affected 250 human cases. It took place in a school.

These outbreak data are summarised in the Community and EU Summary Report on Trend and Sources of Zoonoses and Food-borne Outbreaks in 2004–2009 [9–11, 13–15].

Table 7 Reported food- and waterborne *E. coli* outbreaks to EFSA in accordance with Directive 2003/99/EC in 2004–2009

STEC/VTEC/EHEC outbreaks	2009	2008	2007	2004–2006
Food-borne outbreaks	75	75	61	195
Waterborne outbreaks	5	4	4	5
Human cases in food-borne outbreaks	595	339	479 (includes only verified outbreaks)	2 345 (data missing from some outbreaks)
Human cases in waterborne outbreaks	12	22	62	26

Table 8 STEC/VTEC in fruits and vegetables and products thereof; data reported in accordance of Directive 2003/99/EC in 2004–2009

Country	Food category	2004		2005		2006		2007		2008		2009	
		N	Po	N	Po	N	Po	N	Po	N	Po	N	Po
Austria	Vegetables			7	0	3	0	1	0				
	Fruits and vegetables									96	0		
	Juice					118	0						
Belgium	Vegetables			76	0								
	Fruits and vegetables			114	0								
Czech Republic[1]	Vegetables					11	0			10	0	5	0
	Fruits					1	0			1	0		
Estonia	Vegetables	4	0										
	Fruits and vegetables	5	0										
	Ready-to-eat salads							2	0				
Germany	Vegetables					179	0						
Italy	Vegetables					9	0	27	0	1	0		
	Fruits[1]									3	0		
Ireland	Vegetables			6	0	3	0						
	Fruits and vegetables	337	0					3	0	7	0	29	0
	Juice			3	0			172	0	5	0	1	0
	Fruits			1	0	2	0						
	Spices and herbs											3	0
Latvia[1]	Sprouted seed			29	0								
Netherlands	Fruits					816	0	1 852	0				
	Vegetables									947	5		
Portugal	Fruits	2	0										
	Fruits and vegetables										1		
Romania[1]	Vegetables									49	0	30	0
	Fruits											7	0
Slovenia	Vegetables					50	0	150	0				
	Fruits and vegetables	100	0										
	Juice	18	0										
	Fruit			67	0	20	0						
	Sprouted seed			45	0	30	0						
Spain	Vegetables	120	0	50	0	51	0	54	0	23	2	2	0
Sweden	Vegetables	75	0	19	0							57	3
	Fruits											2	0
Total		**661**	**0** 0%	**417**	**0** 0%	**1 293**	**0** 0%	**2 261**	**0** 0%	**1 142**	**8** 0.7%	**136**	**3** 2.2%

N=number of samples; Po=number of positive samples
[1] *Batch-based data:*
Spain, 2008: 2 positive for VTEC, unspecified;
Portugal, 2008: 1 positive for VTEC non-O157;
Netherlands, 2008: 5 positive for VTEC O157;
Sweden, 2009: 3 positive for VTEC O157.

5 STEC/VTEC O104 cases reported in humans, food and animals

5.1 STEC/VTEC O104 in humans

The published data for STEC O104 is very scarce as this is a very rare serogroup in humans in Europe and the entire world. According to the information reported to ECDC, a total of nine cases were infected with STEC O104 in the EU Member States in the period 2004–2009. STEC O104 cases have been reported from Austria (one case in 2010), Belgium (one case in 2008), Denmark (one case in 2008), Finland (one case in 2010), France (one case in 2004), Norway (one case in 2006 and three cases in 2009), and Sweden (one case in 2010) (Annex, Table 9). Based on the known data, 56% of the cases were male and the age ranged from < 1 year to 76 years. One of the cases (11%) developed HUS. Four of the cases (44%) were travel-related; the countries of origin of infection were Afghanistan (2008), Egypt (2010), Tunisia (2010) and Turkey (2009).

Only two of the STEC O104 cases were of serotype STEC O104:H4 (in Finland in 2010 and in France in 2004). The Finnish case was travel-related with infection acquired in Egypt.

Based on literature, STEC O104:H4 has been isolated twice in Germany in 2001 [16], and once in Korea in 2005 [17]. The case in Korea was a 29-year-old woman who developed HUS, but the source of the infection was unknown.

Other serotypes attributed to human cases have been published for serotypes O104:H2 [18], O104:H21 [19, 20] and O104:H- [21]. An outbreak due to contaminated milk and related to serotype STEC O104:H21 was reported in the USA in 1994 [20].

5.2 STEC/VTEC O104 in animals and food

According to the information provided to EFSA in accordance with Directive 2003/99/EC, the serogroup STEC/VTEC O104 has been reported three times from animals and food by the EU Member States (Annex, Table 9). Two of these isolations are from cattle, older than 1 year old, reported by Austria in 2009, and the serotypes detected were O104:H12 and O104:H21.

In addition, Germany detected O104 serogroup from minced red meat in 2005. Furthermore, based on available literature, STEC/VTEC O104 has been isolated from wild boar (O104/O127) [22] and sheep (O104:H7) [23] in Spain, as well as from sheep in India [24] and young cattle (O104:H7) in Argentina [25]. In food, according to the literature, the USA has reported STEC/VTEC O104 from bovine carcases [26] and New Zealand from unspecified meat and sheep meat [27, 28].

6 Reservoir for STEC/VTEC and contamination of food products with STEC/VTEC

Ruminants (particularly cattle) are recognised as the main natural reservoir of STEC/VTEC, in particular for STEC O157. Comprehensive information on the occurrence of STEC in animals other than cattle is scarce. Pigs and poultry have not been identified to be major sources of STEC for human infection in Europe [7].

In addition to direct contamination of growing sites by animals, fields for growing leafy vegetables and other produce can be contaminated with STEC by indirect means, such as contaminated water, aerosols and dust from livestock production and feeding facilities, and other human activities such as landfills and wastewater treatment sites. Wildlife may play a role in dispersal of pathogens from these sources to fields used for vegetable production [29]. A major source of contamination is organic fertiliser (e.g. manure, municipal sludge) and faecally contaminated water. The specific risk with manure and sewage sludge for organic production has to be acknowledged [30].

7 Conclusions

- As of today, the STEC O104:H4 outbreak in Germany and other EU/EEA countries is so far one the largest reported HUS and STEC outbreak in the world.
- The serotype of outbreak strain STEC O104:H4 is very rare and only few case reports in humans have been diagnosed and reported. Furthermore, the serotype has never been reported in animals/food.
- The outbreak strain has a combination of virulence factors from STEC/VTEC and enteroaggregative *E. coli*, suggesting a transfer of stx2 genes between STEC/VTEC and EAggEC pathogroups. The preliminary data from ongoing genomic investigation suggest that EAggEC has acquired genetic material (stx2) from STEC/VTEC. Further investigation is needed to confirm this and better understand the impact of this finding.
- Among the foodstuffs, STEC/VTEC was most often detected by the EU Member States from bovine and other ruminant meat and raw milk. However, at EU level, the reported proportions of positive samples are at low to very low level. In the animal populations, most reported data on STEC/VTEC is from cattle and sheep, and the reported prevalence is also mostly at low and very low level.
- In animals and food, the proportion of STEC/VTEC positive samples varied widely between Member States, which may be at least partly due to different sampling and analytical methods used.
- Less data have been reported on STEC/VTEC in vegetables and fruits, and STEC/VTEC was seldom detected in these kinds of food. However, vegetables have been implicated as the vehicle of food-borne outbreaks in the EU and some positive findings were reported by the Member States, but the overall prevalence of vegetable contamination at EU level was very low.

References

[1] Beutin L, Steinruck H, Krause G, Steege K, Haby S, Hultsch G, et al. Comparative evaluation of the Ridascreen Verotoxin enzyme immunoassay for detection of Shiga-toxin producing strains of *Escherichia coli* (STEC) from food and other sources. J Appl Microbiol. 2007 Mar;102(3):630-9.

[2] Frank C FM, Askar M, Bernard H, Fruth A, Gilsdorf A, Höhle M, et al. Large and ongoing outbreak of haemolytic uraemic syndrome. Eurosurveillance. 2011 26 May 2011;16(21).

[3] Askar M FM, Frank C, Bernard H, Gilsdorf A, Fruth A, Prager R, et al. Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing *Escherichia coli* (STEC) serotype O104, Germany, May 2011. Eurosurveillance. 2011 1 June 2011;16(22).

[4] Morabito S, Karch H, Mariani-Kurkdjian P, Schmidt H, Minelli F, Bingen E, et al. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. J Clin Microbiol. 1998 Mar;36(3):840-2.

[5] Sarantuya J, Nishi J, Wakimoto N, Erdene S, Nataro JP, Sheikh J, et al. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among E. coli strains causing diarrhea in Mongolian children. J Clin Microbiol. 2004 Jan;42(1):133-9.

[6] Okeke IN, Lamikanra A, Steinruck H, Kaper JB. Characterization of *Escherichia coli* strains from cases of childhood diarrhea in provincial southwestern Nigeria. J Clin Microbiol. 2000 Jan;38(1):7-12.

[7] EFSA (European Food Safety Authority). Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. The EFSA Journal 2007;579,1-61.

[8] EFSA (European Food Safety Authority). Technical specifications for the monitoring and reporting of verotoxigenic Escherichia coli (VTEC) on animals and food (VTEC surveys on animals and food) on request of EFSA. EFSA Journal 2009; 7(11):1366,1-43

[9] EFSA (European Food Safety Authority). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2004. The EFSA Journal 2005,310.

[10] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union in 2005. The EFSA Journal 2006;94,288.

[11] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Food-borne outbreaks in the European Union in 2006. The EFSA Journal 2007;130,2-352.

[12] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the European Union in 2007. The EFSA Journal 2009;223,3-320.

[13] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and foodborne outbreaks in the European Union in 2008. EFSA Journal 2010;8(1):1496,410 pp.

[14] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2009, EFSA Journal 2011;9(3):2090 [378 pp.].

[15] EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The Community Summary Report on Foodborne outbreaks in the European Union in 2007. The EFSA Journal 2009;271,1-128.

[16] Mellmann A, Bielaszewska M, Kock R, Friedrich AW, Fruth A, Middendorf B, et al. Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli*. Emerg Infect Dis. 2008 Aug;14(8):1287-90.

[17] Bae WK, Lee YK, Cho MS, Ma SK, Kim SW, Kim NH, et al. A case of hemolytic uremic syndrome caused by *Escherichia coli* O104:H4. Yonsei Medical Journal. 2006 Jun 30;47(3):437-9.

[18] Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol. 1988 Apr;25(4):237-43.

[19] Beutin L, Krause G, Zimmermann S, Kaulfuss S, Gleier K. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J Clin Microbiol. 2004 Mar;42(3):1099-108.

[20] Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21--Helena, Montana, 1994. Mmwr. 1995 Jul 14;44(27):501-3.

[21] Bockemuhl J, Aleksic S, Karch H. Serological and biochemical properties of Shiga-like toxin (verocytotoxin)-producing strains of *Escherichia coli*, other than O-group 157, from patients in Germany. Zentralbl Bakteriol. 1992 Jan;276(2):189-95.

[22] Sanchez S, Martinez R, Garcia A, Vidal D, Blanco J, Blanco M, et al. Detection and characterisation of O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in wild boars. Vet Microbiol. 2010 Jul 14;143(2-4):420-3.

[23] Blanco J, Blanco M, Blanco JE, Mora A, Gonzalez EA, Bernardez MI, et al. Verotoxin-producing *Escherichia coli* in Spain: prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp Biol Med (Maywood). 2003 Apr;228(4):345-51

[24] Wani SA, Bhat MA, Samanta I, Ishaq SM, Ashrafi MA, Buchh AS. Epidemiology of diarrhoea caused by rotavirus and *Escherichia coli* in lambs in Kashmir valley, India. Small Ruminant Res. 2004 Apr;52(1-2):145-53.

[25] Meichtri L, Miliwebsky E, Gioffre A, Chinen I, Baschkier A, Chillemi G, et al. Shiga toxin-producing *Escherichia coli* in healthy young beef steers from Argentina: prevalence and virulence properties. Int J Food Microbiol. 2004 Nov 1;96(2):189-98.

[26] Arthur TM, Barkocy-Gallagher GA, Rivera-Betancourt M, Koohmaraie M. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. Appl Environ Microbiol. 2002 Oct;68(10):4847-52.

[27] Brett KN, Ramachandran V, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP. stx1c Is the most common Shiga toxin 1 subtype among Shiga toxin-producing *Escherichia coli* isolates from sheep but not among isolates from cattle. J Clin Microbiol. 2003 Mar;41(3):926-36.

[28] Bennett J, Bettelheim KA. Serotypes of non-O157 verocytotoxigenic *Escherichia coli* isolated from meat in New Zealand. Comp Immunol Microbiol Infect Dis. 2002 Mar;25(2):77-84.

[29] FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). Microbiological hazards in fresh leafy vegetables and herbs: Meeting Report. Microbiological Risk Assessment Series No. 14. Rome; 2008. 151pp.

[30] EC (European Commission). Risk profile on the microbial contamination of vegetables and fruits eaten raw. Report of the Scientific Committee on Food Health and Consumer Protection Directorate-General. European Commission, Brussels,; 2002.

Annex: Overview of reported STEC/VTEC O104 cases in humans and VTEC/STEC O104 isolations in animals/food with a reference to the outbreak strain

Year	Country	STEC serotype	*stx*[1]	*eae*[2]	No of cases	Gender	Age	HUS	Suspected source	Source
Outbreak strain	Germany	O104:H4	2a	neg.		71%F	88% > 20	470	cucumber, tomato, lettuce	RKI[3]/ Germany [1]
Human cases reported to ECDC										
2010	Austria	O104:H21	UNK[4]	UNK	1	F	58	yes	UNK	ECDC/TESSy[5]
2010	Sweden	O104:NT	2	neg.	1	F	45	no	travel (Tunisia)	ECDC/TESSy
2009	Norway	O104:H-	1	neg.	1	M	<1	no	UNK	ECDC/TESSy
2009	Norway	O104:NT	1	neg.	1	F	11	no	travel (Turkey)	ECDC/TESSy
2009	Norway	O104:NT	1	neg.	1	F	22	no	UNK	ECDC/TESSy
2008	Belgium	O104:H2	2	neg.	1	M	33	no	UNK	ECDC/TESSy
2008	Denmark	O104:H2	2[6]	neg.	1	M	23	no	travel (Afghanistan)	ECDC/TESSy
2006	Norway	O104:NT[7]	2	neg.	1	M	> 70	no/ UNK	UNK	ECDC/EPIS
Other										
2010	Finland	O104:H4[8]	2	neg.	1	M	76	no	travel (Egypt)	THL[9]/Finland
2004	France	O104:H4	2	neg.	1	UNK	UNK	UNK	UNK	EN[10]/ Denmark
Literature										
2005	Korea	O104:H4	NR[11]	NR	1	F	29	yes	UNK	[2]
2001	Germany	O104:H4	2	neg.	1	UNK	UNK	yes	UNK	[3]
1997–1999	Germany	O104:H21	1,2	neg.	2	UNK	UNK	UNK	UNK	[4]
1994	USA	O104:H21	2	neg.	11	67% F	8–63 median 36	no	milk	[5]
1988	United Kingdom	O104:H2	2[12]	NR	1	M	child	NR	UNK	[6]
1987-1990	Germany	O104:H-	2	UNK	1	UNK	2	yes	UNK	[7]

[1]stx = Shiga toxin gene
[2]eae = intimin
[3]RKI = Robert Koch Institut, Germany
[4]UNK = unknown/missing data
[5]TESSy = The European Surveillance System
[6]Also verotoxin 2 production positive
[7]Eagg-pos.
[8]Ampicillin, streptomycin, sulfonamide, tetracyclin, trimethoprim, and nalidixic acid resistant; hly and saa –neg.; saa, aggR, aatA, aap, and aaiC -pos.
[9]THL= Terveyden ja hyvinvoinnin laitos
[10]EN= Enter-net data reported by Statens Serum Institut
[11]NR = not reported
[12]Verotoxin production positive

Year	Country	STEC serotype	*stx*[1]	*eae*[2]	No of isolates	Animal population/ food category	Source
Animal cases reported to EFSA/zoonoses database							
2009	Austria	O104:H12	neg. (no VTEC)	neg.	1	cattle (older than 1 year) at slaughterhouse, official sampling	EFSA/zoonoses database
2009	Austria	O104:H21	1, 2	neg.	1	cattle (older than 1 year) at slaughterhouse, official sampling; enterohaemolysin positive	EFSA/zoonoses database
Literature							
2007/2008	Spain	O104/O127:H-	2	neg	1	wild boar	[8]
2007/2008	Spain	O104/O127:H1 /H12	1	neg	1	wild boar	[8]
unspecified	Spain	O104:H7	NR	NR	9	sheep	[9]
2001/2002	India	O104	NR	NR	1	lamb with diarrhoea negative for rotavirus	[10]
1999/2000	Argentina	O104:H7	2 vh-a	neg	1	young beef steer (14–16 months old)	[11]
Food isolates reported to EFSA/zoonoses database							
2005	Germany	O104	NR	NR	1	red meat/minced meat (frozen), official controls of food	EFSA/zoonoses database
Literature							
1999	USA	O104	NR	NR	8	carcass in processing plant, 3 previsceration, 5 postprocessing (hemolysin *hly*A positive)	[12]
Unspecified	New Zealand	O104:H7	1c	neg	1	unspecified meat	[13]
Unspecified	New Zealand	O104:H-	1c	neg	1	unspecified meat	[13]
1998	New Zealand	O104:H-	1	NR	1	sheep meat (enterohaemolysin negative)	[14]
1998	New Zealand	O104:H7	1	NR	1	sheep meat (enterohaemolysin negative)	[14]
1998	New Zealand	O104:H7	1	NR	2	sheep meat (enterohaemolysin positive)	[14]

References

[1] Askar M FM, Frank C, Bernard H, Gilsdorf A, Fruth A, Prager R, et al. Update on the ongoing outbreak of haemolytic uraemic syndrome due to Shiga toxin-producing *Escherichia coli* (STEC) serotype O104, Germany, May 2011. Eurosurveillance. 2011 1 June 2011;16(22).

[2] Bae WK, Lee YK, Cho MS, Ma SK, Kim SW, Kim NH, et al. A case of hemolytic uremic syndrome caused by *Escherichia coli* O104:H4. Yonsei Medical Journal. 2006 Jun 30;47(3):437-9.

[3] Mellmann A, Bielaszewska M, Kock R, Friedrich AW, Fruth A, Middendorf B, et al. Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli*. Emerg Infect Dis. 2008 Aug;14(8):1287-90.

[4] Beutin L, Krause G, Zimmermann S, Kaulfuss S, Gleier K. Characterization of Shiga toxin-producing *Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J Clin Microbiol. 2004 Mar;42(3):1099-108.

[5] Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21--Helena, Montana, 1994. Mmwr. 1995 Jul 14;44(27):501-3.

[6] Scotland SM, Rowe B, Smith HR, Willshaw GA, Gross RJ. Vero cytotoxin-producing strains of *Escherichia coli* from children with haemolytic uraemic syndrome and their detection by specific DNA probes. J Med Microbiol. 1988 Apr;25(4):237-43.

[7] Bockemuhl J, Aleksic S, Karch H. Serological and biochemical properties of Shiga-like toxin (verocytotoxin)-producing strains of *Escherichia coli*, other than O-group 157, from patients in Germany. Zentralbl Bakteriol. 1992 Jan;276(2):189-95.

[8] Sanchez S, Martinez R, Garcia A, Vidal D, Blanco J, Blanco M, et al. Detection and characterisation of O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in wild boars. Vet Microbiol. 2010 Jul 14;143(2-4):420-3.

[9] Blanco J, Blanco M, Blanco JE, Mora A, Gonzalez EA, Bernardez MI, et al. Verotoxin-producing *Escherichia coli* in Spain: prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. Exp Biol Med (Maywood). 2003 Apr;228(4):345-51.

[10] Wani SA, Bhat MA, Samanta I, Ishaq SM, Ashrafi MA, Buchh AS. Epidemiology of diarrhoea caused by rotavirus and *Escherichia coli* in lambs in Kashmir valley, India. Small Ruminant Res. 2004 Apr;52(1-2):145-53.

[11] Meichtri L, Miliwebsky E, Gioffre A, Chinen I, Baschkier A, Chillemi G, et al. Shiga toxin-producing *Escherichia coli* in healthy young beef steers from Argentina: prevalence and virulence properties. Int J Food Microbiol. 2004 Nov 1;96(2):189-98.

[12] Arthur TM, Barkocy-Gallagher GA, Rivera-Betancourt M, Koohmaraie M. Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. Appl Environ Microbiol. 2002 Oct;68(10):4847-52.

[13] Brett KN, Ramachandran V, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP. stx1c Is the most common Shiga toxin 1 subtype among Shiga toxin-producing *Escherichia coli* isolates from sheep but not among isolates from cattle. J Clin Microbiol. 2003 Mar;41(3):926-36.

[14] Bennett J, Bettelheim KA. Serotypes of non-O157 verocytotoxigenic *Escherichia coli* isolated from meat in New Zealand. Comp Immunol Microbiol Infect Dis. 2002 Mar;25(2):77-84.