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## EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment

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## SCIENTIFIC OPINION

### Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment<sup>1</sup>

EFSA Panel on Biological Hazards (BIOHAZ)<sup>2,3</sup>

European Food Safety Authority (EFSA), Parma, Italy

#### ABSTRACT

During 2007-2010, 13 545 confirmed human VTEC infections and 777 haemolytic uraemic syndrome (HUS) cases were reported in the EU; isolates from 85 % of cases were not fully serotyped and therefore could not be classified using the Karmali seropathotype concept. Seropathotype group D covered 5 % of isolates from fully serotyped cases; 14 cases (0.7 %) belonged to seropathotype group E, defined by Karmali et al. (2003) as non-human only. Isolates from around 27 % of cases could not be assigned. There were no HUS cases reported for the serotypes in groups D and E but 17 HUS cases could not be assigned. The health outcome was reported for only a fraction of confirmed cases. About 64 % of patients presented with only diarrhoea; VTEC infection resulted in HUS in around 10 % of cases. The new ISO/TS 13136:2012 standard improves the detection of VTEC in food. An alternative concept based on the detection of verocytotoxins alone or genes encoding such verocytotoxins does not provide a sound scientific basis on which to assess risk to the consumer because there is no single or combination of marker(s) that fully define a 'pathogenic' VTEC. Strains positive for verocytotoxin 2 gene (*vtx2*)- and *eae* (intimin production)- or [*aaiC* (secreted protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes are associated with higher risk of more severe illness than other virulence gene combinations. The 2011 O104:H4 outbreak demonstrated the difficulty of predicting the emergence of 'new' pathogenic VTEC types by screening only for the *eae* gene or by focusing on a restricted panel of serogroups. A molecular approach utilising genes encoding virulence characteristics additional to the presence of *vtx* genes has been proposed.

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#### KEY WORDS

VTEC, virulence factors, serogroup, seropathotype, detection, isolation, identification.

<sup>1</sup> On request from the Austrian Federal Ministry of Health, Question No EFSA-Q-2012-00576, adopted on 7 March 2013.

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## SUMMARY

Following a request from the Austrian Federal Ministry of Health, the Panel on Biological Hazards (BIOHAZ) was asked by the European Food Safety Authority to deliver a scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. Specifically, EFSA was asked to review the ‘seropathotype’ concept of Karmali and colleagues (2003) – the limitation to “relevant” serotypes O157, O26, O103, O111, O145, O121, O91, O104, O113 and assess whether the pathogenicity can be excluded for defined VTEC serotypes, to justify the statement: ‘*seropathotypes D and E are not HUS-associated and are uncommon in man or only found in non-human sources*’ and to assess an alternative concept based on detection of verocytotoxins or genes encoding for verocytotoxins in isolates. EFSA was also asked to assess the contribution by VTEC to diarrhoeal cases and to more severe outcomes in the EU, based on hazard identification and characterisation, and under-reporting in EU and the public health risk associated with the contamination of ready-to-eat (RTE) foods with VTEC, considering either the seropathotype concept or the detection of verocytotoxins or genes encoding the production of such toxins in isolates.

The 2003 Karmali seropathotype model classifies verocytotoxin-producing *Escherichia coli* (VTEC) into seropathotypes. Serotypes responsible for haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), O157:H7 and O157:NM, were assigned to seropathotype A. Seropathotype B strains have been associated with outbreaks and HUS, but less commonly than those of seropathotype A and included O26:H11, O103:H2, O111:NM, O121:H19 and O145:NM. Seropathotype C serotypes were associated with sporadic HUS cases but not epidemics. The serotypes in group C were O91:H21, O104:H21, O113:H21, O5:NM, O121:NM and O165:H25. Seropathotype D serotypes have been associated with diarrhoea but not with outbreaks or HUS cases; seropathotype E serotypes comprised VTEC serotypes that had never been associated with human disease and had been isolated only from animals. Seropathotypes D and E included multiple serotypes, 12 serotypes for seropathotype D and 14 for seropathotype E.

The approach adopted entailed a summary of the types of pathogenic *E. coli* which have been associated with cases of human disease, and the putative virulence factors therein; the use of data from the European Surveillance System (TESSy data) as provided by the ECDC (European Centre for Disease Prevention and Control) and data available in the EU Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011 for assessing the current situation regarding human infections with VTEC in the EU; a review of methods for the isolation and identification of VTEC, including detection of virulence factors and characterisation and typing of VTEC strains and virulence genes therein; hazard characterisation, including illnesses associated with VTEC and identification of predictive factors for VTEC that may contribute to human disease; evaluation of the seropathotype concept using the Karmali approach, a modification of the Karmali approach based on the health outcome of reported confirmed human VTEC cases in the EU during 2007-2010, and a molecular approach based on the identification of known or putative colonization genes and additional toxins; and finally exposure assessment, including EU monitoring data on occurrence of VTEC in RTE food.

The BIOHAZ Panel concluded that the Karmali seropathotype classification does not define pathogenic VTEC nor does it provide an exhaustive list of pathogenic serotypes. Instead it classifies VTEC based on their reported frequency in human disease, their known association with outbreaks and their severity of the outcome including HUS and HC. During 2007-2010, 13 545 confirmed human VTEC infections were reported in Europe; isolates from 85 % of these cases were not fully serotyped and could therefore not be classified using the seropathotype concept. The D group covered 5 % of human cases that were fully serotyped. Fourteen cases (0.7 %) were assigned to seropathotype group E, defined by Karmali et al. (2003) as non-human only. Around 27 % of the cases could not be assigned to a seropathotype group as these were not listed in the 2003 Karmali paper. There were no HUS cases reported for the serotypes included in groups D and E, but there were 17 HUS cases reported that could not be assigned to a group. The health outcome has been reported for only a fraction (for diarrhoea: 53 % of cases; and for HUS: 59 % of cases) of the reported confirmed VTEC

cases in the EU between 2007 and 2010. Most patients (ca. 64 %) presented with only diarrhoea. VTEC infection resulted in HUS in around 10 % of cases. Thus pathogenicity can neither be excluded nor confirmed for a given VTEC serogroup or serotype based on the seropathotype concept or analysis of the public health surveillance data.

Detection of VTEC is highly dependent on the methods applied to clinical specimens and these vary between different Member States (MSs). The degree of under-estimation (including under-ascertainment and under-reporting) of VTEC O157 infections has been estimated in seven EU MSs. Disease-multipliers differ widely between EU countries, ranging from 13 to 87. The 2011 O104:H4 German outbreak has clearly demonstrated the difficulty of predicting the emergence of 'new' pathogenic VTEC types by only looking at the presence of the *eae* gene or by focusing on a restricted panel of serogroups. The new ISO/TS 13136:2012 standard improves the strategy for detecting VTEC in the food by enlarging the scope of the previous standard to all VTEC.

The BIOHAZ Panel further concluded that it is not possible to fully define human pathogenic VTEC or to identify factors for VTEC that absolutely predict the potential to cause human disease. The detection of verocytotoxins alone, or of genes encoding for such verocytotoxins is not a sound scientific basis for assessing the disease risk to the consumer. There is no single or combination of marker(s) that defines a 'pathogenic' VTEC. Strains positive for verocytotoxin 2 gene (*vtx2*)- and *eae* (intimin production)- or [*aaiC* (secreted protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes are associated with a higher risk of more severe illness than other virulence gene combinations. Other virulence gene combinations and/or serotypes may also be associated with severe disease in humans, including HUS.

A modification of the Karmali seropathotype model was proposed based on the health outcome of reported confirmed human VTEC cases in the EU during 2007-2010. In cases when full serotyping has been undertaken all serotypes associated with severe disease (HUS) could be categorised as seropathotype group 'haemolytic uraemic syndrome (HUS)-associated serotype(s)' or HAS. By this modified approach, in cases when full serotyping has been undertaken all serotypes associated with severe disease are automatically categorised in the HAS group.

A molecular approach, utilising genes encoding virulence characteristics additional to the presence of *vtx* genes, is proposed. This molecular approach must be regarded as provisional because screening VTEC for the presence of *eae*, *aaiC* or *aggR* genes is not routinely undertaken. This scheme has the advantage of overcoming problems associated with the lack of flagella 'H' antigen typing. The performance of this proposed approach needs to be verified with well-characterised isolates from cases of human infection and from food-producing animals and foods.

VTEC has been recovered from a range of different animal species and food categories. 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 serotypes of VTEC.

On the basis of the proposed provisional molecular classification scheme, any RTE product contaminated with an isolate of one of the VTEC serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *vtx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, current available data do not allow any inference regarding potential risks.

The BIOHAZ Panel made a series of recommendations relating to public health investigation of VTEC infection, verification and periodic revision of the proposed molecular approach for the categorisation of VTEC strains. The inclusion of *aaiC* and *aggR* genes in this approach is due to the 2011 outbreak, which was caused by a highly virulent strain. This was an exceptional event and future surveillance will provide data that may be used to review the inclusion of these virulence factors. Thus

screening VTEC for the presence of *aaiC* and *aggR* genes should be performed on isolates from human, food and animal sources, to address this question. Finally, international harmonisation of nomenclature of VTEC and its virulence factors was suggested.

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## BACKGROUND AS PROVIDED BY THE AUSTRIAN FEDERAL MINISTRY OF HEALTH

Verocytotoxin-producing *Escherichia (E.) coli* (VTEC) are an important cause of cases of acute gastroenteritis in Austria and around the world. These bacteria are strongly associated with severe forms of infection including haemorrhagic colitis (bloody diarrhoea, haemorrhagic colitis (HC)) and haemolytic uraemic syndrome (HUS).

Previously routine diagnostics were lacking in the detection of the major pathogenic factor: the production of verocytotoxins. When serotype O157 has been identified as the major cause of HUS in children, as a consequence test systems (sorbitol agar) were implemented in routine clinical microbiology to identify VTEC O157 in stool samples. Following the availability of test kits for verocytotoxins in food, outbreaks and sporadic cases of VTEC infections have been found to be associated with a growing number of different VTEC serotypes.

*“Simple methods for identification of VTEC O157 strains and improved techniques for O26, O103, O111 and O145 may have led to a degree of overestimation of the prevalence and importance of these serotypes”.*<sup>4</sup>

*“The concept of seropathotype classifies VTEC into groups based on the incidence of serotypes in human disease, associations with outbreaks versus sporadic infections, their capacity to cause HUS or HC, and the presence of virulence markers”.*<sup>4</sup>

### **Austrian experts think that this concept is not adequate to support food safety considerations:**

- Outbreaks *versus* sporadic infections: the dimension of a food-borne outbreak and the number of infected persons depends on the kind of food involved (ready-to-eat, RTE, food supporting growth, distribution ...) and not only on the pathogenicity of the microorganism.
- The capacity to cause HUS or HC: HUS or HC are severe complications, but in connection with VTEC not the primary food safety criterion. Bacteria that cause solely diarrhoea already constitute a food safety concern.
- Serotypes are phenotypes, useful for epidemiological purposes, whereas pathogenicity of VTEC is characterised by the ability to produce verocytotoxins and other virulence factors.
- The incidence of serotypes in human disease is questionable: massive underreporting - due to routine use of test systems designed to find only VTEC O157 and generally scarce testing in clinical microbiology - leads to an invalid database. Therefore plausible incidences of serotypes in human disease cannot be calculated on the basis of historical data only, without considering underreporting.

Data from Austrian and German reference laboratories as well as results reported by EFSA and ECDC (Annex) demonstrate an unacceptable high level (10 – 50 %) of VTEC “others” than the serotypes mentioned in the “seropathotype concept” causing diarrhoea, severe illness and HUS.

In December 2011 Austrian experts were involved in intensive discussions. VTEC O27:H30 VT2 pos., culture-positive, were detected in RTE food and an identical serotype, VT2 pos. strain occurred in a sick child in 2010. The pathogenicity of the bacteria was doubted, based on a seropathotype concept<sup>5</sup>:

*“A restricted range of serotypes (i.e. O157, followed by O26, O103, O91, O145 and O111) are associated with public health risks, however isolates of these serotypes are not necessarily pathogenic when recovered from food or live animals”.*

<sup>4</sup> EFSA Journal 2011;9(11):2424.

<sup>5</sup> EFSA Journal 2007;579:1-31.



At the time of the outbreak caused by VTEC in Germany, VTEC O104 was not covered by the “seropathotype concept”<sup>5</sup>. Fenugreek seeds tested positive for VTEC O104 before May 2011 would not have been considered a “public health risk” on the basis of the relevant EFSA Opinion. Nevertheless this concept and a pathogenicity concept based on detection of verocytotoxins were discussed equally by EFSA<sup>4</sup>.

Based on a seropathotype concept only a few serotypes are considered in a recent discussion paper distributed by the European Commission and in the relevant method (amending Regulation (EC) No. 2073/2005<sup>6</sup> on microbiological criteria for foodstuffs as regards of microbiological criteria for sprouted seeds):

1.29	Sprouted seeds (ready to eat)	<i>Shiga toxin producing E. coli</i> (STEC) O157, O26, O111, O103, O145 and O104	5	0	Absence in 25 grams	CEN ISO 13136 <sup>7</sup>	Products placed on the market during their shelf-life
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**Most likely the limitation to a small number of serotypes will be the basis for future problems in the EU when another serotype will be identified as the cause of a food-borne outbreak.** Food business operators might feel encouraged to place RTE food (sprouted seeds and other) on the market, contaminated with VTEC “other than the relevant serotypes”.

The Austrian Federal Ministry of Health cannot accept an approach resulting in the next outbreak (which will be only a matter of time given the intensified and improved diagnostic methods in the human health area) being the cause for simply adding another serotype to the list, while waiting for the next outbreak.<sup>8</sup>

Furthermore, if the method (CEN ISO 13136 – an ISS paper/EU ref. laboratory in Rome + O104 amendment) will be implemented in EU Member States laboratories, **other VTEC than the six types mentioned (European Commission) will not be isolated from food samples any longer.** According to this method isolation of VTEC will only be performed if the PCR for the six types is positive, causing severe consequences for outbreak investigations and monitoring<sup>7</sup>.

<sup>6</sup> Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1-26. The amendment was introduced by Commission Regulation (EU) No 209/2013 of 11 March 2013 amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and sampling rules for poultry carcasses and fresh poultry meat. (OJ L 68, 12.3.2013, p. 19-23).

<sup>7</sup> ISO/PRF TS 13136. Microbiology of food and animal feeding stuffs – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) belonging to O157, O111, O26, O103 and O145 serogroups. International Organization for Standardization. This standard has been amended since receipt of the request on 4 May 2012 and has been published as ISO/TS 13136 “Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups”.

<sup>8</sup> Recital 12 of Regulation (EU) 209/2013 of 11 March 2013 clarifies the basis for the decision to limit to six serogroups in the proposal which received the support of the Member States. Recital 12 of the Regulation states that “*Certain STEC serogroups (namely O157, O26, O103, O111, O145 and O104:H4) are recognized to be those causing the most of the Haemolytic Uremic Syndrome (HUS) cases occurring in the EU. Furthermore serotype O104:H4 caused the outbreak in May 2011 in the Union. Therefore microbiological criteria should be considered for these six serogroups. It cannot be excluded that other STEC serogroups may be pathogenic to humans as well. In fact, such STEC may cause less severe forms of disease such as diarrhoea and or bloody diarrhoea or may also cause HUS and therefore represent a hazard for the consumer's health.*”

## TERMS OF REFERENCE AS PROVIDED BY THE AUSTRIAN FEDERAL MINISTRY OF HEALTH

The Austrian Federal Ministry of Health requests that EFSA provides a scientific opinion on:

- a discussion on the scientific evidence of the following concepts, based on valid data and recent literature:
  - the “seropathotype concept” – the limitation to “relevant” serotypes O157, O26, O103, O111, O145, O121, O91, O104, O113<sup>4</sup>. The database and literature justifying the statement: “*seropathotypes D and E are not HUS-associated and are uncommon in man or only found in non-human sources*”<sup>4</sup>
  - versus a concept based on detection of verocytotoxins (relevant pathogenicity factor) in isolates;
  - and the consequences for food safety: is it acceptable to concentrate on most severe complications and VTEC causing the predominant number of these complications, ignoring VTEC causing HUS or HC in fewer cases and neglect considering the clinical picture of diarrhea?
- including a statement concerning the assessment of pathogenicity of all types of VTEC found in RTE food. Can pathogenicity be excluded for defined serotypes? Are VTEC (including *vtx*-pos., *eae*-neg., all serotypes) on RTE food generally a risk for consumers? If this is confirmed: is a seropathotype concept<sup>4</sup> sufficient for food safety issues?

### Revision of the Terms of Reference

Following discussion with the Austrian Federal Ministry of Health services, the Terms of Reference of the mandate have been revised and confirmed by the Austrian Federal Ministry of Health in an e-mail dated 23/11/2012.

The Austrian Federal Ministry of Health requests that, based on valid data and recent literature, EFSA provides a Scientific Opinion on:

1. The ‘seropathotype’ concept – the limitation to “relevant” serotypes O157, O26, O103, O111, O145, O121, O91, O104, O113<sup>4</sup> i.e., can pathogenicity be excluded for defined VTEC serotypes?;
2. justification of the statement: ‘*seropathotypes D and E are not HUS-associated and are uncommon in man or only found in non-human sources*’<sup>4</sup>;
3. an alternative concept based on detection of verocytotoxins, or genes encoding for verocytotoxins, in isolates;
4. the contribution by VTEC to diarrhoeal cases and to more severe outcomes in the EU, based on hazard identification and characterisation, and under-reporting in EU;
5. the public health risk associated with the contamination of RTE foods with VTEC, considering either the seropathotype concept or the detection of verocytotoxins or genes encoding the production of such toxins in isolates.

## APPROACH TAKEN

- Hazard identification, including a summary of the types of *Escherichia coli* pathogenic for humans and the putative virulence factors therein amongst serotypes from cases of human infection; the use of TESSy data (ECDC) for assessing the current situation regarding human infections with verocytotoxin-producing *E. coli* (VTEC) in the EU;
- Review of methods for the isolation and identification of VTEC, including detection of virulence factors and characterisation and typing of VTEC strains and virulence genes therein;
- Hazard characterisation, including illnesses associated with VTEC and identification of predictive factors for VTEC that may contribute to human disease;
- Evaluation of the seropathotype concept using the Karmali approach, a modification of the Karmali approach based on the health outcome of reported confirmed VTEC cases in the EU during 2007-2010, and a new approach based on serogroup information and utilising molecular virulence characteristics additional to the presence of *vtx* genes;
- Exposure assessment, including EU monitoring data on occurrence of VTEC in ready-to-eat (RTE) food.

## ASSESSMENT

### 1. Introduction

Illnesses associated with verocytotoxin-producing *Escherichia coli* (VTEC<sup>9</sup>) range from mild to bloody diarrhoea through to haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS), and thrombocytopenia. Such symptoms are common to VTEC infections worldwide.

To assist in assessing the clinical and public health risks associated with different VTEC strains an empirical VTEC seropathotype classification, based on their reported frequency in human disease, their known association with outbreaks and the severity of the outcome including HUS and HC was proposed by Karmali and colleagues in 2003 (Karmali et al., 2003). This classification system, presented in Table 1, utilises a gradient ranging from seropathotype A – high risk – to seropathotypes D and E – minimal risk. This approach has been of considerable value in defining pathogenic VTEC serotypes of importance in cases of human infection (Caprioli et al., 1997; Coombes et al., 2011; EFSA, 2007) and also for VTEC isolates from ruminants (for review, see Gyles (2007)).

**Table 1:** Classification of VTEC serotypes into seropathotypes (Karmali et al., 2003)

Seropathotype	Relative incidence <sup>(a)</sup>	Frequency of involvement in outbreaks	Association with severe disease <sup>(b)</sup>	Serotypes
A	High	Common	Yes	O157:H7, O157:NM <sup>(c)</sup>
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM <sup>(c)</sup> , O121:H19, O145:NM <sup>(c)</sup>
C	Low	Rare	Yes	O91:H21, O104:H21, O113:H21, other <sup>(d)</sup>
D	Low	Rare	No	Multiple <sup>(e)</sup>
E	Non-human only	NA <sup>(f)</sup>	NA <sup>(f)</sup>	Multiple <sup>(g)</sup>

(a): Reported frequency in human disease.

(b): Haemolytic uraemic syndrome (HUS) or haemorrhagic colitis (HC).

(c): NM = non-motile.

(d): Includes O5:NM, O121:NM, O165:H25.

(e): Includes O7:H4, O69:H11, O103:H25, O113:H4, O117:H7, O119:H25, O132:NM, O146:H21, O171:H2, O172:NM, O174:H8, Orough:H2.

(f): NA = not applicable.

(g): Includes O6:H34, O8:H19, O39:H49, O46:H38, O76:H7, O84:NM, O88:H25, O98:H25, O113:NM, O136:H12, O136:NM, O153:H31, O156:NM, O163:NM.

The most common serotype worldwide associated with both outbreaks and sporadic cases has undoubtedly been *E. coli* O157:H7. Recent developments, and in particular the increasing number of reports of non-O157 VTEC outbreaks and cases, and the major outbreak of the serotype O104:H4, first identified in northern Germany in May 2011 (see section 2.2.2.1.), has focused attention on the applicability or otherwise of the Karmali seropathotype concept.

In response to a request from the Austrian Federal Ministry of Health, this Opinion presents an assessment of the validity of the Karmali seropathotype concept in relation to food safety, for the most part based on the use of data from the European Surveillance System (TESSy data) as provided by the ECDC<sup>10</sup> (European Centre for Disease Prevention and Control) for assessing human infections with VTEC in the EU from 2007 to 2010. TESSy data for 2011 were not available for use in this Opinion.

<sup>9</sup> Verocytotoxin-producing *Escherichia coli* is also known as verotoxigenic *E. coli*, verocytotoxigenic *E. coli*, verotoxin producing *E. coli* and Shiga toxin-producing *Escherichia coli* (STEC).

<sup>10</sup> ECDC, TESSy Release on 01/11/2012. ECDC has no responsibility for the results and conclusions when disseminating the results of the work employing TESSy data supplied by ECDC.

Reference to human VTEC data for 2011 was therefore based on the information available in the EU Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2011 (EFSA and ECDC, 2013).

## 2. Hazard identification

### 2.1. Pathogenic *Escherichia coli*, including VTEC

*Escherichia coli* strains, which form part of the flora of the intestine, can cause enteric/diarrhoeogenic or extra-intestinal (ExPEC) infections in humans. ExPEC infections are primarily urinary tract (caused by uropathogenic *E. coli* – UPEC) and sepsis/meningitis (particularly neonatal meningitis). Only the enteric *E. coli* will be covered in this Opinion.

Traditionally enteric *E. coli* that cause disease have been divided into six pathotypes (for review, see Clements et al. (2012)): (i) verocytotoxigenic *E. coli* (VTEC), which is synonymous with the term ‘STEC’ (Shiga toxin-producing *E. coli*), and also includes the enterohaemorrhagic *E. coli* (EHEC) category; (ii) enteropathogenic *E. coli* (EPEC); (iii) enterotoxigenic *E. coli* (ETEC); (iv) enteroaggregative *E. coli* (EAEC); (v) enteroinvasive *E. coli* (EIEC) and (vi) diffuse adherent *E. coli* (DAEC) (Table 2). Of these, isolates belonging to VTEC pathotypes and one EAEC pathotype (EAEC O104:H4) are of particular importance in the context of food safety.

VTEC are characterised by the production of verocytotoxins (Vtx) (because of their cytotoxicity to Vero cells), and are also known as Shiga toxins (Stx), because of their similarity with the toxin produced by *Shigella dysenteriae*.

EHEC are a subset of VTEC, that in addition to the *vtx*-encoding genes, usually carry the attaching and effacing gene (*eae*, intimin-coding) and thereby have the ability to cause attaching and effacing (A/E) lesions in infected cells. The ability to cause A/E lesion is mediated by the locus of enterocyte effacement (LEE) pathogenicity island (PAI). EHEC strains are typically isolated from cases of severe disease.

EPEC carry the *eae* gene but do not produce Vtx. They are subdivided into typical and atypical strains based on the presence (or absence) of the EPEC Adherence Factor (EAF) plasmid. Typical EPEC carry this plasmid, which includes the bundle forming pili (*bfp*) operon encoding the pili required for localised adherence on epithelial cells. ETEC are associated with traveller’s diarrhoea. ETEC adhere to the epithelium of the small intestine using one or more colonisation factor antigens (CFA), and produce heat-stable (ST) and/or heat-labile (LT) enterotoxins.

EAEC are characterised by their ability to aggregatively adhere to tissue culture cells in a distinct ‘stacked and brick-like’ manner which is mediated by aggregative adherence fimbriae (AAF). They usually produce an enteroaggregative heat-stable toxin (EAST1) encoded by the plasmid-borne *astA* genes.

EIEC invade gut epithelial cells in a process mediated by invasion plasmid antigens (Ipa) encoded in the *ipa* operon that is carried on a 220 kilobase (kb) virulence plasmid. Illness is characterised by the appearance of blood and mucus in the faeces.

DAEC are comprised of a heterogenous group of *E. coli* with variable virulence. They are identified by their adherence to HEp-2 cells in a diffuse pattern.

Different *E. coli* serogroups or serotypes may belong to more than one pathotype group. For example O26 may be an EPEC or a VTEC and the major 2011 outbreak *E. coli* O104:H4 strain had characteristics of both the VTEC and EAEC categories.

**Table 2:** Summary of virulence factors expressed by the human enteric *E. coli* pathotypes (adapted from Clements et al. (2012))

Pathotype	Adhesin	Toxin	T3SS <sup>(a)</sup>	SPATE <sup>(b)</sup>	Symptomology/Illness/Disease
<b>VTEC</b>	Aggregative adherence fimbriae (AAF) IrgA homologue adhesin (Iha) STEC autoagglutinating adhesion (Saa)	Verocytotoxin (Vtx) <sup>(c)</sup>	-	Pic Pet	Mild to severe bloody diarrhoea through to HC, HUS, and thrombocytopenia
<b>Including EHEC<sup>(d)</sup></b>	Intimin Paa Toxin B (ToxB) <i>E. coli</i> factor for adherence (Efa)-1 LPF Saa <i>E. coli</i> immunoglobulin-binding protein (EibG) EHEC autotransporter encoding gene A (EhaA) Outer membrane protein A (OmpA) Iha	Vtx <sup>(c)</sup>	LEE encoded	EspP	As above
<b>DAEC</b>	Afimbrial (Afa) or fimbrial (Dr) adhesins	-	-	Sat	Acute diarrhoea (<5 years old)
<b>EPEC</b>	Intimin Bundle forming pili (BFP) Paa LPF Iha EhaA	-	LEE encoded	EspC	Infant diarrhoea
<b>ETEC</b>	Colonization factors (CF) Porcine A/E associated adhesin (Paa)	Heat-labile enterotoxin (LT) Heat-stable enterotoxin (ST) Cytolysin A (ClyA)	-	ETEC autotransporter A (EatA)	Acute watery diarrhoea (<5 years old) Travellers' diarrhoea
<b>EAEC</b>	AAF (I, II, III, Hda) Toxigenic invasion loci A (Tia)	EAEC heat-stable enterotoxin 1 (EAST1) <i>Shigella</i> enterotoxin (ShET)1 Haemolysin E (HlyE)	+/- <sup>(e)</sup>	Plasmid-encoded toxin (Pet) Protein involved in intestinal colonization (Pic) Secreted autotransporter toxin (Sat) <i>Shigella</i> IgA-like protease homology (SigA) <i>E. coli</i> -secreted protein (Esp)P	Travellers' diarrhoea Infant diarrhoea
<b>EIEC (Shigella)</b>	-	ShET1/2	pINV encoded	<i>Shigella</i> extracellular protein (Sep)A SigA	Shigellosis

(a): Type three secretion system.

(b): SPATE = serine protease autotransporter of *Enterobacteriaceae*.

(c): Verocytotoxin (Vtx) is also known as Shiga toxin (Stx).

(d): EHEC may also be subgroup of EAEC.

(e): One potentially functional but as yet uncharacterised T3SS (ETT2) was found in the genome sequence of EAEC O42 (and remnants of a second).



Infections with *E. coli* occur through consumption of contaminated food products (e.g. undercooked meat, or fresh produce such as salad leaves), drinking water contaminated with animal or human waste, contact with animals, the environment, or through direct person-to-person or animal-to-person spread. In the developing world ETEC, EPEC and EAEC appear to be major causes of infantile diarrhoea with potentially fatal consequences when untreated. In contrast, in the developed world such infections are mild and self-limiting and VTEC are the main *E. coli* pathogens associated with food-poisoning outbreaks (Pennington, 2000; Whittaker et al., 2009; Willshaw et al., 2001a), with institutional outbreaks involving person-to-person transmission (Devakumar et al., 2013), and with outbreaks associated with open farms (Underwood et al., 2013).

Various factors and toxins contribute to the virulence of VTEC. Verocytotoxin type 2 (Vtx2) is more often associated with cases of human disease, and those strains producing this toxin are more frequently associated with severe illness. Strains that produce Vtx2 and more specifically Vtx2c subtype c (Vtx2c), have been suggested to be more likely to cause HUS than those that produce Vtx1 alone (Bosilevac and Koochmarai, 2011; Friedrich et al., 2002). Except for the intimin protein encoded by the LEE PAI and the AAF encoded by the EAEC plasmid (for review see Kaper et al. (2004)), to our knowledge there are no other adherence factors that have been consistently associated with the virulence of EHEC and VTEC respectively (Nataro and Kaper, 1998; Nataro et al., 1998).

## 2.2. EU monitoring data on VTEC in humans

### 2.2.1. Sporadic human cases

The case classification of a confirmed case of STEC/VTEC is defined in Decision (EC) No 2012/506/EU<sup>11</sup> as ‘any person meeting the clinical criteria (for STEC/VTEC diarrhoea: any person with at least the following two: diarrhoea or abdominal pain; and for HUS: any person with acute renal failure and at least one of the following two: microangiopathic haemolytic anaemia or thrombocytopenia) and at least one of the four laboratory criteria: (1) isolation of an *Escherichia coli* strain that produces Shigatoxin (*Stx*) or harbours *stx1* or *stx2* gene(s); (2) isolation of non-sorbitol-fermenting (NSF) *Escherichia coli* O157 (without *Stx* or *stx* gene testing); (3) direct detection of *stx1* or *stx2* gene(s) nucleic acid (without strain isolation); (4) direct detection of free *Stx* in faeces (without strain isolation)’. In the Annex of this Decision, it is explained that a confirmed case means a case classified as confirmed for reporting purposes. Confirmed cases are laboratory-confirmed and may or may not fulfil the clinical criteria as described in the case definition.

Detection of VTEC is highly dependent on the methods applied to clinical specimens. Such methods vary markedly between different EU Member States (MSs) and, VTEC O157 is more readily detected than non-O157 VTEC. Thus data relating to non-O157 VTEC probably represent a substantive under-estimation of its true incidence, both for the EU as a whole and particularly for those MSs where molecular detection methods are not as yet fully utilised.

A total of 4 000 confirmed human VTEC cases were reported from 25 EU MSs in 2010 through TESSy; the EU notification rate being 0.83 cases per 100 000 population (EFSA and ECDC, 2012). In 2011, due to the O104:H4 outbreak (see section 2.2.2.1.), a large increase was observed and 9 485 confirmed VTEC cases were reported from 23 MSs (EFSA and ECDC, 2013).

Full serotype data on VTEC isolates were reported for 1 288 and 686 (32 % and 7.2 %) of confirmed infections whereas data on the ‘O’ (lipopolysaccharide) antigen were reported for 68 % and 55.9 % of such infections in 2010 and 2011 respectively. In 2010 almost half of the reported ‘O’ serogroups were O157 (41.1 %) (Table 3). In 2011, the most commonly reported ‘O’ serogroups were O157 (41.2 %) followed by O104 (20.1 %). The latter was due to the O104:H4 outbreak. Only two cases of serogroup O104 were reported in 2010.

<sup>11</sup> Commission Implementing Decision (EC) No 2012/506/EU of 8 August 2012 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. OJ L 262, 27.09.2012, p. 1-57.



**Table 3:** Reported confirmed VTEC cases in humans in the EU<sup>(a)</sup> by serogroup, 2007-2011, EFSA and ECDC (2010, 2011, 2012, 2013)

2011			2010			2009			2008			2007		
Serogroup	<i>n</i>	% <sup>(d)</sup>	Serogroup	<i>n</i>	% <sup>(d)</sup>	Serogroup	<i>n</i>	% <sup>(d)</sup>	Serogroup	<i>n</i>	% <sup>(d)</sup>	Serogroup	<i>n</i>	% <sup>(d)</sup>
<b>Total typed</b>	<b>4 499</b>	<b>85.0</b>	<b>Total typed</b>	<b>2 413</b>	<b>66.1</b>	<b>Total typed</b>	<b>2 553</b>	<b>71.5</b>	<b>Total typed</b>	<b>2 340</b>	<b>74.1</b>	<b>Total typed</b>	<b>2 062</b>	<b>71.0</b>
O157	2 185	48.5	O157	1 501	62.2	O157	1 848	72.4	O157	1 673	71.5	O157	1 571	76.2
O104	1 064	23.6	O26	257	10.7	O26	192	7.5	O26	166	7.1	O26	136	6.6
O26	287	6.4	O103	90	3.7	O103	82	3.2	O103	88	3.8	O103	77	3.7
O103	141	3.1	O145	61	2.5	O91	48	1.9	O145	49	2.1	O91	43	2.1
O91	116	2.6	O91	57	2.4	O145	47	1.8	O91	50	2.1	O145	31	1.5
O145	76	1.7	O63	42	1.7	O146	31	1.2	O111	43	1.8	O111	23	1.1
O128	53	1.2	O111	41	1.7	O128	26	1.0	O128	28	1.2	O128	21	1.0
O111	52	1.2	O128	29	1.2	O111	25	1.0	O146	25	1.1	O113	16	0.8
O146	48	1.1	O146	28	1.2	O113	22	0.9	O117	20	0.9	O146	14	0.7
Other <sup>(b)</sup>	484	10.7	Other <sup>b</sup>	307	12.7	Other <sup>b</sup>	232	9.1	Other <sup>b</sup>	198	8.5	Other <sup>b</sup>	130	6.3
<b>NT<sup>(c)</sup></b>	<b>795</b>	<b>15.0</b>	<b>NT<sup>c</sup></b>	<b>1 238</b>	<b>33.9</b>	<b>NT<sup>c</sup></b>	<b>1 020</b>	<b>28.5</b>	<b>NT<sup>c</sup></b>	<b>819</b>	<b>25.9</b>	<b>NT<sup>c</sup></b>	<b>842</b>	<b>29.0</b>
<b>Grand total</b>	<b>5 301</b>		<b>Grand total</b>	<b>3 651</b>		<b>Grand total</b>	<b>3 573</b>		<b>Grand total</b>	<b>3 159</b>		<b>Grand total</b>	<b>2 904</b>	

(a): Austria, Belgium, Czech Republic (only 2011), Cyprus (only 2008), Denmark, Estonia, Finland (not 2011), France, Germany, Greece (only 2011 and 2010), Hungary (not 2008), Ireland, Italy, Luxembourg, Malta, Poland (not 2009), Romania (only 2011, 2010 and 2008), Slovakia, Slovenia, Spain, Sweden, the Netherlands and the UK.

(b): Other is other than top nine.

(c): NT = untyped/untypeable and cases where 'O' antigen was reported as unknown.

(d): The percentage for the serogroups is using the total typed as denominator.

The clinical outcome of the confirmed VTEC cases in the EU in 2007-2010 by serotype, based on the data as provided by the ECDC<sup>10</sup>, is presented in Appendix A, Table 1. In the period 2007-2010, 13 545 confirmed VTEC infections were reported to ECDC. For the majority of these cases, the clinical outcome was not reported: the case fatality was not reported for 52 % of these cases, hospitalisation was not reported for 90 % and HUS status was unknown for 41 %. Data on hospitalisation have only been collected for the last two years (2009 and 2010). The clinical manifestation (expressed as bloody diarrhoea, diarrhoea or asymptomatic) was not reported for 47 % of cases.

The virulence characteristics of the reported confirmed VTEC serogroups, in terms of presence of the *eae* gene as well as *vtx1* and *vtx2* genes, are listed in Table 4. These virulence characteristics were reported for isolates from 7 278 (54 %) out of 13 545 cases. Most cases (around 60 %), for which information was reported on virulence factors, were *eae,vtx2*-positive and this was particularly common for serogroup O157 (71 % of cases; 3 833 out of 5 412 cases) (Table 4). For VTEC O157, *eae* in combination with both verocytotoxin genes was also common (28 % of cases). Most isolates from cases associated with serogroups O26, O103 and O111 were *eae*- and *vtx1*-positive. Approximately 11 % of serogroups did not carry the *eae* gene (770 out of 7 278 cases). All but two reported cases caused by serogroup O91 – for which information was provided on virulence and virulence-associated factors – were *eae*-negative. The majority of reported cases by O117 and O146 serogroups were also *eae*-negative. The 2011 O104 outbreak strain was *eae*-negative and *vtx2*-positive (EFSA and ECDC, 2013).

**Table 4:** Virulence characteristics of reported confirmed VTEC serogroups from cases of human infection from 2007-2010 (based on TESSy data as provided by ECDC)

Serogroup	Total	Virulence <sup>(a)</sup> characteristics					
		<i>eae,vtx1</i>	<i>eae,vtx2</i>	<i>eae,vtx1,vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1,vtx2</i>
O157	5 412	31	3 833	1 530	1	13	4
O26	309	205	63	30	9	2	0
O103	176	168	4	2	1	1	0
O145	108	25	81	1	0	1	0
O91	69	0	1	1	35	5	27
O111	67	37	13	13	1	2	1
O117	64	2	0	1	60	1	0
O146	62	0	1	1	3	30	27
O128	61	1	7	5	7	19	22
O121	49	1	45	1	1	1	0
O113	35	0	5	0	2	18	10
O63	29	0	25	4	0	0	0
O174	23	0	0	0	1	12	10
O156	21	6	0	0	13	0	2
O76	21	2	0	0	15	1	3
O5	18	9	0	1	4	1	3
O55	14	3	4	0	7	0	0
O8	11	0	2	1	0	7	1
O153	11	0	7	0	1	2	1
O84	10	8	0	0	0	2	0
O181	10	0	0	0	4	0	6
Other <sup>(b)</sup>	231	26	51	10	42	80	22
NT <sup>(c)</sup>	467	88	112	41	88	89	49
<b>Total</b>	<b>7 278</b>	<b>612</b> <b>(8.4 %)</b>	<b>4 254</b> <b>(58.5 %)</b>	<b>1 642</b> <b>(22.6 %)</b>	<b>295</b> <b>(4.1 %)</b>	<b>287</b> <b>(3.9 %)</b>	<b>188</b> <b>(2.6 %)</b>

(a): *eae* = intimin-coding gene; *vtx1* = verocytotoxin 1 gene; *vtx2* = verocytotoxin 2 gene.

(b): Includes other serogroups than already listed. This group includes 83 serogroups.

(c): NT = untyped/untypeable and cases where 'O' antigen was reported as unknown.

During 2007-2010 the most commonly reported serotype was O157:H7 (774 out of 2 140 fully serotyped cases) followed by O157:H- (273) and O103:H2 (131). In 2011 the most commonly reported serotype was O104:H4 (118 out of 686 fully serotyped isolates), followed by O157:H- (117) and O157:H7 (114) (Table 5).

**Table 5:** VTEC O:H serotypes most commonly reported and confirmed in cases of human infection from 2007-2010 (based on TESSy data as provided by ECDC) and in 2011 (based on EFSA and ECDC (2013))

2007-2010			2011		
Serotype	No. of cases	% of cases	Serotype	No. of cases	% of cases
O157:H7	774	36.2	O104:H4 <sup>(a)</sup>	118	17.2
O157: H- <sup>(b)</sup>	273	12.8	O157: H-	117	17.1
O103:H2	131	6.1	O157:H7	114	16.6
O26:H11	107	5.0	O26:H11	39	5.7
O117:H7	55	2.6	O103:H2	30	4.4
O91:H-	44	2.1	O146:H21	16	2.3
O145:H-	33	1.5	O111:H-	14	2.0
O63:H6	31	1.4	O26: H-	13	1.9
O128:H2	30	1.4	O145:H-	9	1.3
O111:H-	29	1.4	O145:H34	8	1.2
O146:H21	27	1.3	O128:H2	7	1.0
O121:H19	23	1.1	O91:H14	5	0.7
O26:H-	20	0.9			
Other	563	26.3	Other	196	28.6
<b>Total</b>	<b>2 140</b>	<b>100</b>	<b>Total</b>	<b>686</b>	<b>100</b>

(a): This serotype O104:H4 was common in 2011 due to the extensive 2011 O104:H4 outbreak.

(b): H- = the flagellar or 'H' antigen was analysed but was absent.

### 2.2.2. EU food-borne outbreaks

EFSA coordinates the annual reporting of zoonoses, zoonotic agents, antimicrobial resistance, food-borne outbreaks and animal populations in the EU under the Directive 2003/99/EC,<sup>12</sup> as well as analysing and summarising the data collected.

The zoonoses reports also include data on food-borne outbreaks and has been mandatory for EU MSs since 2005. Starting in 2007, harmonised specifications on the reporting of these outbreaks at the EU level have been applied<sup>13</sup>. Since 2010, revised reporting specifications for food-borne outbreaks were implemented and the distinction between 'verified' and 'possible' food-borne outbreaks was changed to 'strong' or 'weak' based on the strength of evidence implicating a suspect food vehicle.

Food-borne outbreak investigation systems at the national level are not harmonised between MSs. Consequently, the differences in the numbers and types of reported outbreaks, as well as the causative agents, may not reflect differences in food safety between MSs but may be more indicative of differences in the efficiency and sensitivity of the national monitoring systems for identifying and investigating food-borne outbreaks. Nevertheless this zoonosis reporting represents the most comprehensive set of data for the EU.

<sup>12</sup> 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.

<sup>13</sup> EFSA (European Food Safety Authority), 2007. Report of the Task Force on Zoonoses Data Collection on harmonising the reporting of foodborne outbreaks through Community reporting system in accordance with Directive 2003/99/EC. EFSA Journal, 123, 1-16.

Food-borne outbreak data from 2007 to 2011 have been extracted and used for this Opinion. In this period, 306 food-borne outbreaks caused by human pathogenic *E. coli* were reported in the EU (Table 6). Most outbreaks ( $n = 197$ ) were ‘weak evidence’ outbreaks (or possible outbreaks). Seventy-three outbreaks were supported by ‘strong evidence’ (or verified outbreaks) and involved 5 212 confirmed cases, of which 4 250 were reported in 2011. In 2011, twelve MSs reported a total of 60 food-borne outbreaks caused by pathogenic *E. coli*. This represents 1.1 % of the total number of reported food-borne outbreaks in the EU. Fourteen *E. coli* outbreaks (23.3 %) were supported by ‘strong evidence’. The term ‘pathogenic *E. coli*’ is used here since consolidated data on all *E. coli* types causing food-borne outbreaks (including VTEC) are only collected as part of the zoonoses monitoring process.

**Table 6:** ‘Strong’ and ‘weak evidence’ food-borne outbreaks caused by pathogenic *E. coli* (excluding ‘strong evidence’ waterborne outbreaks) in the EU, 2007-2011. Based on EFSA and ECDC (EFSA and ECDC, 2010, 2011, 2012, 2013)

Year	Total outbreaks	‘Strong evidence’ outbreaks <sup>(a)</sup>				‘Weak evidence’ outbreaks <sup>(b)</sup>			
		<i>n</i>	Cases	Hospitalised	Deaths	<i>n</i>	Cases	Hospitalised	Deaths
2007	65	29	541	24	0	-	-	-	-
2008	75	10	135	8	0	65	204	40	0
2009	75	18	228	62	0	57	367	33	0
2010	31	2	58	2	0	29	131	28	0
2011	60	14	4 250	2 495	54	46	226	29	0
<b>Total</b>	<b>306</b>	<b>73</b>	<b>5 212</b>	<b>2 591</b>	<b>54</b>	<b>197</b>	<b>928</b>	<b>130</b>	<b>0</b>

(a): Reported as verified outbreaks in 2007-2009.

(b): Reported as possible outbreaks in 2008-2009, not reported in 2007.

The clinical outcome of cases was also different in the last year of reporting. In the period 2007-2010, 96 cases out of the 962 (10.0 %) cases were hospitalised with no case fatalities. In contrast in 2011, 2 495 cases were hospitalised and 54 fatal cases occurred. The 2011 O104:H4 outbreak was responsible of 89.2 % of cases, 94.3 % of hospitalisations and 98.1 % of deaths related to ‘strong evidence’ outbreaks due to pathogenic *E. coli*.

Detailed information (i.e. the number of cases, hospitalisations and deaths) on the ‘strong evidence’ outbreaks in the EU in 2007-2011 is provided in Appendix B, Table 1.

Twelve of the 14 ‘strong evidence’ pathogenic *E. coli* outbreaks reported in 2011 were due to VTEC. Different serogroups/types were reported: VTEC O157 in seven outbreaks, VTEC O27:H30 in one, and *E. coli* O104:H4 in four outbreaks, all of which were related to the original outbreak reported by Germany (see section 2.2.2.1.). ‘Vegetables, juices and other products thereof’ were involved in seven outbreaks (50.0 %) and three of those seven were caused by the consumption of sprouted imported fenugreek seeds (i.e. the 2011 O104:H4 outbreak in Germany and related outbreaks in Denmark and the Netherlands). The remaining three were linked to imported sugar peas, mixed salad, raw leeks and raw potatoes. France reported an outbreak associated to ‘vegetables and juices and other products thereof’ without any additional foodstuff information.

In 2010 a ‘strong evidence’ outbreak of VTEC O26 with contaminated cheese the implicated food was reported by Germany. In an outbreak in Spain in 2010 the implicated food vehicles were crustaceans, shellfish, molluscs and products thereof. In previous years the reported outbreak strains were VTEC O157:H7 (German outbreak in 2008 with raw milk as implicated food), VTEC O157 (Irish outbreak in 2007 with well-water implicated), VTEC O76 (Swedish outbreak in 2007 with cheese as the implicated food), and VTEC O26:H- (outbreak in Denmark in 2007 with organic sausage as the implicated food).

Within the O26 serogroup isolates of O26:H11 are classified as seropathotype B. Only the O76:H7 serotype was included in Karmali et al. (2003), and was assigned to seropathotype E. Full serotyping information was reported in only six VTEC outbreaks during the period of 2007-2011; this was the

case for one outbreak by VTEC O27:H30, one by O157:H7, one by O26:H- and in 2011 four by VTEC O104:H4. Of these, only O157:H7 was included in Karmali et al. (2003) and was assigned to seropathotype group A.

Although undoubtedly caused by pathogenic VTEC, the lack of reporting the 'O' antigen in outbreaks, and the lack of reporting the 'H' antigen in the outbreaks described above (several by O157, one by O26, one by O76), exemplifies a critical weakness of classifying all human VTEC outbreak isolates using the seropathotype concept.

#### 2.2.2.1. The 2011 *E. coli* O104:H4 outbreak

The most recent example of a major outbreak caused by a non-O157 VTEC was the O104:H4 outbreak, first identified in northern Germany in May 2011 (Frank et al., 2011a). This outbreak resulted in 4 321 confirmed of VTEC infection and 852 of HUS, with 54 deaths reported in 14 EU countries, the USA and Canada when the epidemic was declared to be over at the end of July 2011 (Buchholz et al., 2011; Karch et al., 2012). The outbreak was unusual because of the high proportion of adult patients (ca. 25 %) presenting with HUS, plus the frequent development of neurological symptoms in these patients (Frank et al., 2011b). These clinical characteristics were thought to be due to the unique combination of traits carried by the pathogen, which included features typical of EAEC, together with the capacity to produce Vtx (Frank et al., 2011b). The strain also has a distinct set of additional virulence and antibiotic resistance genes (Rasko et al., 2011). Whole genome sequence analysis has suggested that the clinical characteristics of the outbreak strain were due to the unique combination of virulence factors carried by the pathogen and acquired by horizontal gene transfer (Frank et al., 2011a; Frank et al., 2011b).

#### 2.2.3. Under-estimation considerations

The above monitoring data should be interpreted with care. Such data are largely based on passive surveillance and as such underestimate the true incidence of human VTEC infections. In line with ECDC (ECDC, 2011) under-ascertainment refers specifically to cases or exposure in the community which are not recorded by a notification or surveillance system, because health care advice may not have been sought. Under-reporting refers more specifically to cases where healthcare advice is sought but the infection status is misdiagnosed, misclassified, miscounted or the information summarised, meaning that full details are not passed on to national monitoring. In short, under-ascertainment occurs within the community and under-reporting occurs within institutes and involves physicians, hospitals, laboratories, governmental organisations and networks. Furthermore, detection will be highly dependent on the methods applied to clinical specimens. Such methods vary markedly between different MSs, and consequently VTEC O157 is more readily detected than non-O157 VTEC.

There have been attempts to more accurately estimate the true prevalence of VTEC infection in both the EU and the USA. To estimate the true number of illnesses due to VTEC in the EU, the notified number of cases can be multiplied with a 'disease-multiplier', which is a hazard-specific value that expresses the degree of under-reporting and under-ascertainment. Haagsma et al. (2012) have developed a transparent model to reconstruct the surveillance pyramid for seven pathogens that cause gastroenteritis, including VTEC O157, in seven EU MSs (see Table 7). Furthermore, they estimated differences in the proportions of patients within the community, consulting a General Practitioner (GP) and becoming hospitalised. Disease-multipliers for VTEC O157, including both under-ascertainment and under-reporting, differed widely between countries, and ranged from 13 to 87 (Table 7). The authors considered that the substantive differences in disease-multipliers were mainly due to the differences in the proportion of patients visiting a GP and in the proportion of patients submitting a stool sample.

**Table 7:** Median values of disease-multipliers, incidence rates and annual numbers of VTEC O157 infections (Haagsma et al., 2012)

	Germany	Denmark	the Netherlands	Sweden	UK
Disease-multiplier <sup>(a)</sup>	23	33	87	13	34
Population <sup>(b)</sup>	2.2 (2 400)	85 (4 600)	76 (12 000)	15 (1 360)	50 (25 000)
GP <sup>(b),(c)</sup>	1.1 (890)	37 (2 000)	19 (3 000)	5.1 (450)	6.8 (3 400)
Hospital <sup>(b)</sup>	0.2 (180)	0.9 (50)	0.3 (40)	1.6 (140)	3.1 (1 500)

(a): Includes both under-ascertainment and under-reporting.

(b): Incidence rate per 100 000 person-years (median annual number of infections).

(c): GP = General Practitioner.

In the USA, disease-multipliers are available for both VTEC O157 and non-O157 VTEC (Scallan et al., 2011). For VTEC O157, the disease-multiplier is 26.1, while for non-O157 VTEC it is 106.8. This multiplier is only composed of under-ascertainment as it is stated that there is no under-reporting for these pathogens in the USA, in the Food-borne Diseases Active Surveillance Network (Food-Net). Based on the USA figures the disease-multipliers for the EU as a whole are estimated as 51.2 for VTEC O157 and 209.6 for non-O157 VTEC. This approach used to calculate these multipliers assumes that the relative degree of under-reporting between hazards is the same in the USA and EU. Detailed information can be found in a previous EFSA Opinion (EFSA Panel on Biological Hazards, 2013).

The estimated disease-multipliers are presented in Table 8 and the estimated true number of illnesses is calculated by the product of the disease-multiplier and the notified number of cases of VTEC O157, non-O157 VTEC and ‘untyped/untypeable’ VTEC. The yearly true number of VTEC cases (based on data from 2007-2010) is therefore estimated as 446 101 of which 85 222 (19.1 %) are due to VTEC O157 and 149 445 (33.5 %) to non-O157 VTEC (the remainder due to ‘untyped/untypeable’ VTEC). When the estimation is based on data that also includes 2011, the yearly true number of VTEC cases is somewhat higher (509 680 cases) as a result of a higher incidence of non-O157 VTEC. It should be noted that in 2011 the multipliers, in particular for non-O157 VTEC, may have been overestimated because of active case seeking due to the O104 outbreak. For comparison, the yearly true number of cases of salmonellosis and campylobacteriosis in the EU were estimated at around 6 and 9 million, respectively (EFSA Panel on Biological Hazards, 2012b, 2012c; Havelaar et al., 2013). VTEC infections are more severe. Havelaar et al. (2012) estimated that the burden per case (in Disability Adjusted Life Years (DALY)) was approximately 3-fold higher for VTEC compared to salmonellosis and campylobacteriosis.

**Table 8:** Estimated true number of illnesses per year in the EU by VTEC, VTEC O157 and non-O157 VTEC based on the application of disease-multipliers

Serotype	Estimated disease-multipliers in EU <sup>(a)</sup>	Notification per year <sup>(b)</sup> , average 2007-2010 (average 2007-2011)	Estimated true incidence data at the EU level <sup>(c)</sup> , average 2007-2010 (average 2007-2011)
VTEC O157	51.2	1 665 (1 768)	85 222 (90 522)
non-O157 VTEC	209.6	713 (1 034)	149 445 (216 684)
VTEC NT <sup>(d)</sup>	209.6	1 009 (966)	211 434 (202 474)
Total VTEC		3 386 (3 768)	446 101 (509 680)

(a): Disease-multipliers for each pathogen based on the estimates published by Scallan et al. (2011) and anchored to the *Salmonella* disease-multiplier estimated at the EU level by Havelaar et al. (2012).

(b): Reported confirmed VTEC cases in humans in EU.

(c): Estimated true number of illnesses in the EU per year calculated by the product of the *Salmonella* based disease-multiplier and the notified number of cases as reported to ECDC database TESSy (The European Surveillance System).

(d): VTEC NT group (untyped/untypeable and cases where ‘O’ antigen was reported as unknown). It is assumed that the disease-multiplier for this group is the same as the one for non-O157 VTEC.



Cohort studies have provided insight in the degree of under-reporting and under-diagnosis of diseases caused by gastrointestinal pathogens. Amongst 395 patients with diarrhoea-associated HUS in the UK and Ireland who were ill between 1985-1988 and 1997-2001, VTEC was detected in 330 (84 %), of which there was evidence for *E. coli* O157 in 329; the remaining patient was infected with *E. coli* O26 (Lynn et al., 2005). Of the remaining 65 patients, no infective agent was detected in 59 and a range of other pathogens were detected in the remaining six. In this study presumptive *E. coli* O157 isolates were confirmed biochemically as *E. coli* and as O157 by serotyping. Confirmed O157 isolates were tested for *vtx* genes by DNA hybridisation or PCR; serum samples were tested for antibodies to *E. coli* O157 lipopolysaccharide (LPS).

The number of asymptomatic cases is extremely difficult to estimate since healthy people rarely have samples of faeces examined for VTEC. In a study in Scotland between 1999 and 2008 (Locking et al., 2011), of the 2 228 individuals where VTEC O157 had been isolated (including contacts of cases), 202 (9.1 %) individuals were asymptomatic.

Two large prospective, population-based studies of infectious intestinal disease (IID) incidence and aetiology have been conducted in the UK in 1993–1996 (IID1) and in 2008–2009 (IID2). Both culturing for VTEC O157 as well as PCR-based procedures for the detection of *vtx* genes were applied (Tam et al., 2012; Tompkins et al., 1999). In the latter study, within patients with diarrhoea VTEC O157 was detected by culture in one out of 866 patients and non-O157 VTEC in seven out of 866 patients. The IID1 study isolated non-O157 VTEC more frequently from asymptomatic controls than from cases. VTEC O157 was isolated from a small number of cases, but not from asymptomatic controls (Tompkins et al., 1999). Both the IID 1 and IID 2 studies have allowed an estimate of the degree of under-reporting and under-diagnosis in the UK.

Because of phenotypic diversity, there are no simple, generally applicable culture-based tests for the detection of non-O157 VTEC in faecal specimens. The UK Health Protection Agency National Standard Methods,<sup>14</sup> that are widely used in clinical microbiology laboratories in England and Wales for diagnosis and identification of *E. coli* O157, are not effective for the detection of VTEC generally. A small number (<15) of isolates of non-O157 VTEC are identified annually in England and Wales by diagnostic testing using a combination of PCR and culture of bacteria from faeces associated with cases of HUS and bloody diarrhoea but their true incidence is unknown. In a recent evaluation of the European Union Reference Laboratory (EU-RL-VTEC) guidelines, a real-time PCR for the detection of VTEC in faeces (Jenkins et al., 2012) was applied to 500 stool samples from patients with diarrhoea. PCR detected *vtx* genes in 62 samples, of which VTEC was recovered by culture in 36, 23 were O157, and 13 were other 'O' types (O26, O103, O104, O111, O117 and O186).

In other countries, non-O157 VTEC has been identified as a significant cause of infectious intestinal disease, including HUS (Mellmann et al., 2008; Tozzi et al., 2003), and its incidence has been found to exceed that of VTEC O157. This may reflect both actual prevalence and differences in testing methods. Recent advice for the USA (Gould et al., 2009) has recommended that all stools submitted for routine testing from patients with acute community-acquired diarrhoea (regardless of patient age, season of the year, or presence or absence of blood in the stool) be simultaneously cultured for *E. coli* O157:H7 and tested with an assay that detects verocytotoxins or toxin genes to detect non-O157 VTEC.

VTEC infections and HUS occur in persons of all ages, but the incidence of VTEC infection is highest in children aged <5 years, as is the risk of HUS (Lynn et al., 2005). The true incidence of non-O157 VTEC infections is probably underestimated because standard stool culture methods routinely used in many clinical laboratories do not detect these bacteria. Surveillance of paediatric HUS provides valuable information on human infection with VTEC. In a prospective survey of paediatric HUS from 1985 to 1988 in the British Isles, the average annual incidence was 79 per 100 000 children under 16 years of age (Lynn et al., 2005). In the intervening years, the number of laboratory-confirmed cases of

<sup>14</sup> <http://www.hpa-standardmethods.org.uk/>



VTEC O157 in England and Wales increased from 50 in 1985 to 1 087 in 1997 (Lynn et al., 2005). Similar increases were seen in Scotland and Ireland. Some of the increase in VTEC O157 might reflect improved laboratory techniques, improved detection or reporting of milder cases, and a greater awareness of the need to investigate diarrhoeal disease for VTEC O157 and VTEC O111, which have a high likelihood of infection at low infectious exposures (<100 organisms) (EC, 2003). The pathogenic potential of other serogroups in England and Wales is not known.

### 2.3. Conclusions

The Karmali seropathotype model does not define pathogenic VTEC or provide an exhaustive list of pathogenic serotypes. Instead it classifies VTEC based on their reported frequency in human disease, their known association with outbreaks and their severity of the outcome including HUS and HC.

EHEC are a subset of VTEC that, in addition to the verocytotoxin-encoding genes, usually carry the attaching and effacing intimin-coding (or *eae* gene) and thereby have the ability to cause attaching and effacing lesions governed by the LEE PAI. Such strains are typically isolated from cases of severe disease.

In the period 2007-2010, for the majority of the 13 545 confirmed VTEC cases, the clinical outcome was not reported: the case fatality was not reported for 52 % of these cases, hospitalisation was not reported for 90 % and HUS status was unknown for 41 %. The clinical manifestation (expressed as bloody diarrhoea, diarrhoea or asymptomatic) was not reported for 47 % of the cases.

Full serotyping information was reported for only six VTEC outbreaks (period 2007-2011). Of these, only O157:H7 could be assigned to a seropathotype group using the Karmali classification, as the other strains were not included in the study by Karmali et al. (2003).

The degree of under-estimation (including under-ascertainment and under-reporting) of VTEC O157 infections has been estimated in seven EU MSs. Disease-multipliers differ widely between countries, ranging from 13 to 87. Assuming that the relative degree of under-reporting between hazards is the same in the USA and EU, the disease-multipliers for the EU as a whole are estimated as 51.2 for VTEC O157 and 209.6 for non-O157 VTEC.

## 3. Microbiological methods for VTEC

Methods for the isolation and identification of VTEC (both O157 and non-O157) from food, water and environmental (FW&E) samples, the detection of virulence factors and the characterisation and typing of VTEC strains and virulence genes therein are discussed below. Of note are changes in detection strategy and of identification of the factors contributing to virulence following the 2011 outbreak of *E. coli* O104:H4.

### 3.1. Methods for isolation and identification of VTEC

Detection of VTEC in complex matrices represents a challenge, and may be affected by different types of clinical specimens or FW&E samples.

Analysis of clinical specimens for the detection of VTEC is in principle less problematic than their detection in FW&E samples. In cases of VTEC disease, the pathogen often constitutes the principal bacterial population in the specimen and the identification of the presence of the *vtx*-encoding genes or the Vtx is enough to come to a correct diagnosis. Moreover, selection of the specimens to be included in the screening for such pathogens may be operated according to the symptoms shown by the patient. Nevertheless, VTEC can cause diseases characterised by a broad range of symptoms many of which can be also ascribed to infections caused by a number of other enteric pathogens. Incorrect differential diagnosis may therefore occur, and together with the general lack of scientific and technical skills required for managing DNA- or toxin-based assays, may contribute to the low application of routine screening programmes for VTEC in clinical microbiology laboratories.

The screening of FW&E samples for the presence of bacterial pathogens is intended to be proactive in protecting people from acquiring the infections. This implies the availability of markers with a high predictive value associated with pathogenicity. While for some bacterial pathogens the identification of the presence of microorganisms belonging to a given species is regarded as a risk for the consumer in its own right (e.g. *Salmonella* spp.), this is not applicable to *E. coli*.

VTEC differ from commensal *E. coli* by the presence of the *vtx* genes and, in addition, by a complex array of accessory genetic determinants associated with virulence, including those encoding factors involved in colonization, other toxins and immunomodulators (Imamovic et al., 2010; Morabito et al., 2003; Tozzoli et al., 2005). The virulence genes are heterogeneously distributed among VTEC strains but only a sub-population of strains have been associated with the most severe forms of infections and contain a complete set of virulence genes. Other strains contain only part of the virulome, are infrequently or never associated with severe forms of infection, but still possess the *vtx* genes.

The 2011 O104:H4 outbreak (see section 2.2.2.1.) raised questions on the efficacy of using a strategy for the detection of VTEC in food based on the identification of the virulence and serogroup-associated genes for characterising VTEC considered as pathogenic according to the previous definitions (EFSA, 2007, 2009). The outbreak strain did not fit in this scheme and yet was possibly the most pathogenic VTEC ever described. The 2011 O104:H4 outbreak strain had unusual genetic features, e.g. the lack of the *eae* gene and presence of enteroaggregative adhesion determinants, and as such did not belong to the typical VTEC serogroups associated with HUS. Its appearance determined a shift in the perception of the hazard represented by VTEC and made the definition of pathogenic strains problematic, thereby leading to changes in the methodologies proposed for testing food.

### 3.1.1. Isolation of VTEC O157

The methodology for the detection and isolation of *E. coli* O157 in food and feedstuffs is the subject of the international standard ISO 16654:2001<sup>15</sup>. The protocol is based on the selective enrichment of the food samples in a standard culture medium. Part of the enrichment culture is then treated with an O157-specific immuno-magnetic separation (IMS) reagent based on paramagnetic beads coated with antibodies against the LPS antigen of this *E. coli* serogroup and plated onto a solid medium containing sorbitol instead of lactose.

The method described above is very effective in isolating *E. coli* O157 from complex matrices and is highly sensitive. Nevertheless there are some concerns. For example, isolated colonies have still to be confirmed as VTEC by demonstrating the presence of *vtx* genes or the production of verocytotoxins. Moreover, sorbitol-fermenting *E. coli* O157 has recently emerged as a public health problem.

### 3.1.2. Isolation of non-O157 VTEC

The isolation of non-O157 VTEC is hampered by the lack of the differential selection offered by the Sorbitol-MacConkey (CT-SMAC) medium as these are generally more susceptible to inhibition from the antimicrobials used in the ISO 16654 method than the typical VTEC O157. The metabolic and antimicrobial resistance features of 'typical' *E. coli* O157 are not always present in non-O157 VTEC, which are phenotypically indistinguishable from the other commensal *E. coli*. This has hindered the development of specific media for the detection of non-O157 VTEC strains.

VTEC belonging to the O26 serogroup exhibit, as a common feature, the inability to ferment rhamnose. This characteristic may be useful to distinguish this VTEC serogroup from the other *E. coli*, being infrequent in the latter strains. Although useful, this feature is not linked, in VTEC O26, to antimicrobial resistance characteristics that can be exploited for their selection onto a specific agar medium.

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<sup>15</sup> ISO 16654:2001. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Escherichia coli* O157. International Organization for Standardization.

One promising strategy is based on a two-step method and involves the use of a solid medium with selective and differential properties, followed by plating of the presumptive VTEC colonies on a second confirmation medium (Possé et al., 2008). This medium showed some discrimination between non-O157 VTEC and other microflora and to be effective in the exclusivity testing against a panel of *Enterobacteriaceae*, *Pseudomonas* and Gram-positive bacteria, but showed high false-positive ratios for the different VTEC serogroups.

In order to reduce the number of false positive results, Possé et al. (2008) have proposed the use of a second passage onto a second agar medium where the suspected colonies from the first plates are streaked. Despite the good outcome in the exclusivity testing and the decrease in the false positive results, the proposed strategy remains time-consuming, bringing to three to four days the time needed for isolating the suspected colonies, which still have to be confirmed by molecular methods for the presence of the main virulence genes. Moreover, the study of Possé et al. (2008) reported the performances of the proposed media based on their use with pure cultures of VTEC or mixed cultures from pure strains cultured in the laboratory and have not been tested with enrichment cultures from complex matrices such as food samples. Finally, the preparation of the media is labour-intensive and since such media is not available as pre-assembled commercial formulations, its use is limited in the routine clinical or food testing laboratories.

Immuno-magnetic separation (IMS) can be carried out before plating using either commercially available IMS beads, available for the serogroups O26, O45, O103, O111 (Bonardi et al., 2007; OIE (World Organisation for Animal Health), 2008) or in-house prepared IMS beads (Fratamico et al., 2011). A suspension of the recovered beads is then spread on selective or chromogenic agar (Kalchayanand et al., 2013) and colonies screened by serum agglutination by serogroup-specific antisera.

### 3.1.3. Identification of VTEC

#### 3.1.3.1. Available standard methods

Until recently ISO 16654:2001<sup>15</sup> was the only published international standard for the detection of *E. coli* strains belonging to a VTEC serogroup in food or feed. The standard describes a method for the isolation of *E. coli* O157 based on specific features of bacteria belonging to this serogroup, including their capability to grow in presence of novobiocin, cefixime and potassium tellurite as well as the inability to ferment sorbitol. Despite the attempt to identify features characterising the growth of non-O157 VTEC, the development of cultural standards for the detection and isolation of strains belonging to these other VTEC serogroups has been hampered by the lack of clear-cut metabolic and antimicrobial resistance features allowing the specific enrichment and discrimination from the ubiquitous and commensal *E. coli*.

VTEC are distinguished from the other *E. coli* mainly on a genetic basis. The choice of virulence characteristics to be included as targets for a molecular detection methodology has been based on the assumption that pathogenic VTEC were characterised by the presence, in addition to the *vtx*-encoding genes, of the *eae* gene. Additionally, the serogroups that had been most frequently associated with severe human disease in the EU, in particular HUS, belonged to O157, O26, O111, O103, and O145, also regarded as the “top five”. Therefore the grid of features identifying VTEC causing severe disease in humans was proposed to be made up of a number of targets including the *vtx* and *eae* genes as well as genes associated with the above-mentioned five serogroups (EFSA, 2007, 2009).

This scheme represented the core of a detection method developed under the coordination of the EU-RL VTEC and proposed as an international standard to the working group on microbial contaminants (WG6) of the “Food analysis-Horizontal methods” technical committee (TC 275) of the European Committee of Standardization (CEN). The proposed detection methodology was based on the screening by real-time PCR of enrichment cultures from food samples for the detection of the proposed grid of VTEC virulence and serogroup-associated genes. The laboratory procedure has been

approved by the CEN TC275 WG6 as a Technical Specification (CEN ISO/TS 13136) entitled “*Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of foodborne pathogens – Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) belonging to O157, O111, O26, O103 and O145 serogroups*”.

The new standard includes sequential application of the screening for *vtx* genes followed, in the positive samples, by screening for the *eae* gene. All samples in which the *vtx* and *eae* genes have been detected are then subjected to screening for serogroup-associated genes. This latter step had the purpose of indicating the presence of a VTEC with the potential to cause the more severe forms of disease in the food sample and to select the positive samples for the isolation by serogroup-specific IMS.

The 2011 outbreak caused by O104:H4 raised questions on the efficacy of the grid adopted for the screening of food samples according to the draft CEN ISO/TS 13136. The appearance of such an unusual VTEC in the food chain required the modification of the general approach upon which the CEN ISO/TS 13136 was based.

The previous standard was based on the use of PCR and was sufficiently flexible to be adapted to the new scenario. The amended standard has been published as ISO/TS 13136<sup>16</sup> “*Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of foodborne pathogens – Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups*”.

The new ISO/TS 13136:2012 version requires an attempt to isolate VTEC from all samples where *vtx* genes are detected; the former CEN ISO/TS 13136 version recommended proceeding towards isolation only after the identification, in *vtx*-positive samples, of the presence of the *eae* and the serogroup-associated genes.

The flow charts of the operations of the CEN ISO/TS 13136:2012 standard, including both the screening of the enrichment cultures and the VTEC isolation procedure are included as Appendices C and D.

### 3.1.3.2. Detection of additional virulence factors

For the detection of additional virulence characteristics, a molecular approach has advantages. Primarily it allows a rapid identification of negative test samples. In general the methods based on the molecular detection of genetic traits have a rapid implementation and, in particular for VTEC, the methodology requires 24-27 hours to complete the screening step. The presence of any VTEC in the food sample can be excluded at this stage. In the case of positivity, the sample is suspected to contain a VTEC (presumptive positivity).

The approaches based on the indirect evidence of a pathogen in a food vehicle also have drawbacks. In the case of VTEC, since the indirect evidence of the presence of virulence genes presumptively determines the presence of the bacteria, the isolation of the strain is needed to confirm the presence of *vtx* genes in addition to relevant virulence factors in the same live cell whilst excluding the presence of free DNA or free *vtx* phages in the enrichment culture. This step can delay identification because of difficulties in developing culture media specifically or differentially allowing the growth of VTEC.

Vtx-producing EAEC can be effectively detected by the ISO/TS 13136:2012 procedure (see section 3.1.3.1.) since they are positive for the presence of *vtx* genes. For epidemiological purposes, the scheme for strain characterisation should be upgraded by incorporating additional genes beside the *eae*

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<sup>16</sup> ISO/TS 13136:2012. Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of foodborne pathogens – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups. International Organization for Standardization.

gene and serogroup-associated genes specific for this pathotype. Genes associated with enteroaggregative adhesion (AA) appear to represent the best choice. The gene encoding the regulator AggR (Dudley et al., 2006) is a good target for detecting EAEC and has long been used for this purpose since it regulates the AAF PAI, governing the enteroaggregative adhesion along with those specifying other AA-associated plasmid-encoded factors (Dudley et al., 2006). Because *aagR* is plasmid-located and may not be detected in the event of plasmid loss, the concomitant detection of the chromosomal gene *aaiC*, encoding a secreted protein of EAEC and harboured by the PAI AAI (Taniuchi et al., 2012), has been proposed by the authors to circumvent this possibility.

The 2011 *E. coli* O104:H4 outbreak strain forced a change in the procedures developed for the identification of VTEC causing severe disease. Vtx-producing EAEC do not possess the LEE PAI common to most pathogenic VTEC and have a genetic background typically present in the classical EAEC. A PCR procedure for the identification of EAEC has therefore been developed. The procedure is based on the detection of two genes typically present in the EAEC strains isolated from human disease: *aaiC*, encoding a secreted protein of EAEC and harboured by the AAI PAI (Taniuchi et al., 2012), and the gene encoding the AggR activator, which is located on the pAA plasmid and is a general regulator influencing the transcription of the genes present on the AAI PAI along with those specifying other pAA plasmid-encoded factors (Dudley et al., 2006).

The use of two targets in a multiplex assay ensures the maximum coverage of EAEC strains, which are characterised by a high degree of diversity in respect to the composition of the virulome. The procedure can be used in combination with the approach used to detect *vtx* genes and described in the international standard for the detection of VTEC in food, ISO/TS 13136:2012, to efficiently detect the Vtx-producing EAEC.

### **3.2. Characterisation and typing of VTEC strains**

#### **3.2.1. Serotyping**

Strains of *E. coli*, including VTEC, are classically identified by a scheme comprising over 180 'O' types (lipopolysaccharide) and 56 'H'-types (flagella). The combination of the 'O' and 'H' types constitute the serotype of an *E. coli* strain. Both the 'O' and 'H' types can be determined by immunological methods such as agglutination with antisera. These techniques have been described in a previous Opinion (EFSA, 2007). PCR methods for the determination of 'O' types are being continuously developed. Methods for the identification by PCR of the 'O' types associated with the most severe forms of VTEC infections have been collected and published on the EU-RL-VTEC website<sup>17</sup>.

#### **3.2.2. Typing of virulence factors and genes**

A number of different investigations applying meta-analysis of the VTEC O157 genome have indicated that at least two different lineages of VTEC O157 have developed, and that one of these lineages is more commonly associated with human disease than the other (Yang et al., 2004; Zhang et al., 2007b). Studies based on subtyping of *vtx* from VTEC O157 isolated from human patients and healthy cattle have furthermore indicated that there are differences among the relative frequency of the seropathotypes that predominate among patients with severe disease (HUS and HC) and pathotypes that predominate in the bovine reservoir (Roldgaard et al., 2004). The same observation has been made for VTEC O26. Strains of VTEC O26:H11 that cause HUS are usually identified as *vtx*- and *eae*-positive, whereas infections caused by *vtx*1- and *eae*-positive VTEC O26:H11 are usually characterised by causing relatively mild diarrhoeal symptoms in most patients (Ethelberg et al., 2004).

Classification of VTEC based on phenotypic differences, biological activity, and hybridisation properties has been recommended by O'Brien et al. (1994). These toxin attributes are clinically relevant since some subtypes or variants of Vtx2 seem to be associated with serious sequelae, namely

<sup>17</sup> <http://www.iss.it/vtec/work/cont.php?id=152&lang=2&tipo=3>



HUS (Bielaszewska et al., 2006; Friedrich et al., 2002; Persson et al., 2007), while others are primarily associated with a milder course of disease (Bielaszewska et al., 2006; Friedrich et al., 2002; Persson et al., 2007).

Vtx2e is primarily associated with oedema disease in pigs (Marques et al., 1987) and is rarely a cause of diarrhoeal disease in humans (Pierard et al., 1991; Scheutz and Ethelberg, 2008; Zweifel et al., 2006) or HUS (Thomas et al., 1994). The differences in Vtx specificity may be due to variation in the genes encoding the B subunits which are responsible for binding of the toxin to the eukaryotic cell receptor (Jacewicz et al., 1986; Lindberg et al., 1987). Vtx2 variants associated with human disease bind primarily to the receptor globotriosyl ceramide found in human kidney cells. In contrast the receptor for Vtx2e is globotetraosyl ceramide, more commonly found in porcine tissues (DeGrandis et al., 1989; Samuel et al., 1990).

Consistent nomenclature and subtyping strategies are thus of primary importance for surveillance and for predicting the risks associated with particular VTEC infections. A PCR protocol for the subtyping of the *vtx*-encoding genes has been developed and evaluated by means of a multicenter study (Scheutz et al., 2012). The primer sequences and the amplification conditions for Vtx gene subtyping are shown in Appendix E, Table 1.

### 3.2.3. Phage typing

VTEC O157 strains can be differentiated into about 90 types, termed phage types, according to their resistance to the superinfection by a panel of 16 bacteriophages (Khakhria et al., 1990). The methodology has been described in a previous EFSA Opinion (EFSA, 2007), together with the significance of the approach for the epidemiology of the infections.

### 3.2.4. Subtyping

Pulsed field gel electrophoresis (PFGE) for the subtyping of VTEC is widely used to compare strains for epidemiological purposes and probably still represents the gold standard for the investigation of outbreaks and source-tracing (Barrett et al., 1994; Willshaw et al., 2001b).

Sequence-based typing methods such as the multi-locus variable number of tandem repeat analysis (MLVA) have been evaluated as an alternative to PFGE for VTEC O157 (Hyytia-Trees et al., 2006; Noller et al., 2003). Although effective for subtyping VTEC O26 (Miko et al., 2010) its use with other non-O157 VTEC serogroups has as yet not been evaluated.

Both methodologies have been discussed in a previous EFSA Opinion (EFSA, 2007).

Whole genome sequencing (WGS) has recently been used to investigate the phylogeny of non-O157 VTEC, and in particular the 2011 outbreak strain of enterohaemorrhagic O104:H4 (Mellmann et al., 2011) and to investigate and characterise human and animal isolates of *E. coli* O157 associated with a major outbreak in the UK associated with an open farm (Underwood et al., 2013). There is little doubt that with the increased availability of WGS methodology and equipment, rapid next-generation sequencing will be increasingly used for the subtyping of both O157 and non-O157 VTEC.

## 3.3. Conclusions

The definition of the sub-population of VTEC causing severe disease has been challenged by the 2011 O104:H4 outbreak. The appearance of such an unusual VTEC strain has clearly demonstrated the impossibility of predicting the emergence of 'new' pathogenic VTEC types based on the presence of the *eae* gene, or by focusing on a restricted panel of serogroups.

Vtx-producing EAEC do not possess the LEE locus common to most pathogenic VTEC and have a genetic background typically present in the classical EAEC. A PCR procedure for the identification of EAEC based on the detection of two genes typically present in the EAEC strains isolated from human disease: *aaic*, encoding a secreted protein of EAEC and the gene encoding the AggR activator, which

is a general regulator influencing the transcription of the genes present on the AAI PAI is being evaluated. The procedure can be used in combination with the approach used to detect *vtx* genes and described in the international standard for the detection of VTEC in food, ISO/TS 13136:2012, to efficiently detect the Vtx-producing EAEC.

The new published ISO/TS 13136:2012 improves the methodology for detecting VTEC in food vehicles by enlarging the scope to the detection of all VTEC.

#### **4. Hazard characterisation**

##### **4.1. Illness associated with VTEC**

Illnesses associated with Vtx-producing *E. coli* range from mild diarrhoea to bloody diarrhoea to HC, HUS, and thrombocytopenia. Some individuals may excrete the organism in the faeces but remain asymptomatic. *E. coli* O157:H7 is the VTEC serotype which has been most often associated with the more severe forms of disease, and in the USA is estimated to cause 63 000 illnesses, 2 100 hospitalisations, and 20 deaths each year (Scallan et al., 2011). At least 80 % of childhood HUS is attributable to infection with VTEC – mainly serogroup O157, although other serogroups are implicated, with considerable differences between countries (Lynn et al., 2005). The peak incidence of HUS is in children under five years of age (Fitzpatrick, 1999).

Isolates belonging to numerous non-O157 VTEC serogroups have also been linked to illnesses and disease outbreaks (Scallan et al., 2011). In the USA non-O157 VTEC have been estimated to cause diarrhoea at frequencies similar to those of other important enteric bacterial pathogens, such as *Salmonella* and *Shigella* (Tillman et al., 2012), and also cause infections resulting in HUS and outbreaks (Brooks et al., 2005).

There is increasing recognition worldwide of over 150 non-O157:H7 serotypes that may cause human illness, some of which can cause outbreaks and severe disease such as HUS and HC. For example in a outbreak in Norway in 2006, 17 persons were diagnosed as infected with *E. coli* O103:H25. Ten of the patients, all children, developed HUS and one died. MLVA of the patient isolates showed that all had identical profiles. Identical profiles were also detected in isolates from several lot-numbers of the incriminated dry-cured sausage products made of mutton, and later in sheep flocks (Schimmer et al., 2008; Sekse et al., 2009). In contrast some VTEC strains have been associated with only mild diarrhoea or with no reported human disease (Bettelheim, 2007; Bettelheim, 2012; Blanco et al., 2001; Coombes et al., 2011; Coombes et al., 2008; EFSA and ECDC, 2012; Johnson et al., 2006). These observations are supported by the clinical outcome of reported VTEC cases in the EU from 2007-2010 (see section 4.3.).

##### **4.2. Commonality with isolates from beef cattle and beef products**

To provide a global assessment of the beef cattle role in human infection with VTEC, published reports on VTEC from beef and beef cattle from 1984 to 2007 were evaluated by Hussein (2007). The prevalence rates of *E. coli* O157 ranged from 0.1 to 54.2 % in ground beef, from 0.1 to 4.4 % in sausage, from 1.1 to 36.0 % in various retail cuts, and from 0.01 to 43.4 % in whole carcasses. The corresponding prevalence rates of non-O157 VTEC were 2.4 to 30.0 %, 17.0 to 49.2 %, 11.4 to 49.6 %, and 1.7 to 58.0 %, respectively. Of the 162 VTEC serotypes isolated from beef products, 43 were detected in HUS patients and 36 are known to cause other human illnesses. Thus only 79 (48.8 %) of 162 serotypes had been identified in cases of human infection. With regard to beef cattle, the prevalence rates of *E. coli* O157 ranged from 0.3 to 19.7 % in feedlots and from 0.7 to 27.3 % on pasture. The corresponding prevalence rates of non-O157 VTEC were 4.6 to 55.9 % and 4.7 to 44.8 %, respectively, often depending on the sensitivity of the methods used. Of the 373 VTEC serotypes isolated from cattle faeces or hides, 65 were detected in HUS patients and 62 are known to cause other human illnesses. Thus only 123 (33.0 %) of 373 VTEC serotypes from faeces or hides had been associated with human illness. The author concluded that the prevalence of a large number of pathogenic VTEC serotypes in beef and beef cattle emphasised the critical need for control measures



to assure beef safety. From these studies it is apparent that the human pathogenic potential of many VTEC serotypes is as yet unknown.

#### 4.3. Clinical outcome of reported human cases

In the period 2007-2010 the clinical presentation (both HUS and diarrhoea) of the VTEC human cases was reported for only 5 405 (39.2 %) of the human cases in the EU. Results, presented in Table 9, and illustrate that 65 % (or 3 536 out of 5 405 patients with known clinical presentation) had diarrhoea and did not develop HUS. In HUS cases, bloody diarrhoea was also frequently reported.

**Table 9:** Clinical presentation of reported confirmed<sup>(a)</sup> human VTEC infections in the EU in the period 2007-2010. Based on TESSy data as provided by ECDC

Clinical manifestation	HUS <sup>(b)</sup>			Total
	Yes	No	Unknown	
Bloody diarrhoea	180	1 265	737	2 182
Diarrhoea	98	3 536	953	4 587
Asymptomatic	3 <sup>(c)</sup>	323	26	352
Unknown	496	2 019	3 909	6 424
Total	777	7 143	5 625	13 545

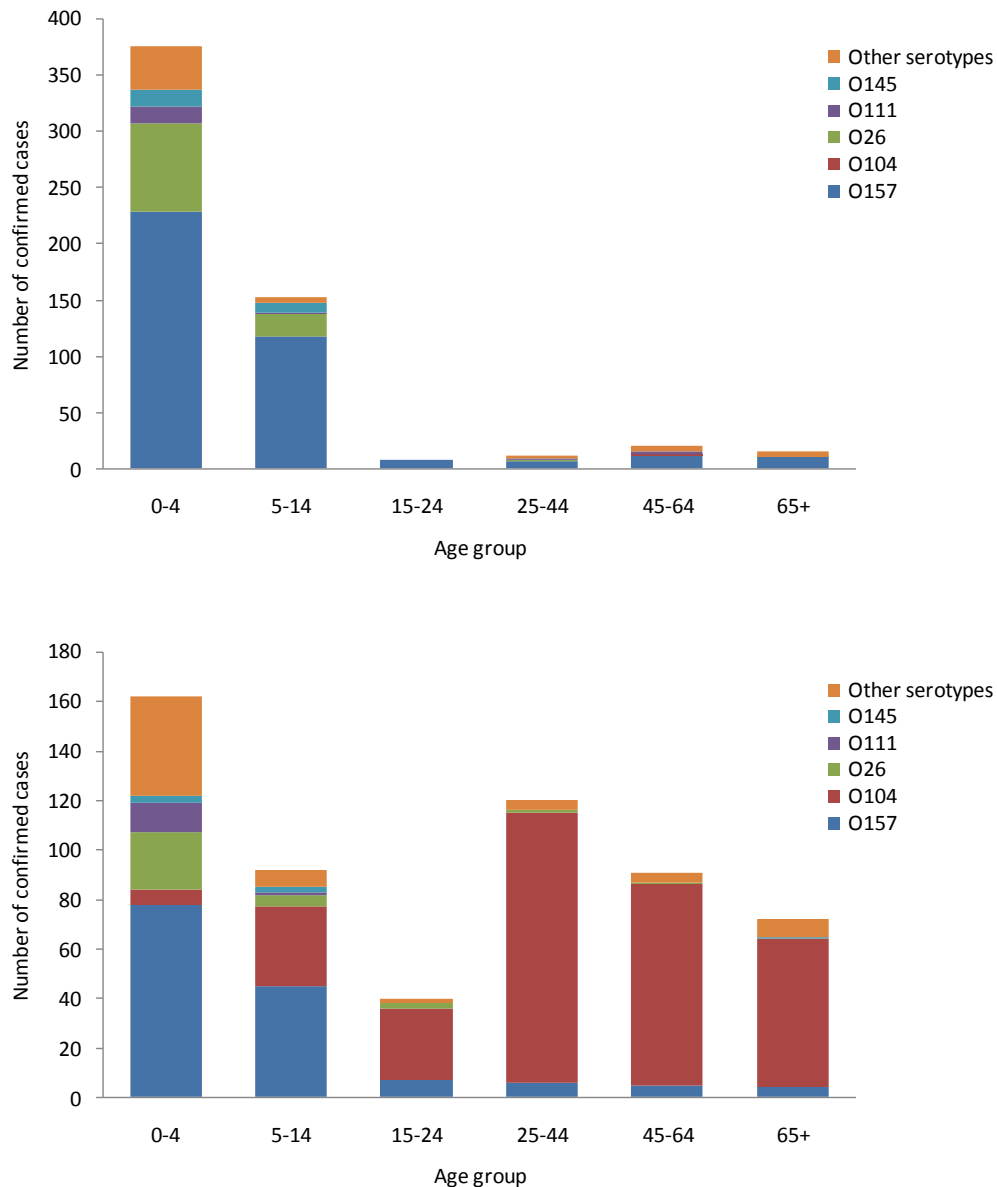
(a): Confirmed cases are laboratory confirmed and may or may not fulfil the clinical criteria as described in the case definition.

(b): HUS = haemolytic uraemic syndrome.

(c): Data as reported to ECDC.

By age group, in the period 2007-2010, provided for 584 HUS cases with known serogroup, 64.2 % of the HUS cases were reported in children up to 4 years of age and 26.0 % in the 5-14 years age group. Of those groups, VTEC O157 was identified in 65.7 % of cases, followed by VTEC O26 in 18.6 % of cases.

In 2011, a total of 1 006 confirmed cases developed HUS, based on EFSA and ECDC (2013) data. Of these cases 318 were reported to be due to the O104:H4 outbreak strain, but the majority of the 411 HUS cases with unknown serogroup reported from Germany are believed to have been caused by the outbreak strain. By age group (provided for 577 HUS cases with known serogroup), 28.1 % of the HUS cases were reported in children up to 4 years of age, followed by 20.8 % in the 25-44 age group. O157 was the most commonly reported VTEC serogroup in the 0-14 year old age groups while O104 was the predominant serotype of confirmed cases in the remaining age groups (Figure 1).



**Figure 1:** Haemolytic uraemic syndrome (HUS) by age and serogroup in reporting MSs. Top: 2007-2010 ( $n = 584$ ) (based on TESSy data as provided by ECDC); bottom: 2011 ( $n = 577$ ) (EFSA and ECDC, 2013).

#### 4.4. Predictive markers for VTEC that may cause human disease

##### 4.4.1. Classification by seropathotype

Using the 2003 Karmali seropathotype model, the predominant serotypes responsible for HUS and HC, O157:H7 and O157:NM, were assigned to seropathotype A (Table 1). Seropathotype B strains have been associated with outbreaks and HUS, but less commonly than those of seropathotype A; serotypes within seropathotype B included O26:H11, O103:H2, O111:NM and O145:NM. Seropathotype C serotypes were associated with sporadic HUS but not epidemics. The serotypes in group C were O91:H21, O104:H21, O113:H21, O5:NM, O121:NM, and O165:H25. Seropathotype D serotypes have been associated with diarrhoea but not with outbreaks or HUS; seropathotype E serotypes comprised VTEC serotypes that had never been associated with human disease and had been isolated only from animals. Seropathotypes D and E included multiple serotypes, 12 serotypes for seropathotype D and 14 for seropathotype E.

Information about seropathotype and disease based on the clinical outcome of confirmed VTEC cases in humans in the EU from 2007-2010 by serotype (TESSy data), as provided by ECDC, has been presented in Appendix A, Table 1.

#### 4.4.1.1. Virulence factors

The ECDC epidemiological data would appear to broadly fit the Karmali seropathotype model in terms of the presence of the *vtx2-eae* gene combination. The percentage of cases, hospitalisations and HUS associated with specific *eae-vtx* gene combinations is shown in Table 10. Most HUS cases (almost 90 %), for which information was reported on virulence factors, were either *eae,vtx2*-positive or *eae,vtx1,vtx2*-positive.

Tables 1-3 in Appendix F list the virulence characteristics by sero(patho)type for all reported confirmed cases (Appendix F, Table 1), hospitalised cases (Appendix F, Table 2) and HUS cases (Appendix F, Table 3). Considering the confirmed VTEC cases, 98.5 % (769/781) of the seropathotype A strains carried the *vtx2* (and *vtx1*) gene and 99.2 % (763/769) of these were *eae*-positive. 99.0 % (297/300) of seropathotype B strains also behaved as predicted by the Karmali concept and were *eae*-positive. Seropathotype B strains from VTEC cases were mostly *vtx1*-positive (81.0 % or 243/300).

All seropathotype A strains causing HUS (77 cases) were *vtx2*-positive and 98.7 % of these were *eae*-positive. The 17 HUS cases caused by strains of the seropathotype B group were all *eae*-positive; 14 were *vtx2*-positive, two *vtx1,vtx2*-positive and one *vtx1*-positive. None of the 124 reported cases due to O103:H2, of which all but one were *eae,vtx1*-positive, caused HUS.

**Table 10:** Virulence characteristics of reported confirmed VTEC cases in 2007-2010 including all cases, hospitalised cases only and haemolytic uraemic syndrome (HUS) cases only (based on TESSy data as provided by ECDC)

Cases	Virulence <sup>(a)</sup> characteristics					
	<i>eae,vtx1</i>	<i>eae,vtx2</i>	<i>eae,vtx1,vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1,vtx2</i>
All <sup>(b)</sup> ( <i>n</i> = 7 278)	612 (8.4)	4 254 (58.5)	1 642 (22.6)	295 (4.1)	287 (3.9)	188 (2.6)
Hospitalised <sup>(b),(c)</sup> ( <i>n</i> = 313)	22 (7.0)	185 (59.1)	85 (27.2)	4 (1.3)	10 (3.2)	7 (2.2)
HUS <sup>(b)</sup> ( <i>n</i> = 371)	10 (2.7)	294 (79.2)	37 (10.0)	2 (0.5)	24 (6.5)	4 (1.1)

(a): *eae* = intimin-coding gene, *vtx1* = verocytotoxin 1 gene, *vtx2* = verocytotoxin 2 gene.

(b): The percentage (between brackets) is calculated using the corresponding total number of cases (either 7 218, 313 or 371) as denominator.

(c): Data on hospitalisation have only been collected for the last two years (2009 and 2010).

The question therefore arises as to whether or not the presence of specific virulence factors or combinations thereof are good predictors of the public health significance of a particular VTEC serotype. The presence of Vtx is necessary, but is not sufficient to cause HUS. While it is unclear precisely which virulence factors make a VTEC pathogenic for humans the presence of multiple virulence genes in non-O157 VTEC serotypes suggests that the additive effects of a variable repertoire of virulence genes contribute to disease severity (Wickham et al., 2006). Furthermore, *vtx* gene variants have been shown to be expressed by a number of enteric pathogens (Mauro and Koudelka, 2011). Thus these genes are broadly distributed among bacteria, and Vtx-producing organisms are abundant in a variety of terrestrial ecosystems, including farm animals and the farm environment.

There is no single or combination of marker(s) that defines the potential of a VTEC strain to cause human disease. While *vtx2*- and *eae*-positive strains are associated with a high risk of more serious illness other virulence gene combinations and/or serotypes may also be associated with serious

disease, including HUS. Patient-associated (e.g., age, immune status, antibiotic therapy in the pre-infection period), and dose-related factors are also important (Todd and Dundas, 2001).

Table 11 presents the virulence markers present in strains from the five different seropathotypes. While different virulence factor combinations have been associated with disease and the precise genetic composition of a pathogenic VTEC cannot be defined, there are genetic features that are commonly associated with pathogenic strains. Thus, in general, clinically important VTEC contain the LEE (with *eae* often used as a marker for this pathogenicity island) and more severe human illness is associated the *vtx2* and *vtx2c* toxin subtypes (Persson et al., 2007; Zhang et al., 2007a). In the USA over 90 % of VTEC HC cases have been reported to have been caused by strains that are *eae*- and haemolysin A (*hlyA*)-positive (USDA, 2012).

**Table 11:** Virulence markers in the seropathotype concept as proposed by Karmali et al. (2003)

Sero-patho-type	Incidence in human disease <sup>(a)</sup>	in Outbreaks	Association with severe disease <sup>(b)</sup>	Virulence markers		Serotypes
				<i>vtx</i>	<i>eae</i>	
<b>A</b>	High	Common	Yes	<i>vtx2</i> (but may in addition also carry <i>vtx1</i> )	+	O157:H7, O157:NM
<b>B</b>	Moderate	Uncommon	Yes	<i>vtx1</i> and/or <i>vtx2</i>	+	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
<b>C</b>	Low	Rare	Yes	<i>vtx1</i> and/or <i>vtx2</i>	+/-	O91:H21, O104:H21, O113:H21, O5:NM, O121:NM, O165:H25
<b>D</b>	Low	Rare	No	<i>vtx1</i> and/or <i>vtx2</i>	+/-	Multiple <sup>(c)</sup>
<b>E</b>	Non-human only	NA <sup>(d)</sup>	NA <sup>(d)</sup>	<i>vtx1</i> and/or <i>vtx2</i>	+/-	Multiple <sup>(c)</sup>

(a): Reported frequency in human disease.

(b): Haemolytic uraemic syndrome (HUS) or haemorrhagic colitis (HC).

(c): See Table 1.

(d): NA = not applicable.

#### 4.4.2. Evaluation of the seropathotype model

For the purposes of this Opinion the seropathotype model of Karmali et al. (2003) has been evaluated using data reported to ECDC over the period 2007-2010. Two approaches have been used, the original Karmali approach (approach 1), as put forward in the 2003 paper (Karmali et al., 2003), and a modification of this approach (approach 2) based on the health outcome of reported confirmed human VTEC cases in the EU. In an attempt to move towards a molecular-based categorisation scheme, a third approach, based on genes encoding virulence characteristics additional to the presence of *vtx* genes, has been proposed.

A large number of VTEC serotypes from ruminants and foods have rarely, if ever, been reported in human cases, despite being *vtx*-, *eae*- and *hlyA*-positive (Gyles, 2007). This was illustrated in two Irish studies conducted on 32 farms over the course of 12 months, which reported no less than 82 different serotype-virulence profile combinations (Ennis et al., 2012; Monaghan et al., 2011). Of these serotypes O5:H-, O13:H2, O26:H11, O150:H2, O-:H-, O76:H34, O157:H7 and O157:H16 had the necessary known virulence factors to cause disease in humans and yet only O157:H7 and O26:H11 are most frequently isolated from patients. The reasons for these discrepancies are as yet unknown, but could result from a combination of factors such as differences in detection and identification methods in different MSs, under-estimation problems (see section 2.2.3.), and lack of full information on serotypes. For example isolates from cases reported as O76:HNT (Appendix A, Table 1), and

therefore not classified, could have been O76:H19 or O76:H34 and therefore classifiable if full serotyping had been undertaken. Furthermore some VTEC strains might not be pathogenic.

#### 4.4.2.1. Original Karmali seropathotype approach (approach 1)

During 2007-2010, 13 545 confirmed VTEC infections were reported to ECDC. These cases have been assigned to seropathotypes based on the list provided by Karmali et al. (2003) and related to outcome. In Table 12 and Figure 2, the clinical outcome of the confirmed VTEC cases during 2007-2010 is shown and categorised by their classification into seropathotypes using the original Karmali seropathotype approach. Of these 13 545 cases, 11 488 (or 85 %) of the implicated strains were not fully serotyped and could therefore not be classified into seropathotypes.

These cases included 1 047 (51 % of fully serotyped cases) where the isolated VTEC belonged to seropathotype A; 323 (16 %) belonged to seropathotype B, 24 (1 %) to seropathotype C, 104 (5 %) to seropathotype D and 14 (0.7 %) to seropathotype E. For 545 cases (27 %) the serotypes were not reported in the Karmali paper and therefore could not be included in the Karmali classification. The majority of cases reporting a severe clinical outcome were associated with seropathotypes A and B. This included the majority (71 %) of the deaths, 76 % of the hospitalisations, 66 % of the HUS cases and 88 % of the cases with bloody diarrhoea.

Considering only those cases with reported outcome and when comparing the classification into seropathotypes, – 1.2 % of the total (five cases) were reported with a fatal outcome for seropathotype A and 0.5 % (two cases) for the group fully serotyped but not listed by Karmali et al. (2003) (Not Listed by Karmali or NLK group), respectively. The fatal cases reported in this latter group were associated with strains typed as O105:H18 and O17:H41. Hospitalisation rates for the different seropathotypes were: A, 59 %; B, 37 %; C, 100 % (only two cases); D, 75 % (only three cases); E, 100 % (only one case); and NLK, 37 %. Similarly HUS and bloody diarrhoea was reported from: seropathotype A, 16 % and 52 % respectively; seropathotype B, 6 % and 12 %; seropathotype C, 6 %, and 0 %; seropathotype D, 0 % and 1.4%, seropathotype E, 0 % and 0.3%; and NLK, 4 % developed HUS and 11 % reported bloody diarrhoea.

Of the 777 HUS cases reported, 645 cases (83 %) were not fully serotyped and could therefore not be classified. Of the remaining 132 cases, 115 (87 %) belonged to seropathotypes A-C and 17 (13 %) were not included in the Karmali paper. The latter included the following serotypes: O145:H28, O91:H10, O111:H8, O128:H2, O121:H2, O76:H19, O174:H21, O174:H2, O1:H42, O86:H27, O80:H2, O123:H2, O105:H18, and O7:H6.

The ECDC epidemiological data covering 2007-2010 would appear to broadly fit the Karmali seropathotype model in terms of incidence and severity. This classification, based on the serotypes listed by Karmali et al. (2003), has the drawback that the number of serotypes is limited (only 39 serotypes listed). As a result, for 545 cases (or 27 % of the confirmed VTEC infections) that were reported to ECDC in the period 2007-2010, classification was not feasible. This group caused 26.5 % of all cases, 29 % of the fatal cases, 17 % of the hospitalisations, 13 % of the HUS cases and 11 % of the cases with bloody diarrhoea.

In addition there are problems in serotyping (e.g., availability of sera for both 'O' and 'H' typing in different countries, strains which do not react with available sera, lack of resources, etc.), which combine to result in incomplete strain identification such as is required for the Karmali model. In the above assessment, incomplete serotype identification has resulted in the exclusion of 85 % of reported VTEC cases. Such exclusions could give rise to a biased outcome. For example, about half of the isolates with missing 'H' antigen information are from cases of O157 infection (5 610 cases). Assuming that these would have been typed as O157:H7 or O157:H-, many of these cases would have been assigned to seropathotype group A, thereby expanding this group to 6 657 cases or 87 % of cases, 78 % of fatal cases, 91 % of the hospitalisations, 91 % of the HUS cases and 95 % of the cases with bloody diarrhoea.

In the USA the top seven serogroups are O157, O26, O45, O103, O111, O121 and O145 (USDA, 2012). While the majority of strains belonging to these serogroups are *eae*- and *hlyA*-positive, there are exceptions and it is not possible to absolutely and definitively distinguish between pathogenic and non-pathogenic VTEC based on either serogroup or virulence factor profile. Thus the original Karmali seropathotype approach is not absolute in categorising all serotypes falling into groups other than A, B or C as not being associated with severe disease.

There are also important exceptions reported in the scientific literature. Before the 2011 outbreak, O104:H4 had a low incidence in human disease and as such was not outbreak-associated (seropathotype D); in the 2011 outbreak 3 816 human cases were reported (including 54 deaths) and of those, 845 cases developed HUS (EFSA and ECDC, 2013). In addition, of the six food-borne VTEC outbreaks (period 2007-2011) where full serotyping data was reported (Appendix B, Table 1), only O157:H7 could be assigned to a seropathotype group (group A), as the other strains were not included in the list of Karmali et al. (2003).

Classification by seropathotype may also be affected by differences in the relative occurrence of some serogroups in different countries (Caprioli et al., 1997; EC, 2012). Nevertheless in the USA at least 70 % of the 940 non-O157 VTEC isolates from persons with sporadic illnesses submitted between 1983 and 2002 to the US Centers for Disease Control and Prevention (CDC), belonged to one of the top six VTEC serogroups, namely O26, O111, O103, O121, O45, and O145 (Brooks et al., 2005). In the USA, the seropathotype B group has been extended and currently includes 13 VTEC serotypes O26:H11 and NM; O45:H2 and NM; O103:H2, H11, H25, and NM; O111:H8 and NM; O121:H19 and H7; and O145:NM (Bosilevac and Koohmaraie, 2011).

#### 4.4.2.2. 'Modified' Karmali seropathotype approach (approach 2)

To take into account serotypes other than those specifically mentioned in the Karmali paper of 2003 a modified seropathotype approach based on the health outcome of reported confirmed VTEC cases in the EU has been developed. This second approach was applied to the data reported to ECDC during 2007-2010. Published literature and older data have not been considered in this assessment.

In this 'modified' approach VTEC serotypes in seropathotype groups A, B and C were combined with those associated with HUS in the 2007 to 2010 data and designated 'HUS-associated serotypes' (HAS). In Table 13 and Figure 3, the clinical outcome of the confirmed VTEC cases during 2007-2010 is shown and categorised by their classification using this 'modified' Karmali seropathotype approach.

With this 'modified' approach 1 340 (86 % of fully serotyped cases) VTEC cases have been classified as seropathotype group HAS. The majority of infections with the most severe forms of the disease were associated with Karmali seropathotypes A/B/C. Under the new scheme, the HAS group now include the serotypes causing the majority (86 %) of the deaths, 71 % of the hospitalisations, 100 % of the HUS cases (due to the concept design) and 86 % of the cases with bloody diarrhoea.

By this 'modified' approach, in cases when full serotyping has been undertaken, all serotypes associated with severe disease are automatically categorised in the HAS group. Furthermore, as new information becomes available, serotypes may be re-classified and the model updated.



**Table 12:** Health outcome of reported confirmed<sup>(a)</sup> human VTEC cases during 2007-2010 as categorised by the seropathotype concept of Karmali et al. (2003). Based on TESSy data as provided by ECDC

Seropathotype	Total	Death		Hospitalisation		HUS		Clinical manifestation		
		Yes (%)	No	Yes (%)	No	Yes (%)	No	Bloody diarrhoea	Diarrhoea	Asymptomatic
<b>A<sup>(b)</sup></b>	1 047 [50.9]	5 (1.23) [71.4]	400	46 (59.0) [55.4]	32	96 (16.3) [72.7]	493	286 (52.1) [79.0]	236 (43.0) [26.3]	27 (4.9) [35.1]
<b>B<sup>(c)</sup></b>	323 [15.7]	0 (0) [0]	283	17 (37.0) [20.5]	29	18 (6.2) [13.6]	273	31 (11.7) [8.6]	222 (84.1) [24.7]	11 (4.2) [14.3]
<b>C<sup>(d)</sup></b>	24 [1.2]	0 (0) [0]	16	2 (100) [2.4]	0	1 (5.6) [0.8]	17	0 (0) [0]	15 (93.8) [1.7]	1 (6.3) [1.3]
<b>D<sup>(e)</sup></b>	104 [5.1]	0 (0) [0]	80	3 (75.0) [3.6]	1	0 (0) [0]	88	5 (6.3) [1.4]	70 (88.6) [7.8]	4 (5.1) [5.2]
<b>E<sup>(f)</sup></b>	14 [0.7]	0 (0) [0]	12	1 (100) [1.2]	0	0 (0) [0]	10	1 (9.1) [0.3]	9 (81.8) [1.0]	1 (9.1) [1.3]
<b>NLK<sup>(g)</sup></b>	545 [26.5]	2 (0.48) [28.6]	414	14 (36.8) [16.9]	24	17 (3.8) [12.9]	430	39 (9.3) [10.8]	346 (82.8) [38.5]	33 (7.9) [42.9]
<b>NFT<sup>(h)</sup></b>	11 488	11 (0.21)	5 296	439 (38.3)	707	645 (10.0)	5 832	1 820 (31.5)	3 689 (63.8)	275 (4.8)
<b>Total</b>	13 545	18 (0.28)	6 501	522 (39.7)	793	777 (9.8)	7 143	2 182 (30.6)	4 587 (64.4)	352 (4.9)

(a): Confirmed cases are laboratory confirmed and may or may not fulfil the clinical criteria as described in the case definition. For the majority of these confirmed VTEC cases, the clinical outcome was not reported: the case fatality was not reported for 52 % of these cases, hospitalisation was not reported for 90 % and HUS (haemolytic uraemic syndrome) status was unknown for 41 %. The clinical manifestation (expressed as bloody diarrhoea, diarrhoea or asymptomatic) was not reported for 47 % of the cases. Percentages of cases are given between brackets based on rows ( ) and based on columns [ ].

(b): Includes O157:H7, O157:NM.

(c): Includes O26:H11, O103:H2, O111:NM, O121:H19, O145:NM.

(d): Includes O91:H21, O104:H21, O113:H21, O5:NM, O121:NM, O165:H25.

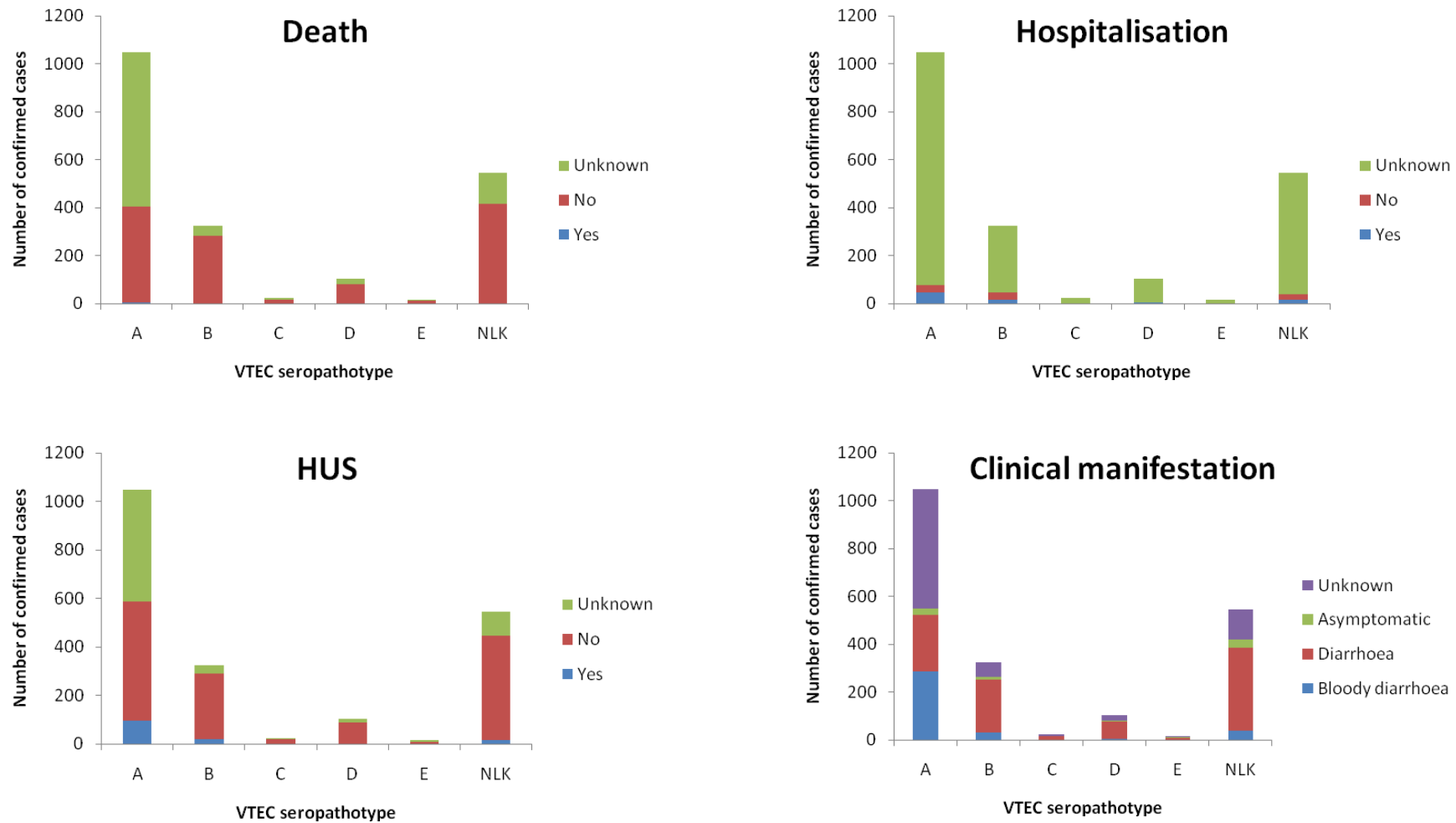
(e): Includes O7:H4, O69:H11, O103:H25, O113:H4, O117:H7, O119:H25, O132:NM, O146:H21, O171:H2, O172:NM, O174:H8, Orough:H2.

(f): Includes O6:H34, O8:H19, O39:H49, O46:H38, O76:H7, O84:NM, O88:H25, O98:H25, O113:NM, O136:H12, O136:NM, O153:H31, O156:NM, O163:NM.

(g): NLK = serotypes that were fully serotyped but were not listed by Karmali et al. (2003).

(h): NFT = strains that were not fully serotyped.





**Figure 2:** Health outcome of reported confirmed human VTEC cases during 2007-2010 as listed by Karmali et al. (2003) and categorised accordingly. NLK includes these serotypes that were fully serotyped but not listed by Karmali et al. (2003). Based on TESSy data as provided by ECDC.

**Table 13:** Health outcome of reported confirmed human VTEC cases during 2007-2010 as categorised based on the reported haemolytic uraemic syndrome (HUS) cases of human VTEC in EU in 2007-2010 (grouped as HAS (A/B/C)). Based on TESSy data as provided by ECDC<sup>a</sup>

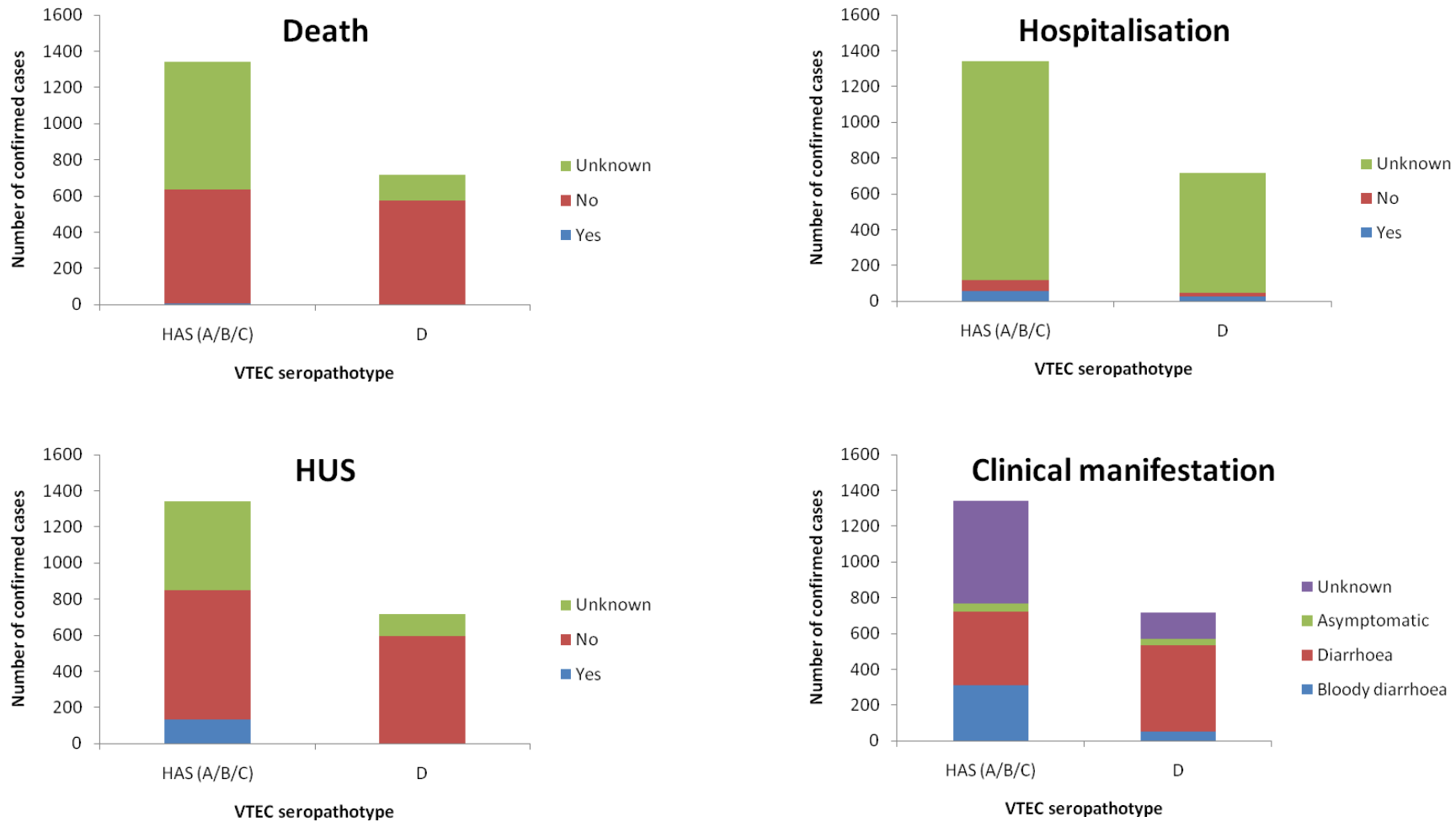
Seropathotype	Total	Death		Hospitalisation		HUS		Clinical manifestation		
		Yes (%)	No	Yes (%)	No	Yes (%)	No	Bloody diarrhoea	Diarrhoea	Asymptomatic
<b>A/B/C (HAS)<sup>(b)</sup></b>	1 340 [65.1]	6 (0.94) [85.7]	631	59 (49.2) [71.1]	32	132 (15.6) [100.0]	717	311 (40.6) [85.9]	413 (53.9) [46.0]	43 (5.6) [55.8]
<b>D<sup>(c)</sup></b>	717 [34.9]	1 (0.17) [14.3]	574	24 (49.0) [28.9]	0	0 (0) [0]	594	51 (9.0) [14.1]	485 (85.1) [54.0]	34 (6.0) [44.2]
<b>NFT<sup>(d)</sup></b>	11 488	11 (0.21)	5 296	439 (38.3)	707	645 (10.0)	5 832	1 820 (31.5)	3 689 (63.8)	275 (4.8)
<b>Total</b>	13 545	18 (0.28)	6 501	522 (39.7)	793	777 (9.8)	7 143	2 182 (30.6)	4 587 (64.4)	352 (4.9)

(a): Confirmed cases are laboratory confirmed and may or may not fulfil the clinical criteria as described in the case definition. For the majority of these confirmed VTEC cases, the clinical outcome was not reported: the case fatality was not reported for 52 % of these cases, hospitalisation was not reported for 90 % and HUS (haemolytic uraemic syndrome) status was unknown for 41 %. The clinical manifestation (expressed as bloody diarrhoea, diarrhoea or asymptomatic) was not reported for 47 % of the cases. Percentages of cases are given between brackets based on rows ( ) and based on columns [ ].

(b): HAS = HUS-associated serotypes. Includes the serotypes that have been associated with reported confirmed HUS cases of human VTEC in EU in 2007-2010: O157:H7, O157:H-, O121:H19, O26:H11, O174:H2, O111:H-, O145:H-, O145:H28, O1:H42, O128:H2, O111:H8, O104:H21, O174:H21, O7:H6, O76:H19, O80:H2, O86:H27, O121:H2, O123:H2, O105:H18, O91:H10.

(c): Includes the serotypes that have been fully serotyped but have not been associated with the reported confirmed HUS cases of human VTEC in EU in 2007-2010.

(d): NFT = strains that were not fully serotyped.



**Figure 3:** Health outcome of reported confirmed human VTEC cases during 2007-2010 as categorised based on the reported haemolytic uraemic syndrome (HUS) cases of human VTEC in EU in 2007-2010 (grouped as HAS (A/B/C)). Based on TESSy data as provided by ECDC

#### 4.4.2.3. Molecular approach (approach 3)

There is insufficient data to perform a quantitative risk assessment relating the presence of a particular combination of virulence genes and/or serogroup to a particular disease outcome. Achieving a balance between specificity and sensitivity of virulence prediction is therefore difficult. The ‘modified’ Karmali approach does not resolve the underlying problem with strains that have not been fully serotyped. Furthermore classification based solely on the presence of *vtx* genes is inadequate. To overcome these problems a third approach, utilising genes encoding virulence characteristics additional to the presence of *vtx* genes, is proposed.

The additional virulence-associated genes suggested for this classification are *eae* (intimin production), *aaiC* (secreted protein of EAEC) and *aggR* (plasmid-encoded regulator) genes (see section 3.1.3.2.). The intimin protein encoded by the LEE PAI (*eae* gene) is, to our knowledge, the only adherence factor that has been consistently associated with clinical isolates of VTEC, but with some notable exceptions such as the O104:H4 outbreak strain in 2011, which was *eae*-negative.

In principle this approach delivers a new scheme that describes the categorisation of VTEC according to potential risk for consumers’ health. These ‘risks’ have been categorised as group I (high potential risk) through to group III (unknown risk) (see Table 14).

**Table 14:** Proposed<sup>(a)</sup> molecular approach for the categorisation of VTEC (*vtx* present)

Group	Genes <sup>(b)</sup>	Serogroups	Potential risk <sup>(c)</sup>	
			Diarrhoea	HUS/HC <sup>(d)</sup>
I	<i>eae</i> -positive or ( <i>aaiC</i> and <i>aggR</i> )-positive	O157, O26, O103, O145, O111, O104	High	High
II	<i>eae</i> -positive or ( <i>aaiC</i> and <i>aggR</i> )-positive	Any other	High	Unknown
III	<i>eae</i> -negative and ( <i>aaiC</i> plus <i>aggR</i> )-negative	Any other	Unknown	Unknown

(a): As yet this proposed molecular approach must be regarded as provisional. This is because screening VTEC for the presence of *eae*, *aaiC* and *aggR* genes is not routinely undertaken by all laboratories reporting data to TESSy.

(b): Additional to the presence of *vtx* genes. *eae* = intimin-coding gene, *aaiC* = chromosomally-encoded gene encoding secreted protein of EAEC, *aggR* = plasmid-encoded regulator gene.

(c): Needs epidemiological studies for confirmation.

(d): HUS = haemolytic uraemic syndrome, HC = haemorrhagic colitis.

The listed serogroups under group I reflect the top-5 (O157, O26, O103, O145, O111) generally recognised as most frequently associated with human clinical cases, with the addition of O104. The proposal for the inclusion of *aaiC* and *aggR* genes is due to the 2011 outbreak, which was caused by a highly virulent strain. This was an exceptional event and future surveillance will provide data that may be used to review the inclusion of these virulence factors in this molecular approach.

VTEC strains falling under group I should be regarded as representing a higher risk. For VTEC that would fall under group II there is still uncertainty whether or not they are able to cause HUS due to as yet unknown additional virulence mechanisms. For VTEC that would fall under group III there is uncertainty whether or not they are able to cause disease and we are unable to make a scientific judgement based on current knowledge of virulence characteristics. Routine surveillance that includes molecular testing for known/new virulence genes together with accurate reporting of clinical presentation will help to classify VTEC strains according to risk.

PCR-based methods for the identification and detection of the relevant genes in serogroups assigned to the three risk groups are already available as well as methods to detect the serogroups listed under group I (see section 3.1.3.2.).

This molecular approach must be regarded as provisional. This is because screening VTEC for the presence of *aaiC* and *aggR* genes is not routinely undertaken by all laboratories reporting data to TESSy. As such the relevant *aaiC* and *aggR* gene data are not fully available for all isolates (see Tables 4 and 10). Similarly information on the presence of *eae-vtx* genes is not always available. For example, during 2007-2010 such data were available for only 371 out of 777 (47.7 %) of reported HUS cases. Additionally information on serogroup has not always been reported. For example, for the remaining 371 cases serogroup information was not available for 56 cases.

This proposed molecular approach has the advantage of overcoming problems associated with the lack of flagella 'H' antigen typing. The model needs to be periodically revised in light of new epidemiological information. The performance of this proposed approach needs to be verified with well-characterised isolates from cases of human infection and from food-producing animals and foods, thus accommodating all cases with information on the infecting strain.

#### 4.5. Conclusions

In the period 2007-2010, 13 545 confirmed human VTEC infections were reported to ECDC. 85 % of these cases were not fully serotyped and could therefore not be classified using the seropathotype concept of Karmali et al. (2003).

The human pathogenic potential of many VTEC serogroups is at yet unknown.

The seropathotype D group of the Karmali seropathotype approach covered 5 % of cases that were fully serotyped. Fourteen cases (0.7 %) belonged to the seropathotype E, a group formerly considered to be only found in animals. Twenty-seven percent of the cases could not be assigned to a seropathotype group as these were not listed in Karmali's 2003 paper. There were no HUS cases reported for the serotypes included in seropathotype groups D and E. There were 17 HUS cases reported that could not be assigned to a seropathotype group.

Various virulence factors and toxins contribute to the pathogenesis of VTEC. Vtx2 is the more potent toxin in cases of human disease, and those strains producing this toxin are generally associated with more acute illness. Strains that produce Vtx2 and more specifically, Vtx2 subtype c (Vtx2c) have been suggested to be more likely to cause HUS than those that produce Vtx1 alone. A further important virulence gene is the *eae* gene, which encodes a protein involved in the intimate attachment of *E. coli* to the gut mucosa and is typically found in strains causing serious illness.

There is no single or combination of marker(s) that defines the potential of a VTEC strain to cause human disease. While *vtx2*- and *eae*-positive strains are associated with a high risk of more serious illness other virulence gene combinations and/or serotypes may also be associated with serious disease, including HUS. Patient-associated (e.g., age, immune status, antibiotic therapy in the pre-infection period), and dose-related factors may also be of importance. Alternative concepts based on the detection of verocytotoxins alone or genes encoding such verocytotoxins do not provide a sound scientific basis on which to assess risk to the consumer.

The intimin protein encoded by the LEE PAI (*eae* gene) and the AAF encoded by the EAEC plasmid (*aaiC*) gene are, to our knowledge, the only adherence factors that have been consistently associated with the virulence of EHEC and VTEC respectively. Therefore, any VTEC strains that carry at least one of the genes encoding such products should be regarded as higher risk.

Pathogenicity can neither be excluded nor confirmed for a given VTEC serogroup or serotype based on the Karmali seropathotype concept or analysis of the public health surveillance data.

Using a modification of the Karmali et al. (2003) approach based on the health outcome of reported confirmed human VTEC cases in the EU during 2007-2010, in cases when full serotyping has been undertaken all serotypes associated with severe disease (HUS) could be categorised as seropathotype group HAS. Under the new scheme, the HAS group now includes the serotypes causing the majority

(86 %) of the deaths, 71 % of the hospitalisations, 100 % of the HUS cases and 86 % of the cases with bloody diarrhoea.

By this 'modified' approach, in cases when full serotyping has been undertaken all serotypes associated with severe disease are automatically categorised in the HAS group. Furthermore, as new information becomes available, serotypes may be reclassified and the model updated.

A molecular approach, utilising genes encoding virulence characteristics additional to the presence of *vtx* genes, is proposed. This molecular approach must be regarded as provisional because screening VTEC for the presence of *eae*, *aaiC* and *aggR* genes is not routinely undertaken. This scheme has the advantage of overcoming problems associated with the lack of flagella 'H' antigen typing. The performance of this proposed approach needs to be verified with well-characterised isolates from cases of human infection and from food-producing animals and foods.

## 5. Exposure assessment

Data on VTEC are reported annually on a mandatory basis by EU MSs to the EC and EFSA based on Zoonoses Directive 2003/99/EC<sup>18</sup>. Most MSs have provided data on their VTEC investigations in the past years. When interpreting these data it is important to note that data from different investigations are not directly comparable due to differences in sampling strategies and applied analytical methods. 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 serotypes of VTEC. Thus the proportion of non-O157 VTEC strains may have been largely under-reported.

Most reported data on VTEC are from animals (mainly ruminants) and meat and milk thereof, since these are considered to be main sources of human infections. These data are summarised in the Community and EU Summary Reports on Zoonoses and Food-borne Outbreaks in 2004-2011.

### 5.1. Occurrence of VTEC in ready-to-eat (RTE) food

#### 5.1.1. EU monitoring data

An overview of the data during the years 2007 to 2011 (from EFSA and ECDC (2010, 2011, 2012)) on occurrence of VTEC in RTE food is provided in Table 15.

In food, most information on VTEC was reported on fresh bovine meat. During 2007-2010, overall 0.3 - 2.3 % of fresh bovine meat samples were found positive for VTEC in the reporting MSs, and 0.1 - 0.7 % of these samples were positive for VTEC O157. The other VTEC serogroups reported in bovine meat were O26, O103, O111, and O145, but overall very little information on the serogroups was provided by MSs. The proportion of positive samples varied widely between the MSs. On fresh sheep meat, VTEC were detected in 0 - 8.2 % of the samples: VTEC O157 was not detected. Some data were reported on fresh meat from other animal species at the EU level, where VTEC were detected between <0.1 - 3.2 % of the samples and <0.1 % were positive for VTEC O157.

VTEC was reported from samples of raw cow's milk and cheeses made from cow's milk. VTEC O157 was only recovered from raw milk in 2011 in Belgium in one out of 39 batches of raw milk intended for direct human consumption. VTEC O22 was detected in 2008. In cheeses, VTEC O91 was recovered in soft and semi-soft cheeses made from raw or mild heat-treated cow's milk in 2010.

Fewer VTEC data were provided from other foodstuffs. Nine MSs provided data on VTEC in fruit, vegetables and juices in 2007-2010. Five investigations reported VTEC in 0.5 - 6.5 % of samples and VTEC O157 was detected in three investigations of vegetables, with the proportion positive units at

<sup>18</sup> 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.



0.5 – 5.3 %. Two MSs reported data on VTEC in fishery products, and in one investigation VTEC was detected at 4.2 %. In 2011 more data have been provided on VTEC in seeds, sprouts and vegetables, likely prompted by the O104:H4 outbreak. None of the samples tested positive for VTEC O157, three were positive for non-O157 in sprouted RTE seeds at retail and one in a RTE dish at retail.

As mentioned in a previous EFSA Opinion (EFSA Panel on Biological Hazards, 2012a), when reviewing the EU Summary Reports on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne outbreaks in 2009 and 2010 comparison between MSs is difficult due to the differences in the methods, sampling schemes and reporting systems. For example detection rates of VTEC in fresh bovine meat are available with results based on sampling plans by either surface area or weight (i.e. per 400 cm<sup>2</sup> or 25 g): it is not clear in this dataset to identify if the samples were of carcasses, primary cuts or final products. The sampling stage (i.e. before, after or during chilling) can have important effects on determining VTEC prevalence. In addition there are significant trends in the prevalence of VTEC in fresh bovine meat which may reflect differences in methods, sampling schemes and reporting systems among MSs. For example the reported percentage of samples where VTEC O157 was detected in bovine fresh meat at slaughter, cutting/processing plant in Spain for 2009 was 14.9 %. In contrast the respective prevalence in Spain for the years 2006-2008 was less than 1.3 % whilst in 2010 it was 0 %. For most MS no information has been provided for VTEC serogroups other than O157.

#### **5.1.2. Data from literature**

Table 16 provides an overview of the occurrence of VTEC in RTE food. Depending on the methodologies used, VTEC of various serogroups have been recovered from RTE foods, albeit relatively rarely.

VTEC has been recovered from a range of different animal species and food categories. The most widely used analytical method only aims at detecting VTEC O157, whereas fewer investigations have been conducted on analytical methods aimed at detecting all or selected serotypes of VTEC. At the EU level, O157 has been recovered from fresh bovine meat, fresh sheep meat, raw cows' milk and dairy products as well as other foods, albeit at low prevalences. MSs provided data on the VTEC serogroups other than O157 in 2010, and have detected O26, O91, O103 and O145 from bovine meat, cheeses, cattle, sheep or pigs.

**Table 15:** VTEC in fresh meat, milk and dairy products and other food in EU, 2007-2011 (EFSA and ECDC, 2010, 2011, 2012, 2013)<sup>(a)</sup>

Animal/ food category	2011				2010				2009				2008				2007			
	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)
Fresh bovine meat <sup>(b)</sup>	8	4 347	1.4	0.3	12	8 566	0.5	0.1	13	9 285	2.3	0.7	14	14 810	0.3	0.1	13	14 115	0.3	0.1
Fresh sheep meat	2	220	0	0	3	394	7.4	0	4	248	3.2	0	1	61	8.2	0	4	290	1.7	0
Fresh meat from other animal species <sup>(c)</sup>	3	1 459	<0.1	<0.1	9	5 800	0.6	<0.1	11	248	3.2	0	8	6 660	0.7	<0.1	9	6 374	0.4	0.1
Raw cows' milk <sup>(d)</sup>	4	499	1.6	0.2	4	1 683	3.3	0	3	998	1.2	0.1	4	1 439	1.7	<0.1	5	1 079	0.5	0
Milk and dairy products excl. raw cows' milk <sup>(e)</sup>	3	1 546	<b>1.8</b>	<0.1	5	2 704	0.3	0	7	5 602	0.4	<0.1	4	1 138	1.1	0	7	2 289	0.9	0
Other food <sup>(f)</sup>	8	4 727	0.1	0	4	2 806	1.6	<0.1	5	340	0.9	0.9	4	1 119	0.4	0.4	5	3 459	<0.1	0

(a): Only investigations with  $\geq 25$  samples included; *n* = number of samples; No. MSs = Number of Member States reporting data.

(b): In 2011, Belgium reported on carcasses at slaughterhouses the serotypes VTEC O26 (4), VTEC O103 (3), and VTEC O111 (5) O103 and VTEC O111 (1) and VTEC O145 (2). In 2010, France reported in chilled minced bovine meat the serotypes VTEC O26:H11 (4) and VTEC O145:H28 (1).

(c): Includes meat from pig, broilers, turkey and wild or farmed game - land animals. In 2009 Austria reported in meat from wild or farmed game - land mammal the serotype VTEC O146:H21 (1). In 2008 Germany reported in meat from wild or farmed game - land mammals the serotypes VTEC O146 (2) and VTEC O91 (1). In 2007 Germany reported in meat from wild or farmed game - land mammals the serotypes VTEC O128 (1), VTEC O146 (1), VTEC O8 (1) and VTEC O113 (1).

(d): No additional information on serogroups was provided by MSs except for one investigation from Germany in 2008 in which 3 positive samples were VTEC O22.

(e): In 2010 Germany reported the serotype VTEC O91 (1) in soft and semi-soft cheeses made from raw or low heat treated cow's milk.

(f): Includes fruits and vegetables, juice, fishery products and other processed fruit products and prepared dishes.

**Table 16:** Occurrence of pathogenic *E. coli* in ready-to-eat (RTE) food

Reference	Commodity	Number of samples analysed	Positive samples	Other information
Althaus et al. (2012)	RTE lettuce	142	12 (VTEC), 11 (EPEC)	The VTEC strain was <i>eae</i> negative; non O-157. Screened with multiplex PCR for the <i>vtx</i> and <i>eae</i> genes
	Fresh-cut fruits	64	0	Screened with multiplex PCR for the <i>vtx</i> and <i>eae</i> genes
	Sprouts	27	0	Screened with multiplex PCR for the <i>vtx</i> and <i>eae</i> genes
Castro-Rosas et al. (2012)	RTE-salads (mixed salads with raw vegetables) (restaurants)	130	8 (positive for diarrhoeogenic <i>E. coli</i> )	Generic <i>E. coli</i> tested for virulence factors. Non-O157 VTEC (3 samples), EIEC (2 samples), ETEC (1 sample), non-O157 VTEC and EIEC (2 samples)
Gomez-Govea et al. (2012)	Green onions, parsley, tomatoes, Serrano peppers, jalapeño peppers, cantaloupe (supermarkets)	300	0	Screened for <i>E. coli</i> O157:H7 by VIDAS <sup>(a)</sup>
Santos et al. (2012)	Minimally processed leafy salads: romaine lettuce, spinach; mixed salads (with three or four different ingredients such as endive, radicchio, canonigo, green lettuce, purple lettuce, arugula, carrot, corn, cabbage, chicory and nuts) (retail)	151	0	Screened for <i>E. coli</i> O157:H7 by VIDAS <sup>(a)</sup>
Koseki et al. (2011)	Iceberg lettuce (retail stores)	419	0	Screening with PCR
De Giusti et al. (2010)	RTE salads	699	0	Screened for <i>E. coli</i> O157:H7 by PCR
Oliveira et al. (2010)	Conventional and organic lettuce (farms)	72 × 2 (144)	0	Generic <i>E. coli</i> tested for several virulence genes
Bohaychuk et al. (2009)	Lettuce, spinach, tomatoes, carrots, green onions and strawberries (farmers' markets)	673	0	
Microbial Data Program, Progress Update and 2009 Data Summary ( <a href="http://www.asm.usda.gov/mdp">www.asm.usda.gov/mdp</a> )	Cantaloupe, cilantro, green onions, hot peppers, conventional and organic lettuce, spinach, alfalfa sprouts, round and Roma tomatoes (distribution centres and terminal (wholesale) markets)	15354	51 (presumptive positive by PCR), isolate obtained from 24 samples	13 isolates were characterised as VTEC and 11 as ETEC. None of the VTEC isolates contained <i>eae</i> genes and did not belong to the most common serotypes.

Reference	Commodity	Number of samples analysed	Positive samples	Other information
Microbial Data Program, Progress Update and 2008 Data Summary ( <a href="http://www.asm.usda.gov/mdp">www.asm.usda.gov/mdp</a> )	Cantaloupe, bagged lettuce, spinach, alfalfa sprouts, tomatoes (distribution centres and terminal (wholesale) markets)	10 330	35 (presumptive positive by PCR), isolate obtained from 11 samples	7 isolates were characterised as VTEC and 4 as ETEC. None of the VTEC isolates contained <i>eae</i> genes and did not belong to the most common serotypes
Abadias et al. (2008)	Fresh, minimally processed fruit, vegetables and sprouts (retail)	300	0	Generic <i>E. coli</i> analysed for <i>E. coli</i> O157:H7
Arthur et al. (2007)	Muskmelon, scallions and green onions, organic and conventional leaf lettuce, head lettuce, parsley, cilantro, tomatoes	1 183	0	Enrichment cultures positive for <i>E. coli</i> were assessed for verotoxigenicity
Mora et al. (2007)	Fresh vegetables (markets)	101	4 (positive for <i>E. coli</i> O157)	
Johnston et al. (2006)	Leafy greens, herbs, melons, vegetables (packing sheds)	466	0	Screened for <i>E. coli</i> O157:H7.
Johnston et al. (2005)	Leafy greens, herbs, and cantaloupe	398	0	Screened for <i>E. coli</i> O157:H7
Loncarevic et al. (2005)	Lettuce (farms)	179	0	Screening for <i>E. coli</i> O157 by IMS <sup>(b)</sup>
Sagoo et al. (2003b)	Bagged prepared RTE salads (mostly mixed salads and also single type of salad) at retail	3 820	0	Screened for <i>E. coli</i> O157
Sagoo et al. (2003a)	Open, RTE prepared salads from catering and retail	2 950	0	Screened for <i>E. coli</i> O157
Johannessen et al. (2002)	Lettuce, pre-cut salad, growing herbs, parsley/dill, strawberries (distributors/wholesalers)	703	0	Screened for <i>E. coli</i> O157:H7 by IMS <sup>(b)</sup>
Sagoo et al. (2001)	RTE organic vegetables (grown in close proximity or in contact with soil (e.g. broccoli, cabbage, carrot, cauliflower, celeriac, celery, cress, lettuce, mushrooms, radish, spring onions, watercress), and other salad vegetables, such as cucumber, pepper and tomato) at retail	3 200	0	
Gillespie et al. (2000)	Cold, RTE sliced meat from hotels, public houses, restaurants, cafés and residential care homes	3 349	0	Screened for <i>E. coli</i> O157 by IMS <sup>b</sup>
Little and de Louvois (1998)	Cooked sliced meats from manufacturing butchers' premises	1 491	0	Screened for <i>E. coli</i> O157:H7 by plating
	RTE pies/pasties from manufacturing butchers' premises	637	0	Screened for <i>E. coli</i> O157:H7 by plating
	Other cooked meat products from manufacturing butchers' premises	55	0	Screened for <i>E. coli</i> O157:H7 by plating

(a): Vitek Immunodiagnostic Assay System.

(b): Immuno-magnetic separation.

## 5.2. Occurrence of VTEC in food animals

### 5.2.1. EU monitoring data

An overview of the data reported during 2007 to 2011 (from EFSA and ECDC (2010, 2011, 2012)) on occurrence of VTEC in food animals is provided in Table 17. In cattle, during the years 2007-2011 VTEC was reported in 2.1 - 13.5 % of animals at the EU level, and VTEC O157 was found in 0.2 - 2.3 % of these. The prevalence of VTEC in cattle varied between the MSs from 0 to 53.8 %. The prevalence at herd or holding level ranged between 6.1 to 12.6 % and 1.5 to 13.7 % for VTEC and VTEC O157, respectively. For slaughter batches, the prevalence ranged between 13.0 to 20.2 % for all VTEC, and 5.5 to 20.2 % for VTEC O157. The serogroup of the majority of the VTEC organisms were not specified, except for Austria, which reported a complete list. A wide range of serogroups was identified in those countries where serogrouping was undertaken.

In sheep VTEC was detected in 0.9 - 20.1 % of the animal sampled by the reporting MSs during 2007-2011, and 0 - 4.8 % of these samples were VTEC O157. The specimens taken from animals varied between the MSs and included faeces, ear, hide and fleece samples. Most of the VTEC serotypes were not specified, except for Austria, which reported a complete list. Data from goats were reported only in 2007, 2010 and 2011. The prevalence of VTEC in goats varied between 0 and 11.8 % and for VTEC O157, between 0 and 1.3 %.

In conclusion, among food animals, most reported data on VTEC were from cattle and sheep, in which the reported VTEC and VTEC O157 prevalence varied widely between the MSs. In addition, other serogroups were reported from cattle.

### 5.2.2. Data from literature

Although the most common serogroups associated with human infection (i.e. VTEC O157) have been widely recovered from ruminants, a considerable number of non-O157 serogroups have also been identified. For example, in study of bovine faecal samples and soil samples collected from farms throughout Ireland over a 12-month period from 2007-2008. 107 VTEC isolates were recovered, representing 17 serogroups. O26:H11 and O145:H28 were detected, with O113:H4 being the most frequently isolated. Additionally, serogroups O2:H27, O13/O15:H2, and ONT:H27 carrying *vtx1* and/or *vtx2* and *eae* genes were recovered (Monaghan et al., 2011).

In a further study VTEC was detected by PCR- and culture-based methods in 67 % of 450 beef animal hides and 27 % of a similar number of carcasses screened for over a 12 month period. Forty isolates representing 12 VTEC serotypes (O5:H-, O13:H2, O26:H11, O33:H11, O55:H11, O113:H4, O128:H8, O136:H12, O138:H48, O150:H2, O168:H8 and ONT:H11) and 15 serotype/virulotype combinations were identified (Monaghan et al., 2012).

**Table 17:** VTEC in cattle and sheep in EU, 2007-2011 (EFSA and ECDC, 2010, 2011, 2012, 2013)<sup>(a)</sup>

Animal/ food category	2011				2010				2009				2008				2007			
	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)	No. MS	<i>n</i>	VTEC (%)	VTEC O157 (%)
Cattle (animal/single)	6	2 788	3.7	0.8	9	6 800	<b>13.5</b>	0.2	8	5 239	6.6	<b>2.3</b>	8	4 937	2.1	0.3	9	4 746	2.8	2.1
Cattle (herd/holding)	2	2 617	<b>12.6</b>	1.5	2	2 089	10.0	4.7	3	925	6.1	3.6	1	328	16.2	<b>13.7</b>	2	559	8.1	6.3
Cattle (slaughter batch)	1	402	16.2	5.5	1	53	18.9	18.9	1	258	<b>20.2</b>	<b>20.2</b>	1	167	17.4	17.4	2	408	13.0	13.0
Sheep (animals)	2	1 054	12.3	<b>4.8</b>	5	773	17.5	0	3	324	<b>20.1</b>	0.3	3	671	3.1	1.6	4	533	0.9	0.4
Goats (animals)	1	214	0	0	1	76	<b>11.8</b>	<b>1.3</b>	-	-	-	-	-	-	-	-	2	120	4.2	0

(a): Only investigations with  $\geq 25$  samples included; *n* = number of samples; No. MSs = Number of Member States reporting data.



### 5.3. Assessment of public health risk associated with the contamination of RTE foods with VTEC

For assessing public health risk as part of investigation of outbreaks or incidents of VTEC infection, rapid testing should be performed for the detection of verocytotoxins or genes encoding the production of such toxins in any suspect foods. Clinical outcomes should be used to assess the pathogenic potential of all strains, especially those that are newly-emerging and of 'unusual' genotypes. Assessment of public health risk of foods contaminated with VTEC during outbreak investigations will be greatly assisted by preliminary detection or characterisation results (e.g. serogroup or *vtx* type) which identify similarities between the clinical isolates with those from food, even prior to full compilation of data establishing a common strain. Outbreak control responses may be strongly influenced by the severity of disease, setting, food type, additional microbiological data (particularly the presence of organisms indicative of faecal contamination) and supportive epidemiological evidence.

In the absence of evidence of identification of human infection a different approach for the detection of VTEC in foods may be justified. Recent advances in microbiological detection methodologies allow the detection of *vtx* genes (as well as the presence of verocytotoxin) and this requires attempted isolation of VTEC for all samples where these targets are detected. When VTEC have been isolated, the application of the Karmali, 'modified' Karmali and molecular classifications (see section 4.4.2.) indicate that specific groups should be regarded of high public health risk. These three classification systems may also be useful in assessing the public health risks of other VTEC 'types'. As already stated, it is not possible to fully define human pathogenic VTEC or identify factors for VTEC that absolutely predict the potential to cause human disease. Nevertheless, any RTE product containing one of the VTEC serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *vtx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, current available data do not allow any inference regarding potential risks. Additional epidemiological and microbiological data (particularly the presence of organisms indicative of faecal contamination) is also important in assessing appropriate risk management options.

### 5.4. Conclusions

VTEC has been recovered from a range of different animal species and food categories. 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 VTEC serotypes.

Prevalence data on VTEC from food and animals reported at EU level are not sufficiently comparable to enable any conclusions to be made on trends over the years. Nevertheless VTEC, including some serogroups associated with cases of human infection, have been identified in a range of different animal species and food categories.

On the basis of the proposed molecular classification scheme, any RTE product contaminated with an isolate of one of the VTEC serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *vtx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, current available data do not allow any inference regarding potential risks.

## ANSWERS TO TERMS OF REFERENCE (TORs)

**ToR 1: the ‘seropathotype’ concept – the limitation to “relevant” serotypes O157, O26, O103, O111, O145, O121, O91, O104, O113<sup>4</sup>; i.e., can pathogenicity be excluded for defined VTEC serotypes?**

- The seropathotype classification of Karmali et al. (2003) does not define pathogenic VTEC nor does it provide an exhaustive list of pathogenic serotypes. Instead it classifies VTEC based on their reported frequency in human disease, their known association with outbreaks and their severity of the outcome including haemolytic uraemic syndrome (HUS) and haemorrhagic colitis (HC).
- Pathogenicity can neither be excluded nor confirmed for a given VTEC serogroup or serotype based on the seropathotype concept or analysis of the public health surveillance data.
- It is not possible to fully define human pathogenic VTEC or identify factors for VTEC that absolutely predict the potential to cause human disease.

**ToR 2: justification of the statement: ‘seropathotypes D and E are not HUS-associated and are uncommon in man or only found in non-human sources’<sup>4</sup>;**

- In the period 2007-2010, 13 545 confirmed VTEC infections and 777 HUS cases were reported in the EU. Isolates from 85 % of these cases were not fully serotyped and could therefore not be classified using the Karmali seropathotype concept.
- The Karmali seropathotype D group was associated with 5 % of cases that were fully serotyped. Seropathotype E group, defined by Karmali et al. (2003) as non-human only, included 14 confirmed cases (0.7 %) of human infection. Furthermore approximately 27 % of strains from human cases could not be assigned to a seropathotype group as these were not listed by Karmali et al. (2003).
- There were no HUS cases reported for the serotypes included in seropathotype groups D and E, but there were 17 HUS cases reported that could not be assigned to a seropathotype group.
- Using a modification of the Karmali approach based on the health outcome of reported confirmed human VTEC cases in the EU during 2007-2010, in cases when full serotyping has been undertaken all serotypes associated with severe disease (HUS) could be categorised as seropathotype group ‘haemolytic uraemic syndrome (HUS)-associated serotype(s)’ or HAS. Under the new scheme, the HAS group now includes the serotypes causing the majority (86 %) of the deaths, 71 % of the hospitalisations, 100 % of the HUS cases and 86 % of the cases with bloody diarrhoea.
- By this ‘modified’ approach, in cases when full serotyping of isolates has been undertaken, all serotypes associated with severe disease are automatically categorised in the HAS group. Furthermore, as new information becomes available, serotypes may be reclassified and the model updated.

**ToR 3: an alternative concept based on detection of verocytotoxins or genes encoding for verocytotoxins in isolates;**

- The detection of verocytotoxins alone or genes encoding for such verocytotoxins is not a sound scientific basis for assessing the disease risk to the consumer.
- There is no single or combination of marker(s) that defines a ‘pathogenic’ VTEC. Strains positive for verocytotoxin 2 gene (*vtx2*)- and *eae* (intimin production)- or [*aaiC* (secreted

protein of EAEC) plus *aggR* (plasmid-encoded regulator)] genes are associated with a higher risk of more severe illness than other virulence factor combinations. Other virulence gene combinations and/or serotypes may also be associated with severe disease in humans, including HUS.

- A molecular approach, utilising genes encoding virulence characteristics additional to the presence of *vtx* genes, is proposed. This molecular approach must be regarded as provisional because screening VTEC for the presence of *eae*, *aaiC* or *aggR* genes is not routinely undertaken. This scheme has the advantage of overcoming problems associated with the lack of flagella ‘H’ antigen typing. The performance of this proposed approach needs to be verified with well-characterised isolates from cases of human infection and from food-producing animals and foods.

**ToR 4: the contribution by VTEC to diarrhoeal cases and to more severe outcomes in the EU, based on hazard identification and characterisation, and under-reporting in EU;**

- The health outcome has not been reported for all cases (reported for diarrhoea: 53 % of cases; and for HUS: 59 % of cases) of the reported confirmed VTEC cases in the EU between 2007 and 2010. Most patients (ca. 64 %) presented with only diarrhoea. VTEC infection resulted in HUS in around 10 % of the cases.
- Detection of VTEC is highly dependent on the methods applied to clinical specimens and the strategies for application of these methods vary between different MSs.
- The degree of under-estimation (including under-ascertainment and under-reporting) of VTEC O157 infections has been estimated in seven EU MSs. Disease-multipliers differ widely between EU countries, ranging from 13 to 87.
- The 2011 O104:H4 outbreak has clearly demonstrated the difficulty of predicting the emergence of ‘new’ pathogenic VTEC types by only looking at the presence of the *eae* gene or by focusing on a restricted panel of serogroups.
- The ISO/TS 13136:2012 standard improves the concept of detecting VTEC in food.

**ToR 5: the public health risk associated with the contamination of RTE foods with VTEC, considering either the seropathotype concept or the detection of verocytotoxins or genes encoding the production of such toxins in isolates.**

- VTEC has been recovered from a range of different animal species and food categories. 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 serotypes of VTEC. Based on available data, the genetic diversity of VTEC strains in the food chain appears to be greater than from clinical cases. As a consequence, virulence potential of a given VTEC strain among food chain isolates cannot be accurately predicted.
- Pathogenicity can neither be excluded nor confirmed for a given VTEC serogroup or serotype based on the seropathotype concept or analysis of the public health surveillance data.
- On the basis of the proposed molecular classification scheme, any RTE product contaminated with an isolate of one of the VTEC serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *vtx* and [1] *eae* or [2] *aaiC* and *aggR* genes should be considered as presenting a potentially high risk for diarrhoea and HUS. For any other serogroups in combination with the same genes, the potential risk is regarded as high for diarrhoea, but currently unknown for HUS. In the absence of these genes, current available data do not allow any inference regarding potential risks.

## RECOMMENDATIONS

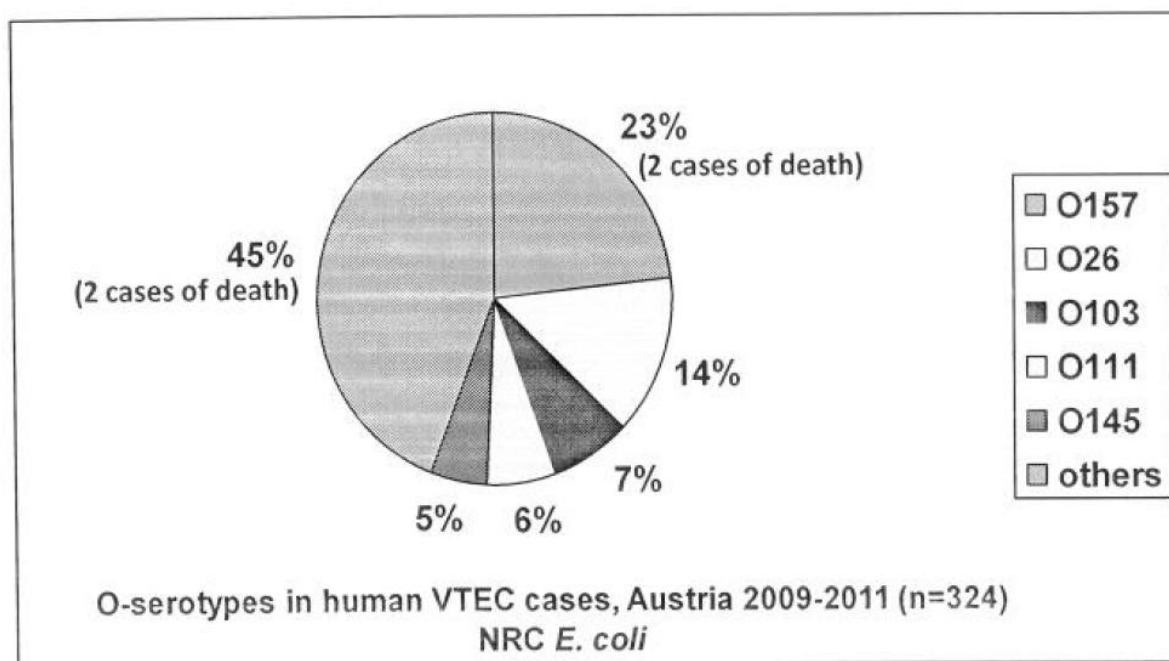
- The inclusion of *aaiC* and *aggR* genes in the proposed molecular approach is due to the O104:H4 outbreak, which was caused by a highly virulent strain. This was an exceptional event and future surveillance will provide data that may be used to review the inclusion of these virulence factors. Thus screening VTEC for the presence of *aaiC* and *aggR* genes should be performed on isolates from human, food and animal sources, to address this question.
- For public health investigation of VTEC infection, clinical and/or food samples should be screened by PCR for the presence of the *vtx* genes. If positive, all efforts should be made to isolate and characterise the causative organism.
- Verification and periodic revision of the proposed molecular approach in light of new epidemiological information.
- In accordance with the ISO specifications, international harmonisation of nomenclature of VTEC and its virulence factors, using STEC instead of VTEC and *stx* instead of *vtx* or *vt* may be considered.

## DOCUMENTATION PROVIDED TO EFSA

1. E-mail dated 26/09/2012 from the Austrian Federal Ministry of Health providing data of the human VTEC cases in Austria in 2009-2012.
2. Annex to the mandate as provided by the Austrian Federal Ministry of Health<sup>19</sup>.

Data: EFSA, ECDC, Germany and Austria regarding the Austrian request: “Verocytotoxin producing *E. coli* (VTEC) – “seropathotype concept” and scientific criteria regarding pathogenicity assessment”. May 2012. Submitted by the Austrian Federal Ministry of Health.

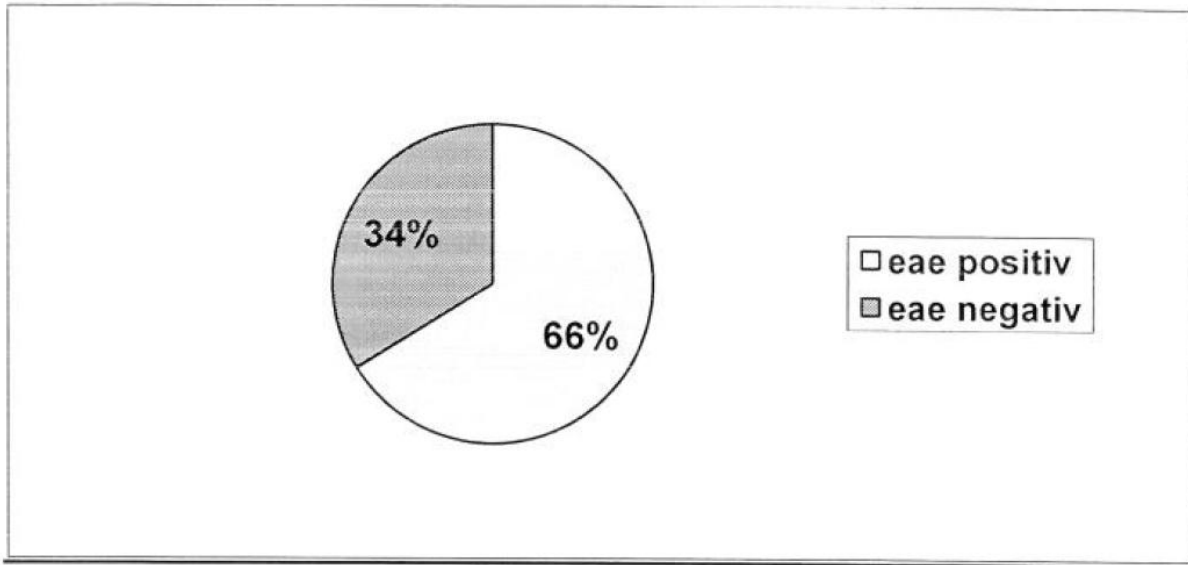
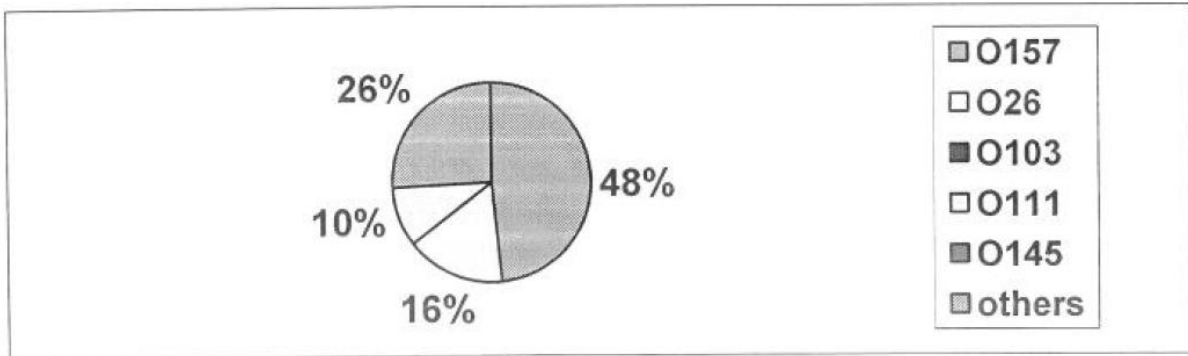
**Austrian data** (National Reference Laboratory for *E. coli*, VTEC data 2009 – 2011, human samples) demonstrate that the cause of the majority of human cases (41 %) are other serotypes than the types mentioned in the EC draft paper (Regulation (EC) No. 2073/2005 proposal 2012) based on EFSA “seropathotype concept”:



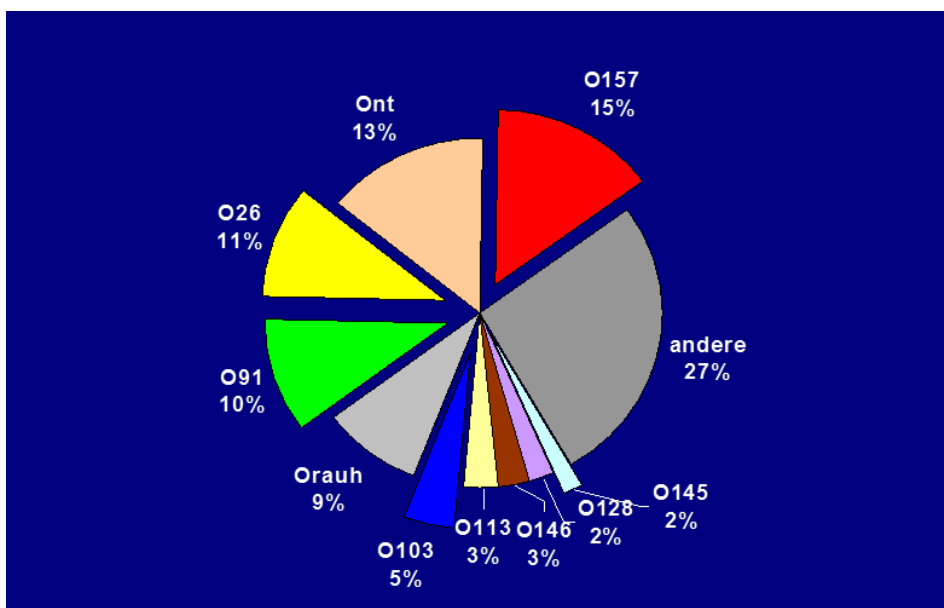
### VTEC HUS cases Austria 2009-2011 (n = 31):

- **23 % of HUS cases** were caused by “other serotypes” → no isolate, no pathogenicity assessment possible according to the ISO method<sup>7</sup>
- **25 % of HUS cases** were caused by *eae* neg. VTEC → no isolate, no pathogenicity assessment possible according to the ISO method<sup>7</sup>

<sup>19</sup> The Annex is shown above in the original format. Explanatory footnotes have been added by EFSA.



**Data, kindly provided from the German NRC for *Salmonella* and other enterics** demonstrate, that in Germany the proportion of “non top five O-serotypes” in human isolates was even higher. It was 67 % in the years 1999-2004 ( $n = 3\,424$ )! The situation is approximately the same now (personal communication).



German data: (German National Reference Center for *Salmonella* and other enterics, VTEC data 1999 – 2004, human samples ( $n = 3424$ ))



**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, Stockholm: ECDC; 2011”:

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

2009		
Serogroup	N	% total
O157	1 848	51.7
NT <sup>1</sup>	1 008	28.2
O26	192	5.4
O103	82	2.3
O91	48	1.3
O145	47	1.3
O146	31	0.9
O128	26	0.7
O111	25	0.7
O113	22	0.6
Other <sup>2</sup>	244	6.8
<b>Total</b>	<b>3 573</b>	

Source: [14]

<sup>1</sup> NT = untyped/untypeable.

Total cases: 3573 minus 1008 cases (untyped/untypeable): 2565 cases (serotyped). **Of these 2565 (serotyped) cases 371 cases belong to other serotypes** (O91, O113, O128, O146, “others”) than the serotypes mentioned in the EC draft paper (O157, O26, O103, O111, O145, O104).

**VTEC: EFSA ECDC, Zoonoses & Outbreaks 2010<sup>20</sup>:**

- “Others” 2010: at least about 10 % of human cases
- Assuming “NT” VTEC are predominantly “others”: about **40 % of human cases caused by “others”!**

<sup>20</sup> EFSA Journal, 10(3):2597, 442 pp.

**Table VT3. Reported confirmed VTEC cases in humans by serogroup (top 10), 2009-2010**

2010			2009		
Serogroup	No. of cases	% total	Serogroup	No. of cases	% total
O157	1,501	41.1	O157	1,848	51.7
NT <sup>1</sup>	1,230	33.7	NT1	1,008	28.2
O26	257	7.0	O26	192	5.4
O103	90	2.5	O103	82	2.3
O145	61	1.7	O91	48	1.3
O91	57	1.6	O145	47	1.3
O63	42	1.2	O146	31	0.9
O111	41	1.1	O128	26	0.7
O128	29	0.8	O111	25	0.7
O146	28	0.8	O113	22	0.6
Other <sup>2</sup>	315	8.6	Other <sup>2</sup>	244	6.8
<b>Total</b>	<b>3,651</b>		<b>Total</b>	<b>3,573</b>	

1. NT = untyped/untypeable.

2. Other included 8 (2010) and 12 (2009) confirmed cases where antigen O was reported as unknown.

Source: Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, Netherlands, Poland, Romania, Slovakia, Slovenia, Spain, Sweden and United Kingdom (N=3,651).

**Many MS (Austria, Denmark, Germany, the Netherlands): number of reported cases caused by “others” greater than cases caused by VTEC O157 – × 3 in Austria and Denmark!**

**Most MS: predominantly O157 → a strong indication for massive underreporting of “others”!**

**Table VT4. VTEC serogroups in humans by country, 2010**

Country	Serogroup										
	O157	NT	O26	O103	O145	O91	O63	O111	O128	O146	Other
Austria	11	4	16	9	3	0	0	6	0	1	38
Belgium	51	1	6	1	4	0	4	2	1	0	14
Denmark	25	5	14	24	6	6	1	3	9	9	73
Estonia	0	5	0	0	0	0	0	0	0	0	0
Finland	1	20	0	0	0	0	0	0	0	0	0
France	39	37	16	0	0	0	0	3	4	0	4
Germany	63	645	58	33	16	37	0	13	6	12	72
Greece	0	0	0	0	0	0	0	0	0	0	1
Hungary	3	2	1	0	0	0	0	0	1	0	0
Ireland	117	3	66	0	4	0	0	2	1	0	4
Italy	8	9	11	0	1	0	0	2	0	0	0
Lithuania	1	0	0	0	0	0	0	0	0	0	0
Luxembourg	2	0	1	1	0	2	0	0	1	0	0
Malta	1	0	0	0	0	0	0	0	0	0	0
Netherlands	40	280	17	9	12	9	37	3	2	4	65
Poland	2	0	0	0	0	0	0	1	0	0	0
Romania	1	0	1	0	0	0	0	0	0	0	0
Slovakia	0	10	0	0	0	0	0	0	0	0	0
Slovenia	2	7	6	1	0	0	0	2	1	0	1
Spain	17	0	0	0	0	0	0	0	0	0	1
Sweden	53	179	26	12	13	3	0	4	3	2	39
United Kingdom	1,064	23	18	0	2	0	0	0	0	0	3
<b>EU Total</b>	<b>1,501</b>	<b>1,230</b>	<b>257</b>	<b>90</b>	<b>61</b>	<b>57</b>	<b>42</b>	<b>41</b>	<b>29</b>	<b>28</b>	<b>315</b>

Without considering the “underreporting bias”, these “others” constitute a certainly considerable and unacceptably high number of serotyped VTEC causing human cases and HUS belonging to “seropathotypes D and E ... not HUS-associated and ... uncommon in man or found only in non-human sources” according to the EFSA opinion 2011<sup>21</sup>:

- About 15 % – EFSA/ECDC data – Technical Report 2009 – considering serotypes discussed by EFSA<sup>20</sup>, or 301 cases (comprises of 244 cases “others”, 47 cases O146 and 26 cases O128)
- 41 % – Austrian data, 25 % of HUS cases
- 67 % – data Germany
- 10 % - 40 % according to EFSA ECDC data 2010<sup>20</sup>

**ECDC – European Centre for Disease Control: 10 VTEC strains were selected for an external quality assurance programme 2010, results published in 2012:**

10 different VTEC were selected, the “most commonly reported strains”, there were no statements regarding restrictions (five strains ISO 13136, six strains in the proposal amending Reg. (EC) No. 2073/2005) concerning pathogenicity.

Relevance of the “seropathotype concept” and ISO 13136: using this concept and in consequence ISO 13136 accredited food laboratories would fail to identify relevant strains distributed by ECDC for quality assurance purposes:

Suggested citation: European Centre for Disease Prevention and Control. External quality assurance scheme for typing of verocytotoxin-producing *E. coli* (VTEC). Stockholm: ECDC;2012.

### 3.2 Selection of strains

Strains were selected for the EQA programme based on three criteria: a) they should represent the most commonly reported strains, b) they should remain stable during the preliminary testing period at the organising

**Table 1. Characteristics of the 10 VTEC strains used in the second VTEC EQA 2009–2010<sup>†</sup>**

Ranking <sup>a</sup>	Strain No.	Serotype	Sorbitol fermentation	β-glucuronidase activity	Haemolysin production	VCA	<i>eae</i>	<i>vtx1</i>	<i>vtx2</i>	<i>ehxA</i>	Subtyping of <i>vtx</i> genes <sup>b</sup>	Other virulence genes	Comments
19	AA1	O174:H 8	pos.	pos.	neg.	pos.	neg.	pos.	pos.	neg.	<i>vtx1c + vtx2b</i>		
23	BB2	O55:H 7	neg.	pos.	neg.	pos.	pos.	pos.	neg.	neg.	<i>vtx1a</i>	<i>astA</i>	
9 <sup>c</sup>	CC3	O128ac:[H2] <sup>d</sup>	pos.	pos.	neg.	pos.	pos.	neg.	neg.	pos.	<i>vtx2f</i>	<i>bfpA, astA</i>	
16	DD4	O177:[H25] <sup>d</sup>	neg.	pos.	Ent.	pos.	pos.	neg.	pos.	neg.	<i>vtx2c + vtx2d</i>		Lactose neg.
7	EE5	O111:[H8] <sup>d</sup>	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	<i>vtx1a + vtx2a</i>		
12	FF6	O113:H4	pos.	pos.	Ent.	pos.	neg.	pos.	pos.	neg.	<i>vtx1c + vtx2b</i>	<i>astA</i>	
2	GG7	O103:H2	pos.	pos.	Ent.	pos.	pos.	pos.	neg.	neg.	<i>vtx1a</i>		
3	HH8	O26:H11	pos.	pos.	neg.	pos.	pos.	pos.	neg.	neg.	<i>vtx1a</i>		
NR	ii9	O41:H26	pos.	pos.	neg.	pos.	neg.	pos.	neg.	neg.	<i>vtx1d</i>		
1 <sup>e</sup>	JJ10	O157:H 7	neg.	neg.	Ent.	(pos.)	pos.	neg.	pos.	neg.	<i>vtx2c</i>	<i>astA</i>	

<sup>21</sup> EFSA Journal, 9(11):2424, 101 pp.

### Austrian data regarding “underreporting bias”

Following a food-borne outbreak in an Austrian province (Tirol) caused by VTEC the local health insurance agreed to paying for VTEC tests in clinical microbiology.

**Result: provinces – e.g. Niederösterreich, Oberösterreich, twice the population compared to Tirol, report about half the cases:**

Table: preliminary data – reported infectious diseases in Austria. “E” (Erkrankungsfälle) stands for “illness”, “T” (Todesfälle) for “deaths”.

Link:[http://bmg.gv.at/cms/home/attachments/2/9/6/CH1258/CMS1314254664782/ja\\_2011\\_vorlaeufig.pdf](http://bmg.gv.at/cms/home/attachments/2/9/6/CH1258/CMS1314254664782/ja_2011_vorlaeufig.pdf) (Austrian Ministry for Health - website)

Jahr 2011		Burgenland	Kärnten	Niederösterreich	Oberösterreich	Salzburg	Steiermark	Tirol	Vorarlberg	Wien	Österreich
	Salmonella spp.	E	85	170	442	328	134	211	240	62	546
	T	-	-	3	-	-	-	-	-	-	3
STEC/VTEC	E	1	22	13	24	15	12	40	14	9	150
	T	-	-	-	-	-	2	-	-	-	2

Statistik Austria: Austrian population, provinces, since 1961:

[http://www.statistik.at/web\\_de/statistiken/bevoelkerung/bevoelkerungsstand\\_und\\_veraenderung/bevoelkerung\\_im\\_jahresdurchschnitt/index.html](http://www.statistik.at/web_de/statistiken/bevoelkerung/bevoelkerungsstand_und_veraenderung/bevoelkerung_im_jahresdurchschnitt/index.html)

#### Jahresdurchschnittsbevölkerung seit 1961 nach Bundesland

Jahr	Österreich	Burgenland	Kärnten	Nieder- österreich	Ober- österreich	Salzburg	Steiermark	Tirol	Vorarlberg	Wien
2009	8.363.040	283.506	560.056	1.606.615	1.411.041	529.314	1.207.588	704.792	368.061	1.692.067
2010	8.387.742	284.363	558.955	1.609.772	1.412.252	530.610	1.209.229	707.485	369.453	1.705.623

Q: STATISTIK AUSTRIA, Statistik des Bevölkerungsstandes. Erstellt am: 19.05.2011.

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APPENDICES

A. CLINICAL OUTCOME OF CONFIRMED HUMAN VTEC CASES DURING 2007-2010 BY SEROTYPE

**Table 1:** Clinical outcome of confirmed VTEC cases in humans in the EU<sup>(a)</sup> by serotype. TESSy data, 2007-2010 as provided by the European Centre for Disease Prevention and Control (ECDC)

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
		<b>O157</b>	<b>6 658</b>	<b>1 361</b>	<b>7</b>	<b>5 290</b>	<b>394</b>	<b>616</b>	<b>5 648</b>	<b>384</b>	<b>2 121</b>	<b>4 153</b>	<b>1 623</b>	<b>1 696</b>	<b>167</b>	<b>3 172</b>
NFT	NFT	O157:HNT	5 610	960	2	4 648	348	584	4 678	288	1 628	3 694	1 337	1 460	140	2 673
A	HAS (A/B/C)	O157:H7	774	200	4	570	26	14	734	69	280	425	172	158	20	424
A	HAS (A/B/C)	O157:H-	273	200	1	72	20	18	235	27	213	33	114	78	7	74
NLK	D	O157:H11	1	1					1			1				1
		<b>ONT</b>	<b>4 035</b>	<b>3 093</b>	<b>7</b>	<b>935</b>	<b>30</b>	<b>20</b>	<b>3 985</b>	<b>191</b>	<b>2 941</b>	<b>903</b>	<b>312</b>	<b>1 535</b>	<b>60</b>	<b>2 128</b>
NFT	NFT	ONT:HNT	3 904	3 010	7	887	24	19	3 861	185	2 852	867	305	1 464	54	2 081
NFT	NFT	ONT:H-	36	31		5	1		35		32	4	2	28	1	5
NFT	NFT	ONT:H7	14	4		10			14		3	11	1	2		11
NFT	NFT	ONT:H2	12	8		4			12	4	8			5	1	6
NFT	NFT	ONT:H19	10	3		7	1	1	8	1	3	6		3		7
NFT	NFT	ONT:H18	7	5		2			7		6	1	2	4		1
NFT	NFT	ONT:H28	6	4		2			6		4	2		4		2
NFT	NFT	ONT:H21	6	3		3	1		5		4	2		4		2
NFT	NFT	ONT:H25	5			5			5	1	1	3		2		3
NFT	NFT	ONT:H16	4	4					4		4		1	3		
NFT	NFT	ONT:H6	4	1		3			4		1	3				4
NFT	NFT	ONT:H10	4	4					4		3	1		3		1
NFT	NFT	ONT:H9	3	1		2			3		1	2		1		2

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
				NFT	NFT	ONT:H45	2	1	1		2		1	1		1
NFT	NFT	ONT:H8	2		2	1	1		2			1	1			
NFT	NFT	ONT:H14	2	1	1	1	1		2				1	1		
NFT	NFT	ONT:H1	2	2			2		2				1	1		
NFT	NFT	ONT:H4	2	1	1	1	1		2			1	1			
NFT	NFT	ONT:H30	2	2			2		2		1	1				
NFT	NFT	ONT:H20	1	1			1		1			1				
NFT	NFT	ONT:H29	1	1			1		1			1				
NFT	NFT	ONT:H12	1	1			1		1			1				
NFT	NFT	ONT:H49	1	1			1		1			1				
NFT	NFT	ONT:H23	1	1			1		1			1				
NFT	NFT	ONT:H5	1	1			1		1			1				
NFT	NFT	ONT:H39	1	1			1		1			1				
NFT	NFT	ONT:H26	1	1			1		1			1				
<b>O26</b>			<b>780</b>	<b>514</b>	<b>1</b>	<b>265</b>	<b>39</b>	<b>77</b>	<b>664</b>	<b>100</b>	<b>514</b>	<b>166</b>	<b>94</b>	<b>336</b>	<b>45</b>	<b>305</b>
NFT	NFT	O26:HNT	650	402	1	247	33	66	551	95	404	151	78	254	41	277
B	HAS (A/B/C)	O26:H11	107	98		9	4	8	95	5	93	9	11	70	4	22
NLK	D	O26:H-	20	11		9	2	3	15		14	6	3	11		6
NLK	D	O26:H7	2	2					2		2		2			
NLK	D	O26:H34	1	1					1		1			1		
<b>O103</b>			<b>370</b>	<b>306</b>		<b>64</b>	<b>14</b>	<b>21</b>	<b>335</b>	<b>7</b>	<b>302</b>	<b>61</b>	<b>31</b>	<b>198</b>	<b>11</b>	<b>130</b>
NFT	NFT	O103:HNT	230	180		50	4	6	220	7	176	47	19	95	6	110
B	D	O103:H2	131	121		10	10	15	106		121	10	11	99	5	16
NLK	D	O103:H-	6	3		3			6		3	3		3		3
NLK	D	O103:H7	1			1			1			1				1

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
NLK	D	O103:H11	1	1				1		1				1		
D	D	O103:H25	1	1				1		1		1				
		<b>O145</b>	<b>220</b>	<b>147</b>	<b>73</b>	<b>10</b>	<b>24</b>	<b>186</b>	<b>23</b>	<b>141</b>	<b>56</b>	<b>25</b>	<b>78</b>	<b>13</b>	<b>104</b>	
NFT	NFT	O145:HNT	147	95	52	8	10	129	19	89	39	18	38	7	84	
B	HAS (A/B/C)	O145:H-	33	24	9	2	1	30	2	20	11	6	16		11	
NLK	HAS (A/B/C)	O145:H28	19	7	12		13	6	2	11	6		5	6	8	
NLK	D	O145:H34	18	18				18		18			18			
NLK	D	O145:H7	2	2				2		2		1	1			
NLK	D	O145:H20	1	1				1		1					1	
		<b>O91</b>	<b>202</b>	<b>169</b>	<b>33</b>	<b>1</b>	<b>2</b>	<b>199</b>	<b>1</b>	<b>175</b>	<b>26</b>	<b>7</b>	<b>106</b>	<b>3</b>	<b>86</b>	
NFT	NFT	O91:HNT	139	119	20	1		138		124	15	3	63		73	
NLK	D	O91:H-	44	37	7		2	42		37	7	3	32	2	7	
NLK	D	O91:H14	11	7	4			11		9	2		7	1	3	
NLK	D	O91:H7	3	3				3		1	2	1			2	
NLK	HAS (A/B/C)	O91:H10	2	1	1			2	1	1			1		1	
C	D	O91:H21	2	1	1			2		2			2			
NLK	D	O91:H26	1	1				1		1			1			
		<b>O111</b>	<b>134</b>	<b>94</b>	<b>1</b>	<b>39</b>	<b>4</b>	<b>4</b>	<b>126</b>	<b>22</b>	<b>89</b>	<b>23</b>	<b>15</b>	<b>63</b>	<b>2</b>	<b>54</b>
NFT	NFT	O111:HNT	97	65	1	31	4	3	90	18	62	17	11	37	2	47
B	HAS (A/B/C)	O111:H-	29	26	3		1	28	3	24	2	2	24		3	
NLK	HAS (A/B/C)	O111:H8	6	3	3			6	1	3	2	2	2		2	
NLK	D	O111:H2	1		1			1			1				1	
NLK	D	O111:H4	1		1			1			1				1	
		<b>O128</b>	<b>108</b>	<b>83</b>	<b>25</b>	<b>1</b>	<b>3</b>	<b>104</b>	<b>5</b>	<b>88</b>	<b>15</b>	<b>5</b>	<b>58</b>	<b>5</b>	<b>40</b>	
NFT	NFT	O128:HNT	65	43	22	1	1	63	4	49	12	3	25	1	36	

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
				NLK	HAS (A/B/C)	O128:H2	30	28	2		2	28	1	28	1	1
NLK	D	O128:H-	13	12	1			13		11	2	1	9	2	1	
		<b>O146</b>	<b>102</b>	<b>81</b>	<b>21</b>	<b>2</b>	<b>2</b>	<b>98</b>		<b>82</b>	<b>20</b>	<b>4</b>	<b>62</b>	<b>7</b>	<b>29</b>	
NFT	NFT	O146:HNT	45	29	16		1	44		32	13	1	20	2	22	
D	D	O146:H21	27	23	4	2		25		25	2	2	21	2	2	
NLK	D	O146:H28	18	17	1			18		14	4		13	2	3	
NLK	D	O146:H-	9	9			1	8		9		1	7	1		
NLK	D	O146:H5	1	1				1		1					1	
NLK	D	O146:H10	1	1				1			1				1	
NLK	D	O146:H31	1	1				1		1			1			
		<b>O117</b>	<b>76</b>	<b>48</b>	<b>28</b>		<b>4</b>	<b>72</b>		<b>58</b>	<b>18</b>	<b>1</b>	<b>49</b>	<b>1</b>	<b>25</b>	
D	D	O117:H7	55	36	19		1	54		41	14		34	1	20	
NFT	NFT	O117:HNT	13	6	7		3	10		10	3		9		4	
NLK	D	O117:H-	6	5	1			6		5	1		5		1	
NLK	D	O117:H11	1		1			1		1		1				
NLK	D	O117:H8	1	1				1		1			1			
		<b>O113</b>	<b>61</b>	<b>43</b>	<b>18</b>	<b>3</b>		<b>58</b>	<b>2</b>	<b>43</b>	<b>16</b>	<b>3</b>	<b>26</b>	<b>3</b>	<b>29</b>	
NFT	NFT	O113:HNT	28	17	11			28	2	16	10	1	6		21	
D	D	O113:H4	16	15	1	1		15		16		2	11	1	2	
C	D	O113:H21	9	4	5	1		8		5	4		3	1	5	
NLK	D	O113:H6	5	5				5		5			5			
E	D	O113:H-	3	2	1	1		2		1	2		1	1	1	
		<b>O121</b>	<b>56</b>	<b>19</b>	<b>37</b>	<b>2</b>	<b>5</b>	<b>49</b>	<b>11</b>	<b>19</b>	<b>26</b>	<b>1</b>	<b>17</b>	<b>2</b>	<b>36</b>	
NFT	NFT	O121:HNT	29	3	26	1	1	27	2	2	25		2		27	
B	HAS (A/B/C)	O121:H19	23	14	9	1	4	18	8	15		1	13	2	7	

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome		Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
C	D	O121:H-	2	2				2		2			2			
NLK	D	O121:H7	1					1			1					1
NLK	HAS (A/B/C)	O121:H2	1					1		1						1
		<b>O63</b>	<b>55</b>	<b>38</b>		<b>2</b>		<b>53</b>	<b>1</b>	<b>43</b>	<b>11</b>	<b>9</b>	<b>30</b>	<b>2</b>		<b>14</b>
NLK	D	O63:H6	31	17		2		29		23	8	1	18	2		10
NFT	NFT	O63:HNT	19	16				19	1	15	3	5	10			4
NLK	D	O63:H7	3	3				3		3		2	1			
NLK	D	O63:H19	1	1				1		1		1				
NLK	D	O63:H10	1	1				1		1			1			
		<b>O76</b>	<b>34</b>	<b>20</b>				<b>34</b>	<b>1</b>	<b>19</b>	<b>14</b>	<b>1</b>	<b>14</b>	<b>1</b>		<b>18</b>
NFT	NFT	O76:HNT	17	6				17		6	11	1	3			13
NLK	HAS (A/B/C)	O76:H19	15	13				15	1	13	1		11	1		3
E	D	O76:H7	2	1				2				2				2
		<b>O156</b>	<b>34</b>	<b>21</b>				<b>34</b>		<b>21</b>	<b>13</b>	<b>3</b>	<b>16</b>			<b>15</b>
NLK	D	O156:H7	14	3				14		3	11	1	2			11
NFT	NFT	O156:HNT	9	8				9		8	1	1	5			3
E	D	O156:H-	5	5				5		5			5			
NLK	D	O156:H25	4	3			1	4		3	1	1	2			1
NLK	D	O156:H34	2	2				2		2			2			
		<b>O174</b>	<b>32</b>	<b>27</b>		<b>3</b>		<b>29</b>	<b>4</b>	<b>25</b>	<b>3</b>	<b>3</b>	<b>13</b>	<b>1</b>		<b>15</b>
NFT	NFT	O174:HNT	12	10				12		10	2	1	1			10
NLK	HAS (A/B/C)	O174:H21	11	9				11	1	9	1	1	8	1		1
NLK	HAS (A/B/C)	O174:H2	5	4		3		2	3	2		1				4
D	D	O174:H8	3	3				3		3			3			
NLK	D	O174:H28	1	1				1		1			1			

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
						<b>O55</b>	<b>31</b>	<b>27</b>	<b>4</b>			<b>31</b>	<b>3</b>	<b>27</b>	<b>1</b>	<b>5</b>
NFT	NFT	O55:HNT	22	20	2			22	3	19		4	9	2	7	
NLK	D	O55:H12	6	4	2			6		5	1		5	1		
NLK	D	O55:H7	3	3				3		3		1	2			
		<b>O5</b>	<b>25</b>	<b>18</b>	<b>7</b>	<b>4</b>		<b>21</b>	<b>1</b>	<b>20</b>	<b>4</b>	<b>1</b>	<b>16</b>	<b>1</b>	<b>7</b>	
NFT	NFT	O5:HNT	15	9	6	4		11	1	11	3	1	7	1	6	
C	D	O5:H-	8	7	1			8		7	1		7		1	
NLK	D	O5:H19	1	1				1		1			1			
NLK	D	O5:H16	1	1				1		1			1			
		<b>O8</b>	<b>20</b>	<b>9</b>	<b>11</b>			<b>20</b>		<b>12</b>	<b>8</b>	<b>2</b>	<b>8</b>	<b>1</b>	<b>9</b>	
NFT	NFT	O8:HNT	14	6	8			14		8	6	2	4	1	7	
NLK	D	O8:H9	2	1	1			2		1	1		1		1	
NLK	D	O8:H8	1	1				1		1			1			
NLK	D	O8:H12	1	1				1		1			1			
NLK	D	O8:H-	1		1			1		1			1			
NLK	D	O8:H25	1		1			1			1				1	
		<b>O78</b>	<b>20</b>	<b>19</b>	<b>1</b>	<b>1</b>		<b>19</b>	<b>1</b>	<b>18</b>	<b>1</b>	<b>1</b>	<b>15</b>		<b>4</b>	
NFT	NFT	O78:HNT	14	13	1	1		13	1	13			10		4	
NLK	D	O78:H-	6	6				6		5	1	1	5			
		<b>O177</b>	<b>19</b>	<b>15</b>	<b>4</b>		<b>1</b>	<b>18</b>		<b>14</b>	<b>5</b>		<b>9</b>	<b>1</b>	<b>9</b>	
NFT	NFT	O177:HNT	12	10	2			12		9	3		5		7	
NLK	D	O177:H-	5	3	2		<b>1</b>	4		3	2		2	1	2	
NLK	D	O177:H45	1	1				1		1			1			
NLK	D	O177:H11	1	1				1		1			1			
		<b>O118</b>	<b>19</b>	<b>13</b>	<b>6</b>			<b>19</b>		<b>14</b>	<b>5</b>	<b>3</b>	<b>7</b>		<b>9</b>	



Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
NFT	NFT	O118:HNT	14	10	4		14		12	2	3	5					6
NLK	D	O118:H16	3	1	2		3		1	2		1					2
NLK	D	O118:H12	1	1			1		1			1					
NLK	D	O118:H4	1	1			1			1							1
		<b>O125</b>	<b>18</b>	<b>17</b>	<b>1</b>		<b>18</b>		<b>17</b>	<b>1</b>	<b>2</b>	<b>10</b>	<b>2</b>			<b>4</b>	
NFT	NFT	O125:HNT	12	11	1		12		11	1	1	6	1			4	
NLK	D	O125:H6	6	6			6		6		1	4	1				
		<b>O2</b>	<b>17</b>	<b>13</b>	<b>4</b>		<b>17</b>		<b>16</b>	<b>1</b>	<b>1</b>	<b>12</b>				<b>4</b>	
NFT	NFT	O2:HNT	8	6	2		8		7	1		4				4	
NLK	D	O2:H6	6	4	2		6		6			6					
NLK	D	O2:H14	1	1			1		1			1					
NLK	D	O2:H20	1	1			1		1			1					
NLK	D	O2:H29	1	1			1		1		1						
		<b>O153</b>	<b>16</b>	<b>4</b>	<b>12</b>		<b>16</b>		<b>6</b>	<b>10</b>		<b>4</b>	<b>1</b>			<b>11</b>	
NFT	NFT	O153:HNT	14	3	11		14		5	9		3	1			10	
NLK	D	O153:H2	1	1			1		1			1					
NLK	D	O153:H25	1		1		1			1						1	
		<b>O166</b>	<b>15</b>	<b>11</b>	<b>4</b>	<b>1</b>	<b>14</b>		<b>12</b>	<b>3</b>	<b>1</b>	<b>9</b>				<b>5</b>	
NFT	NFT	O166:HNT	8	5	3		8		5	3	1	2				5	
NLK	D	O166:H28	5	4	1	1	4		5			5					
NLK	D	O166:H15	1	1			1		1			1					
NLK	D	O166:H30	1	1			1		1			1					
		<b>O84</b>	<b>13</b>	<b>5</b>	<b>8</b>	<b>1</b>	<b>12</b>		<b>8</b>	<b>5</b>	<b>1</b>	<b>5</b>				<b>7</b>	
NFT	NFT	O84:HNT	7	1	6	1	6		2	5						7	
E	D	O84:H-	2	2			2		2		1	1					

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
				NLK	D	O84:H28	2		2		2		2			2
NLK	D	O84:H1	1	1			1		1			1				
NLK	D	O84:H2	1	1			1		1			1				
		<b>O6</b>	<b>13</b>	<b>11</b>	<b>2</b>		<b>1</b>	<b>12</b>	<b>1</b>	<b>9</b>	<b>3</b>	<b>2</b>	<b>5</b>	<b>2</b>	<b>4</b>	
NFT	NFT	O6:HNT	6	5	1		1	5	1	4	1	2	1	1	2	
NLK	D	O6:H-	4	3	1			4		3	1		3		1	
NLK	D	O6:H25	1	1				1		1			1			
NLK	D	O6:H10	1	1				1			1					1
NLK	D	O6:H19	1	1				1		1				1		
		<b>O1</b>	<b>13</b>	<b>13</b>		<b>1</b>		<b>12</b>	<b>2</b>	<b>11</b>			<b>4</b>		<b>9</b>	
NFT	NFT	O1:HNT	11	11				11	1	10			3		8	
NLK	D	O1:H20	1	1				1		1			1			
NLK	HAS (A/B/C)	O1:H42	1	1		1			1							1
		<b>O86</b>	<b>12</b>	<b>11</b>	<b>1</b>			<b>12</b>	<b>1</b>	<b>10</b>	<b>1</b>		<b>6</b>		<b>6</b>	
NFT	NFT	O86:HNT	10	10				10		10			6		4	
NLK	D	O86:H-	1	1				1			1				1	
NLK	HAS (A/B/C)	O86:H27	1		1			1	1							1
		<b>O114</b>	<b>12</b>	<b>10</b>	<b>2</b>			<b>12</b>	<b>2</b>	<b>9</b>	<b>1</b>	<b>1</b>	<b>8</b>		<b>3</b>	
NFT	NFT	O114:HNT	11	9	2			11	2	8	1	1	7		3	
NLK	D	O114:H41	1	1				1		1			1			
		<b>O181</b>	<b>12</b>	<b>12</b>		<b>1</b>		<b>11</b>		<b>11</b>	<b>1</b>		<b>8</b>		<b>4</b>	
NLK	D	O181:H49	7	7		1		6		6	1		3		4	
NLK	D	O181:H16	4	4				4		4			4			
NFT	NFT	O181:HNT	1	1				1		1			1			
		<b>O142</b>	<b>11</b>	<b>7</b>	<b>4</b>			<b>11</b>		<b>7</b>	<b>4</b>		<b>5</b>		<b>6</b>	

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
				NFT	NFT	O142:HNT	8	7	1		8		7	1		5
NLK	D	O142:H34	1		1		1			1				1		
NLK	D	O142:H-	1		1		1			1				1		
NLK	D	O142:H33	1		1		1			1				1		
			<b>11</b>	<b>7</b>	<b>4</b>		<b>11</b>	<b>2</b>	<b>8</b>	<b>1</b>	<b>1</b>	<b>7</b>		<b>3</b>		
NFT	NFT	O80:HNT	6	4	2		6	1	5		1	4		1		
NLK	HAS (A/B/C)	O80:H2	4	2	2		4	1	3			2		2		
NLK	D	O80:H-	1	1			1			1		1				
			<b>10</b>	<b>7</b>	<b>3</b>		<b>10</b>		<b>8</b>	<b>2</b>	<b>1</b>	<b>6</b>		<b>3</b>		
NFT	NFT	O43:HNT	6	4	2		6		5	1	1	4		1		
NLK	D	O43:H2	4	3	1		4		3	1		2		2		
			<b>9</b>	<b>2</b>	<b>7</b>		<b>9</b>	<b>1</b>	<b>4</b>	<b>4</b>	<b>1</b>	<b>1</b>		<b>7</b>		
NFT	NFT	O123:HNT	6	2	4		6		3	3	1	1		4		
NLK	HAS (A/B/C)	O123:H2	2		2		2	1	1					2		
NLK	D	O123:H19	1		1		1			1				1		
			<b>9</b>	<b>9</b>			<b>9</b>		<b>9</b>			<b>5</b>		<b>4</b>		
NFT	NFT	O44:HNT	9	9			9		9			5		4		
			<b>8</b>	<b>6</b>	<b>2</b>	<b>1</b>	<b>7</b>	<b>1</b>	<b>6</b>	<b>1</b>	<b>1</b>	<b>4</b>	<b>1</b>	<b>2</b>		
NLK	D	O20:H45	2	1	1	1	1		2			2				
NLK	D	O20:H49	1	1			1		1			1				
NLK	D	O20:H12	1	1			1		1		1					
NLK	D	O20:H16	1	1			1			1				1		
NFT	NFT	O20:HNT	1	1			1	1						1		
NLK	D	O20:H25	1	1			1		1				1			
NLK	D	O20:H4	1		1		1		1			1				

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
		<b>O104</b>	<b>8</b>	<b>3</b>	<b>5</b>	<b>1</b>	<b>3</b>	<b>4</b>	<b>1</b>	<b>6</b>	<b>1</b>	<b>4</b>	<b>2</b>	<b>2</b>		
NFT	NFT	O104:HNT	4	1	3		2	2		3	1	3		1		
NLK	D	O104:H2	2	1	1			2		2		1	1			
NLK	D	O104:H-	1		1		1			1			1			
C	HAS (A/B/C)	O104:H21	1	1		1			1					1		
		<b>O100</b>	<b>8</b>	<b>8</b>				<b>8</b>		<b>8</b>		<b>2</b>	<b>6</b>			
NFT	NFT	O100:HNT	7	7				7		7		2	5			
NLK	D	O100:H-	1	1				1		1			1			
		<b>ONON-O157</b>	<b>7</b>		<b>7</b>	<b>1</b>		<b>6</b>		<b>1</b>	<b>6</b>		<b>1</b>	<b>6</b>		
NFT	NFT	ONON-O157:HNT	7		7	1		6		1	6		1	6		
		<b>O178</b>	<b>7</b>	<b>7</b>			<b>1</b>	<b>6</b>		<b>7</b>		<b>1</b>	<b>5</b>	<b>1</b>		
NFT	NFT	O178:HNT	3	3			1	2		3			2	1		
NLK	D	O178:H7	3	3				3		3		1	2			
NLK	D	O178:H19	1	1				1		1			1			
		<b>O126</b>	<b>7</b>	<b>6</b>	<b>1</b>			<b>7</b>	<b>2</b>	<b>5</b>		<b>1</b>	<b>1</b>	<b>5</b>		
NFT	NFT	O126:HNT	6	5	1			6	2	4		1	1	4		
NLK	D	O126:H-	1	1				1		1				1		
		<b>O119</b>	<b>6</b>	<b>5</b>	<b>1</b>			<b>6</b>		<b>5</b>	<b>1</b>	<b>3</b>	<b>3</b>			
NFT	NFT	O119:HNT	4	3	1			4		4			2	2		
NLK	D	O119:H-	1	1				1		1			1			
NLK	D	O119:H4	1	1				1			1			1		
		<b>O25</b>	<b>6</b>	<b>5</b>	<b>1</b>			<b>6</b>		<b>6</b>		<b>1</b>	<b>5</b>			
NFT	NFT	O25:HNT	6	5	1			6		6			1	5		
		<b>O165</b>	<b>6</b>	<b>5</b>	<b>1</b>	<b>1</b>		<b>5</b>		<b>5</b>	<b>1</b>	<b>1</b>	<b>3</b>	<b>2</b>		
NLK	D	O165:H-	2	2				2		2			2			

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
				NFT	NFT	O165:HNT	2	2		1	1	2		1		
C	D	O165:H25	2	1	1		2	1	1				1		1	
		<b>O130</b>	<b>5</b>	<b>4</b>	<b>1</b>		<b>5</b>	<b>4</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>		<b>3</b>	
NFT	NFT	O130:HNT	3	3			3	3		1	1				1	
NLK	D	O130:H11	2	1	1		2	1	1						2	
		<b>O18</b>	<b>5</b>	<b>5</b>			<b>5</b>	<b>4</b>	<b>1</b>			<b>1</b>			<b>4</b>	
NFT	NFT	O18:HNT	4	4			4	4				1			3	
NLK	D	O18:H16	1	1			1		1						1	
		<b>O127</b>	<b>5</b>	<b>5</b>			<b>5</b>	<b>1</b>	<b>4</b>		<b>1</b>	<b>1</b>			<b>3</b>	
NFT	NFT	O127:HNT	5	5			5	1	4		1	1			3	
		<b>O101</b>	<b>5</b>	<b>3</b>	<b>2</b>		<b>5</b>		<b>3</b>	<b>2</b>	<b>1</b>	<b>2</b>			<b>2</b>	
NFT	NFT	O101:HNT	2	1	1		2	1	1	1					1	
NLK	D	O101:H9	1	1			1	1				1				
NLK	D	O101:H-	1		1		1			1					1	
NLK	D	O101:H7	1	1			1	1				1				
		<b>O38</b>	<b>5</b>	<b>2</b>	<b>3</b>		<b>5</b>		<b>2</b>	<b>3</b>		<b>1</b>			<b>4</b>	
NFT	NFT	O38:HNT	4	1	3		4	1	3						4	
NLK	D	O38:H26	1	1			1	1				1				
		<b>O112</b>	<b>5</b>	<b>5</b>			<b>5</b>		<b>5</b>			<b>5</b>				
NLK	D	O112:H8	2	2			2	2				2				
NFT	NFT	O112:HNT	1	1			1	1				1				
NLK	D	O112:H18	1	1			1	1				1				
NLK	D	O112:H2	1	1			1	1				1				
		<b>O132</b>	<b>5</b>	<b>5</b>			<b>5</b>		<b>5</b>		<b>1</b>	<b>3</b>			<b>1</b>	
NLK	D	O132:H34	2	2			2	2			1	1				

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation			
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>
NFT	NFT	O132:HNT	1	1				1		1						1
NLK	D	O132:H10	1	1				1		1			1			
NLK	D	O132:H21	1	1				1		1			1			
		<b>O105</b>	<b>5</b>		<b>1</b>	<b>4</b>	<b>1</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>4</b>		<b>1</b>	<b>3</b>	<b>1</b>	
NFT	NFT	O105:HNT	3			3		3			3				3	
NLK	HAS (A/B/C)	O105:H18	2	1	1	1	1	1	1	1			1			1
		<b>O27</b>	<b>5</b>	<b>3</b>		<b>2</b>	<b>1</b>	<b>1</b>	<b>3</b>		<b>3</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>3</b>	
NLK	D	O27:H30	3	3			1		2		3		1	1	1	
NFT	NFT	O27:HNT	2			2		1	1			2				2
		<b>O21</b>	<b>4</b>	<b>3</b>		<b>1</b>			<b>4</b>		<b>3</b>	<b>1</b>		<b>2</b>		<b>2</b>
NFT	NFT	O21:HNT	3	2		1			3		2	1		1		2
NLK	D	O21:H21	1	1					1		1			1		
		<b>O15</b>	<b>4</b>	<b>2</b>		<b>2</b>	<b>1</b>		<b>3</b>		<b>4</b>		<b>1</b>	<b>2</b>	<b>1</b>	
NFT	NFT	O15:HNT	1	1					1		1			1		
NLK	D	O15:H27	1			1	1				1				1	
NLK	D	O15:H-	1			1			1		1		1			
NLK	D	O15:H2	1	1					1		1			1		
		<b>O163</b>	<b>4</b>	<b>3</b>		<b>1</b>			<b>4</b>		<b>2</b>	<b>2</b>		<b>1</b>		<b>3</b>
NFT	NFT	O163:HNT	3	2		1			3		2	1				3
NLK	D	O163:H19	1	1					1			1		1		
		<b>O88</b>	<b>4</b>	<b>4</b>					<b>4</b>		<b>4</b>			<b>4</b>		
E	D	O88:H25	2	2					2		2			2		
NLK	D	O88:H-	2	2					2		2			2		
		<b>O11</b>	<b>4</b>	<b>4</b>					<b>4</b>	<b>1</b>	<b>3</b>		<b>1</b>	<b>1</b>		<b>2</b>
NFT	NFT	O11:HNT	2	2					2	1	1		1			1



Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
NLK	D	O11:H4	2	2				2		2				1			1
		<b>O158</b>	<b>4</b>	<b>4</b>				<b>4</b>		<b>4</b>			<b>1</b>	<b>1</b>			<b>2</b>
NFT	NFT	O158:HNT	4	4				4		4			1	1			2
		<b>O71</b>	<b>4</b>	<b>1</b>	<b>3</b>			<b>4</b>		<b>2</b>	<b>2</b>			<b>1</b>			<b>3</b>
NFT	NFT	O71:HNT	3		3			3		1	2						3
NLK	D	O71:H-	1	1				1		1				1			
		<b>O4</b>	<b>4</b>	<b>3</b>	<b>1</b>		<b>1</b>	<b>3</b>		<b>4</b>				<b>1</b>	<b>1</b>		<b>2</b>
NFT	NFT	O4:HNT	3	2	1		1	2		3						1	2
NLK	D	O4:H-	1	1				1		1				1			
		<b>O75</b>	<b>4</b>	<b>4</b>				<b>4</b>		<b>4</b>				<b>3</b>	<b>1</b>		
NLK	D	O75:H8	2	2				2		2				2			
NFT	NFT	O75:HNT	1	1				1		1						1	
NLK	D	O75:H-	1	1				1		1				1			
		<b>O176</b>	<b>4</b>	<b>3</b>	<b>1</b>			<b>4</b>		<b>4</b>				<b>3</b>			<b>1</b>
NLK	D	O176:H-	3	3				3		3				3			
NFT	NFT	O176:HNT	1		1			1		1							1
		<b>O17</b>	<b>3</b>	<b>2</b>	<b>1</b>			<b>3</b>		<b>2</b>	<b>1</b>			<b>2</b>			<b>1</b>
NLK	D	O17:H41	2	1	1			2		1	1			1			1
NLK	D	O17:H18	1	1				1		1				1			
		<b>O115</b>	<b>3</b>	<b>3</b>				<b>3</b>		<b>3</b>				<b>2</b>			<b>1</b>
NFT	NFT	O115:HNT	2	2				2		2				1			1
NLK	D	O115:H-	1	1				1		1				1			
		<b>O53</b>	<b>3</b>	<b>2</b>	<b>1</b>			<b>3</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>		<b>1</b>
NFT	NFT	O53:HNT	2	2				2	1	1			1	1			
NLK	D	O53:H7	1		1			1				1					1

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
		<b>O182</b>	<b>3</b>	<b>3</b>				<b>3</b>		<b>3</b>				<b>3</b>			
NLK	D	O182:H25	3	3				3		3				3			
		<b>O7</b>	<b>3</b>	<b>1</b>	<b>2</b>			<b>3</b>	<b>1</b>	<b>1</b>	<b>1</b>			<b>1</b>			<b>2</b>
NFT	NFT	O7:HNT	2	1	1			2		1	1			1			1
NLK	HAS (A/B/C)	O7:H6	1		1			1		1							1
		<b>O186</b>	<b>3</b>	<b>3</b>				<b>3</b>		<b>3</b>				<b>3</b>			
NLK	D	O186:H2	2	2				2		2				2			
NLK	D	O186:H-	1	1				1		1				1			
		<b>O98</b>	<b>3</b>		<b>3</b>			<b>3</b>		<b>3</b>			<b>1</b>	<b>2</b>			
NFT	NFT	O98:HNT	2		2			2		2			1	1			
NLK	D	O98:H-	1		1			1		1				1			
		<b>O12</b>	<b>3</b>	<b>1</b>	<b>2</b>			<b>3</b>		<b>1</b>	<b>2</b>			<b>2</b>			<b>1</b>
NFT	NFT	O12:HNT	2		2			2			2			1			1
NLK	D	O12:H-	1	1				1		1				1			
		<b>O54</b>	<b>3</b>	<b>3</b>				<b>3</b>		<b>3</b>			<b>1</b>	<b>1</b>			<b>1</b>
NLK	D	O54:H21	2	2				2		2			1	1			
NFT	NFT	O54:HNT	1	1				1		1							1
		<b>O3</b>	<b>3</b>	<b>1</b>	<b>2</b>		<b>1</b>	<b>2</b>		<b>2</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>
NFT	NFT	O3:HNT	3	1	2		1	2		2	1	1				1	1
		<b>O102</b>	<b>3</b>	<b>3</b>				<b>3</b>		<b>3</b>				<b>2</b>		<b>1</b>	
NLK	D	O102:H6	2	2				2		2				1		1	
NLK	D	O102:H-	1	1				1		1				1			
		<b>O35</b>	<b>3</b>	<b>2</b>	<b>1</b>			<b>3</b>		<b>2</b>	<b>1</b>	<b>2</b>					<b>1</b>
NFT	NFT	O35:HNT	2	1	1			2		1	1	1					1
NLK	D	O35:H19	1	1				1		1				1			

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
		<b>O168</b>	<b>3</b>	<b>2</b>	<b>1</b>			<b>3</b>	<b>3</b>				<b>3</b>				
NLK	D	O168:H8	2	1	1			2	2				2				
NLK	D	O168:H28	1	1				1	1				1				
		<b>O39</b>	<b>3</b>	<b>3</b>				<b>3</b>	<b>3</b>				<b>1</b>	<b>2</b>			
NLK	D	O39:H-	1	1				1	1				1				
NLK	D	O39:H48	1	1				1	1			1					
NLK	D	O39:H21	1	1				1	1				1				
		<b>O89</b>	<b>3</b>	<b>3</b>				<b>3</b>	<b>3</b>				<b>3</b>				
NLK	D	O89:H-	1	1				1	1				1				
NLK	D	O89:H8	1	1				1	1				1				
NLK	D	O89:H4	1	1				1	1				1				
		<b>O40</b>	<b>3</b>	<b>3</b>				<b>3</b>	<b>3</b>				<b>3</b>				
NLK	D	O40:H8	2	2				2	2				2				
NLK	D	O40:H7	1	1				1	1				1				
		<b>O45</b>	<b>3</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>3</b>				<b>1</b>	<b>1</b>	<b>1</b>		
NFT	NFT	O45:HNT	2		2	1	2	3	3				1	1	1		
NLK	D	O45:H2	1	1				1	1				1				
		<b>O74</b>	<b>2</b>	<b>2</b>				<b>2</b>	<b>2</b>								<b>2</b>
NFT	NFT	O74:HNT	2	2				2	2								2
		<b>O51</b>	<b>2</b>		<b>2</b>			<b>2</b>				<b>2</b>					<b>2</b>
NFT	NFT	O51:HNT	1		1			1				1					1
NLK	D	O51:H-	1		1			1				1					1
		<b>O107</b>	<b>2</b>	<b>2</b>				<b>2</b>	<b>2</b>				<b>2</b>				
NLK	D	O107:H21	1	1				1	1				1				
NLK	D	O107:H-	1	1				1	1				1				

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation					
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>		
NFT	NFT	<b>O137</b>	2	2				2		1	1		1				1	
		O137:HNT	2	2				2		1	1		1				1	
NFT	NFT	<b>O141</b>	2	2				2		2				1			1	
		O141:HNT	1	1				1		1							1	
NLK	D	O141:H25	1	1				1		1				1				
NFT	NFT	<b>O22</b>	2	2				2		1	1			1			1	
		O22:HNT	2	2				2		1	1			1			1	
NLK	D	<b>O92</b>	2	2				2		2			1	1				
		O92:H18	1	1				1		1				1				
NLK	D	O92:H-	1	1				1		1			1					
NFT	NFT	<b>O57</b>	2	2				2		1	1			1			1	
		O57:HNT	2	2				2		1	1			1			1	
NLK	D	<b>O87</b>	2	2				2		2				1	1			
		O87:H16	2	2				2		2				1	1			
NLK	D	<b>O24</b>	2	1	1	1		1		2				1	1			
		O24:H18	1	1				1		1				1				
NLK	D	O24:H10	1		1	1				1					1			
NLK	D	<b>O9</b>	2	2				2		2				2				
		O9:H-	2	2				2		2				2				
NLK	D	<b>O109</b>	2	1	1			2		1	1			1			1	
		O109:H5	1	1				1		1							1	
NLK	D	O109:H25	1		1			1				1		1				
NLK	D	<b>O70</b>	2	2				2		2				1			1	
		O70:H7	1	1				1		1							1	
NLK	D	O70:H-	1	1				1		1				1				

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
		<b>O175</b>	<b>2</b>	<b>1</b>	<b>1</b>			<b>2</b>	<b>1</b>	<b>1</b>	<b>1</b>						<b>1</b>
NLK	D	O175:H21	1		1			1		1							1
NLK	D	O175:H16	1	1				1		1			1				
		<b>O136</b>	<b>2</b>	<b>1</b>	<b>1</b>			<b>2</b>	<b>2</b>	<b>1</b>	<b>1</b>		<b>1</b>	<b>1</b>			
NLK	D	O136:H20	2	1	1			2	2	1	1		1	1			
		<b>O139</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>					<b>1</b>			
NLK	D	O139:H-	1	1				1	1					1			
		<b>O124</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>					<b>1</b>			
NFT	NFT	O124:HNT	1	1				1	1					1			
		<b>O106</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>								<b>1</b>
NFT	NFT	O106:HNT	1	1				1	1								1
		<b>O49</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>					<b>1</b>			
NLK	D	O49:H-	1	1				1	1					1			
		<b>O41</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>								<b>1</b>
NFT	NFT	O41:HNT	1	1				1	1								1
		<b>O154</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>					<b>1</b>			
NLK	D	O154:H31	1	1				1	1					1			
		<b>O131</b>	<b>1</b>		<b>1</b>			<b>1</b>			<b>1</b>						<b>1</b>
NFT	NFT	O131:HNT	1		1			1			1						1
		<b>O108</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>					<b>1</b>			
NFT	NFT	O108:HNT	1	1				1	1					1			
		<b>O14</b>	<b>1</b>	<b>1</b>				<b>1</b>	<b>1</b>					<b>1</b>			
NLK	D	O14:H28	1	1				1	1					1			
		<b>O147</b>	<b>1</b>		<b>1</b>			<b>1</b>			<b>1</b>						<b>1</b>

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation					
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>		
NLK	D	O147:H7	1			1			1			1						1
		<b>O138</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
NLK	D	O138:H48	1	1					1		1				1			
		<b>O28</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
NFT	NFT	O28:HNT	1	1					1		1				1			
		<b>O172</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
D	D	O172:H-	1	1					1		1				1			
		<b>O29</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>							<b>1</b>
NFT	NFT	O29:HNT	1	1					1		1							1
		<b>O19</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
NFT	NFT	O19:HNT	1	1					1		1				1			
		<b>O140</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
NFT	NFT	O140:HNT	1	1					1		1				1			
		<b>O77</b>	<b>1</b>			<b>1</b>			<b>1</b>				<b>1</b>					<b>1</b>
NLK	D	O77:H18	1			1			1				1					1
		<b>O116</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
NFT	NFT	O116:HNT	1	1					1		1				1			
		<b>O79</b>	<b>1</b>			<b>1</b>			<b>1</b>		<b>1</b>				<b>1</b>			
NLK	D	O79:H14	1			1			1		1				1			
		<b>O34</b>	<b>1</b>			<b>1</b>			<b>1</b>				<b>1</b>					<b>1</b>
NLK	D	O34:H-	1			1			1				1					1
		<b>O169</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			
NLK	D	O169:H18	1	1					1		1				1			
		<b>O65</b>	<b>1</b>	<b>1</b>					<b>1</b>		<b>1</b>				<b>1</b>			



Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases	Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
				Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
NLK	D	O65:H2	1	1				1		1				1			
		<b>O170</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>			<b>1</b>				
NLK	D	O170:H7	1	1				1		1			1				
		<b>O180</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>				<b>1</b>			
NFT	NFT	O180:HNT	1	1				1		1				1			
		<b>O171</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>							<b>1</b>
D	D	O171:H2	1	1				1		1							1
		<b>O36</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>							<b>1</b>
NLK	D	O36:H-	1	1				1		1							1
		<b>O150</b>	<b>1</b>	<b>1</b>			<b>1</b>			<b>1</b>							<b>1</b>
NFT	NFT	O150:HNT	1	1			1			1							1
		<b>O16</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>							<b>1</b>
NFT	NFT	O16:HNT	1	1			1			1							1
		<b>O23</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>							<b>1</b>
NFT	NFT	O23:HNT	1	1				1		1							1
		<b>O73</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>				<b>1</b>			
NLK	D	O73:H18	1	1				1		1				1			
		<b>O134</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>				<b>1</b>			
NLK	D	O134:H38	1	1				1		1				1			
		<b>O148</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>				<b>1</b>			
NFT	NFT	O148:HNT	1	1				1		1				1			
		<b>O110</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>				<b>1</b>			
NLK	D	O110:H8	1	1				1		1				1			
		<b>O48</b>	<b>1</b>	<b>1</b>				<b>1</b>		<b>1</b>				<b>1</b>			

Classification based on original Karmali seropathotype approach <sup>(b)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(b)</sup>	Serogroup/serotype <sup>(c)</sup>	All cases		Outcome			Hospitalisation			HUS <sup>(d)</sup>			Clinical manifestation				
			All cases	Outcome	Alive	Dead	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	Yes	No	NR <sup>(e)</sup>	BD <sup>(f)</sup>	D <sup>(g)</sup>	Asy <sup>(h)</sup>	NR <sup>(e)</sup>	
NLK	D	O48:H7	1	1					1			1				1		
<b>Grand Total</b>			<b>13 545</b>	<b>6 501</b>	<b>18</b>	<b>7 026</b>	<b>522</b>	<b>793</b>	<b>12 230</b>	<b>777</b>	<b>7 143</b>	<b>5 625</b>	<b>2 182</b>	<b>4 587</b>	<b>352</b>	<b>6 424</b>		

(a): Confirmed cases are laboratory confirmed and may or may not fulfil the clinical criteria as described in the case definition.

(b): NFT = strains that were not fully serotyped. NLK = serotypes that were fully serotyped but were not listed by Karmali et al. (2003). HAS = HUS-associated serotypes. Includes the serotypes that have been associated with reported confirmed HUS cases of human VTEC in EU in 2007-2010.

(c): ONT = the 'O' antigen was untyped/untypeable or reported as unknown. HNT = the 'H' antigen was untyped/untypeable or reported as unknown.

(d): Haemolytic uraemic syndrome.

(e): NR = non reported.

(f): BD = bloody diarrhoea.

(g): D = diarrhoea.

(h): Asy = asymptomatic.

**B. DATA REPORTED IN THE ZOOSES DATABASE ON OCCURRENCE OF STRONG EVIDENCE FOOD-BORNE OUTBREAKS WHERE THE CAUSATIVE AGENT WAS PATHOGENIC *ESCHERICHIA COLI*(2007-2011)**

**Table 1:** Reported strong evidence food-borne outbreaks where the causative agent was pathogenic *Escherichia coli* in the reporting countries in accordance with Directive 2003/99/EC<sup>22</sup>, 2007-2011<sup>(a)</sup>

Zoonotic agent species	Serotype	Year	Country	Food vehicle: more food vehicle information	Type of evidence	Human cases	Hospitalisation	Deaths
Verotoxigenic <i>E. coli</i> (VTEC)	O104:H4	2011	Denmark	<b>Vegetables and juices and other products thereof:</b> fenugreek sprouts	Analytical epidemiological evidence	26	20	0
Verotoxigenic <i>E. coli</i> (VTEC)	O27:H30	2011	Denmark	<b>Vegetables and juices and other products thereof:</b> sugar peas imported	Analytical epidemiological evidence; descriptive epidemiological evidence	87	0	0
Verotoxigenic <i>E. coli</i> (VTEC)	O104:H4	2011	Netherlands	<b>Vegetables and juices and other products thereof:</b> fenugreek	Analytical epidemiological evidence	11	8	0
Verotoxigenic <i>E. coli</i> (VTEC)	O104:H4 <sup>(b)</sup>	2011	France	<b>Vegetables and juices and other products thereof</b> <sup>(c)</sup>	Detection of causative agent in food vehicle or its component - symptoms and onset of illness in outbreak cases	15	15	0
Verotoxigenic <i>E. coli</i> (VTEC)	O104:H4 <sup>(b)</sup>	2011	Germany	<b>Vegetables and juices and other products thereof:</b> sprouted fenugreek seeds	Analytical epidemiological evidence	3 793	2 353	53
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	Netherlands	<b>Bovine meat and products thereof:</b> filet americain	Detection of causative agent in food vehicle or its component - detection of indistinguishable causative agent in humans	3	-	-
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	UK	<b>Vegetables and juices and other products thereof:</b> mixed salad	Descriptive epidemiological evidence	7	2	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	UK	<b>Crustaceans, shellfish, molluscs and products thereof:</b> crab meat	Analytical epidemiological evidence	9	1	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	UK	<b>Vegetables and juices and other products thereof:</b> handling raw leeks, handling raw potatoes	Analytical epidemiological evidence	250	79	1
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	UK	<b>Bovine meat and products thereof:</b> beef curry	Descriptive epidemiological evidence	4	0	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	UK	<b>Other foods:</b> sandwiches	Descriptive epidemiological evidence	6	3	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	UK	<b>Other foods:</b> kebabs	Descriptive epidemiological evidence	12	1	0

<sup>22</sup> 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

Zoonotic agent species	Serotype	Year	Country	Food vehicle: more food vehicle information	Type of evidence	Human cases	Hospitalisation	Deaths
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	Ireland	<b>Tap water, including well water</b>	Detection of causative agent in food vehicle or its component - Detection of indistinguishable causative agent in humans, Descriptive epidemiological evidence	2	0	-
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	Ireland	<b>Tap water, including well water:</b> group water scheme, ground water	Detection of causative agent in food vehicle or its component - Detection of indistinguishable causative agent in humans, Descriptive epidemiological evidence	20	7	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2011	Ireland	<b>Tap water, including well water</b>	Detection of causative agent in food vehicle or its component - Detection of indistinguishable causative agent in humans, Descriptive epidemiological evidence	3	-	-
Verotoxigenic <i>E. coli</i> (VTEC)	O26	2010	Germany	<b>Cheese:</b> different types of cheese, predominantly raw milk cheeses, inclusive semi-hard cheese	Detection of causative agent in food vehicle or its component - Detection of indistinguishable causative agent in humans	4	2	0
Verotoxigenic <i>E. coli</i> (VTEC)	NR	2008	Belgium	<b>Bovine meat and products thereof:</b> minced raw beef meat also mixed with raw pork meat	Analytical epidemiological evidence Laboratory characterisation of food and human isolates Laboratory detection in human cases Laboratory detection in implicated food	6	4	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157:H7	2008	Germany	<b>Milk:</b> raw milk	Analytical epidemiological evidence Laboratory characterisation of food and human isolates Laboratory detection in human cases Laboratory detection in implicated food	23	2	0
Verotoxigenic <i>E. coli</i> (VTEC)	NR	2008	Portugal	<b>Fish and fish products:</b> tuna fish paté	Laboratory detection in implicated food	5	-	0
Verotoxigenic <i>E. coli</i> (VTEC)	NR	2007	Belgium	<b>Dairy products (other than cheeses):</b> ice-cream bought on farm	Laboratory detection in human cases	13	5	0
Verotoxigenic <i>E. coli</i> (VTEC)	O157	2007	Ireland	<b>Tap water, including well water: well water</b>	Laboratory detection in human cases	6	2	0
Verotoxigenic <i>E. coli</i> (VTEC)	O76	2007	Sweden	<b>Cheese</b>	Laboratory detection in human cases	5	0	0
Enterotoxigenic <i>E. coli</i> (ETEC)	NR	2007	Denmark	<b>Unknown</b>	Laboratory detection in human cases	8	0	0
Enterotoxigenic <i>E. coli</i> (ETEC)	O26:H-	2007	Denmark	<b>Bovine meat and products thereof:</b> organic sausage	Analytical epidemiological evidence	18	0	0

Zoonotic agent species	Serotype	Year	Country	Food vehicle: more food vehicle information	Type of evidence	Human cases	Hospitalisation	Deaths
Enterotoxigenic <i>E. coli</i> (ETEC)	NR	2007	Sweden	<b>Mixed or buffet meals:</b> sandwich layer-cake	Laboratory detection in human cases	40	0	0
Enteropathogenic <i>E. coli</i> (EPEC)	NR	2011	Spain	<b>Crustaceans, shellfish, molluscs and products thereof</b>	Detection in a food vehicle or its component in combination with compatible clinical symptoms and onset of illness in outbreak cases	14	0	0
Enteropathogenic <i>E. coli</i> (EPEC)	NR	2007	Poland	<b>Other foods</b>	Analytical epidemiological evidence	3	3	0
Enteropathogenic <i>E. coli</i> (EPEC)	NR	2007	Slovenia	<b>Other foods:</b> French salad	Laboratory characterisation of isolates	92	0	0
Enteropathogenic <i>E. coli</i> (EPEC)	NR	2007	Spain	<b>Cheese</b>	Not specified	6	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2010	Spain	<b>Crustaceans, shellfish, molluscs and products thereof</b>	Detection in a food vehicle or its component in combination with compatible clinical symptoms and onset of illness in outbreak cases	54	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Bovine meat and products thereof</b>	Analytical epidemiological evidence; Laboratory detection in implicated food	5	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Pig meat and products thereof</b>	Analytical epidemiological evidence; Laboratory detection in human cases	30	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Mixed or buffet meals</b>	Analytical epidemiological evidence; Laboratory detection in human cases	13	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Sheep meat and products thereof</b>	Analytical epidemiological evidence; Laboratory detection in implicated food	4	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Bovine meat and products thereof</b>	Analytical epidemiological evidence; Laboratory detection in implicated food	2	2	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Unknown</b>	Analytical epidemiological evidence; Laboratory detection in human cases	5	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Other foods</b>	Analytical epidemiological evidence; Laboratory detection in human cases	19	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Pig meat and products thereof</b>	Analytical epidemiological evidence Laboratory detection in human cases	12	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Mixed or buffet meals</b>	Analytical epidemiological evidence Laboratory detection in human cases	32	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Unknown</b>	Analytical epidemiological evidence Laboratory detection in human cases	3	0	0

Zoonotic agent species	Serotype	Year	Country	Food vehicle: more food vehicle information	Type of evidence	Human cases	Hospitalisation	Deaths
<i>E. coli</i> , pathogenic, unspecified	NR	2009	France	<b>Bovine meat and products thereof</b>	Analytical epidemiological evidence Laboratory detection in human cases	6	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	Romania	<b>Dairy products (other than cheeses)</b>	Laboratory detection in human cases Laboratory detection in implicated food	3	3	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	Romania	<b>Cheese</b>	Analytical epidemiological evidence Laboratory detection in human cases	2	2	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	Romania	<b>Other or mixed red meat and products thereof</b>	Analytical epidemiological evidence; Laboratory detection in implicated food	6	6	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	Romania	<b>Other or mixed red meat and products thereof</b>	Laboratory detection in human cases; Laboratory detection in implicated food	72	32	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	Romania	<b>Cheese</b>	Laboratory detection in human cases; Laboratory detection in implicated food	14	14	0
<i>E. coli</i> , pathogenic, unspecified	NR	2009	Sweden	<b>Tap water, including well water</b>	Laboratory detection in implicated food	4	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	France	<b>Unknown</b>	Analytical epidemiological evidence Laboratory detection in implicated food	4	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	France	<b>Bovine meat and products thereof</b>	Analytical epidemiological evidence Laboratory detection in human cases	8	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	France	<b>Bovine meat and products thereof</b>	Analytical epidemiological evidence Laboratory detection in implicated food	3	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	Spain	<b>Cheese</b>	Epidemiological evidence*	4	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	Spain	<b>Poultry meat</b>	Epidemiological evidence*	2	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	Spain	<b>Bakery product</b>	Epidemiological evidence*	58	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2008	Spain	<b>Other foods</b>	Epidemiological evidence*	22	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Denmark	<b>Cereal products including rice and seeds/pulses (nuts, almonds):</b> boiled wheat salad with raw fennel and black olives from glass	Laboratory detection in human cases	45	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Unknown</b>	Analytical epidemiological evidence	3	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Unknown</b>	Laboratory detection in human cases	4	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Crustaceans, shellfish, molluscs and products thereof</b>	Analytical epidemiological evidence	6	0	0

Zoonotic agent species	Serotype	Year	Country	Food vehicle: more food vehicle information	Type of evidence	Human cases	Hospitalisation	Deaths
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Other or unspecified poultry meat and products thereof</b>	Laboratory detection in implicated food	6	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Fish and fish products</b>	Laboratory detection in human cases	7	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Bovine meat and products thereof</b>	Analytical epidemiological evidence	13	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Other foods</b>	Laboratory detection in implicated food	15	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Broiler meat (<i>Gallus gallus</i>) and products thereof</b>	Laboratory detection in implicated food	20	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Unknown</b>	Analytical epidemiological evidence	30	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Broiler meat (<i>Gallus gallus</i>) and products thereof</b>	Analytical epidemiological evidence	40	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Cheese</b>	Analytical epidemiological evidence	9	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	France	<b>Other foods</b>	Laboratory detection in implicated food	10	10	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Norway	<b>Unknown</b>	Laboratory detection in human cases	2	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Norway	<b>Unknown</b>	Laboratory detection in human cases	4	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Poland	<b>Tap water, including well water</b>	Laboratory detection in implicated food	9	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Poland	<b>Tap water, including well water</b>	Laboratory detection in implicated food	4	2	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Slovenia	<b>Tap water, including well water</b>	Laboratory detection in human cases	43	1	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Spain	<b>Other foods: meat other animal</b>	Not specified	86	0	0
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Spain	<b>Other foods: soups, gravies</b>	Not specified			
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Spain	<b>Other foods: fish</b>	Not specified			
<i>E. coli</i> , pathogenic, unspecified	NR	2007	Spain	<b>Other foods: other salads</b>	Not specified			
Other Bacterial agents - Other Bacterial agents: <i>Escherichia coli</i>	General	2011	Romania	<b>Dairy products (other than cheeses): cream</b>	Detection of causative agent in food vehicle or its component - Symptoms and onset of illness pathognomonic to causative agent	13	13	0

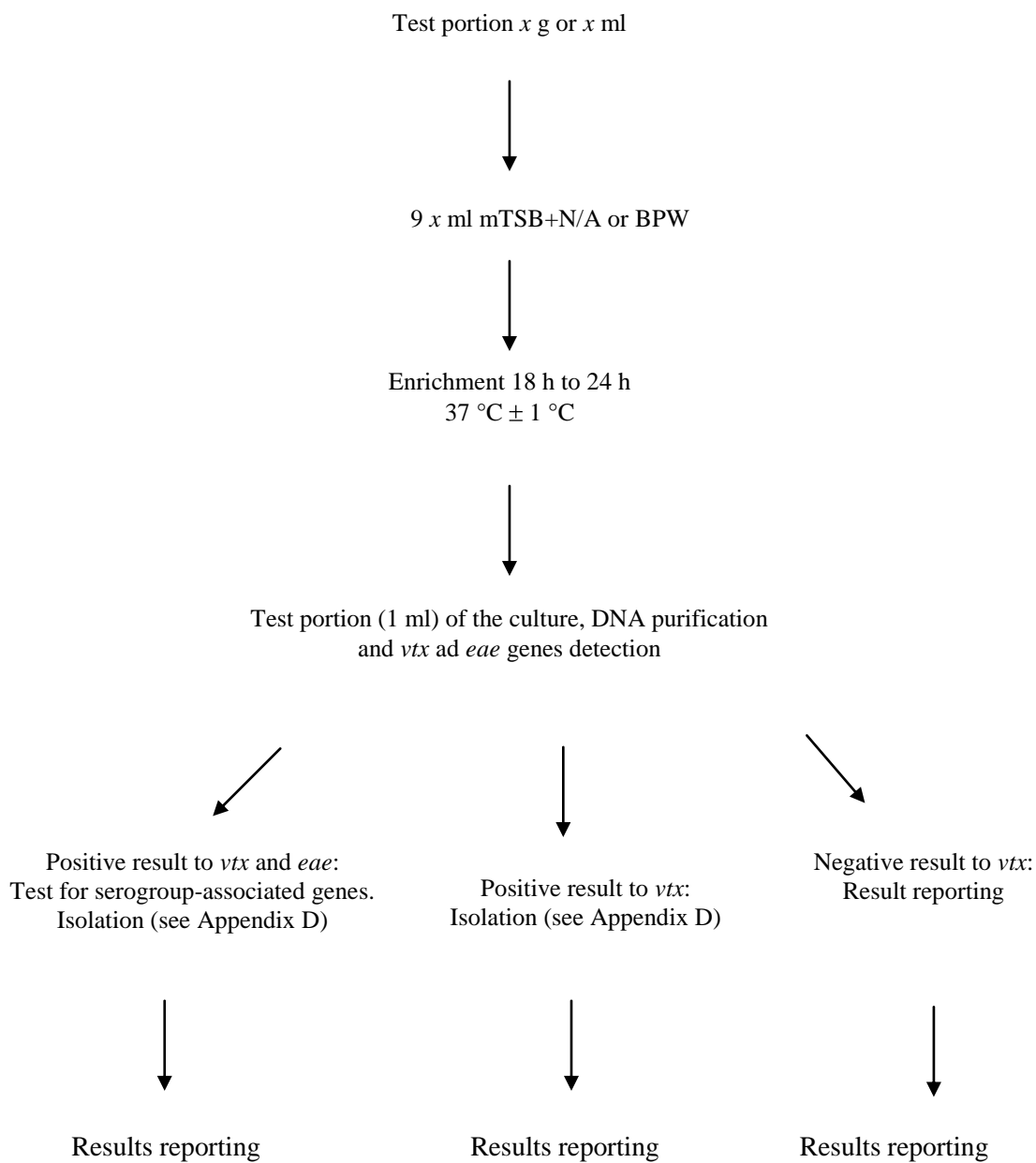
(a): NR or - = not reported.

(b): Enteroaggregative *E. coli*, *vtx2*-positive.

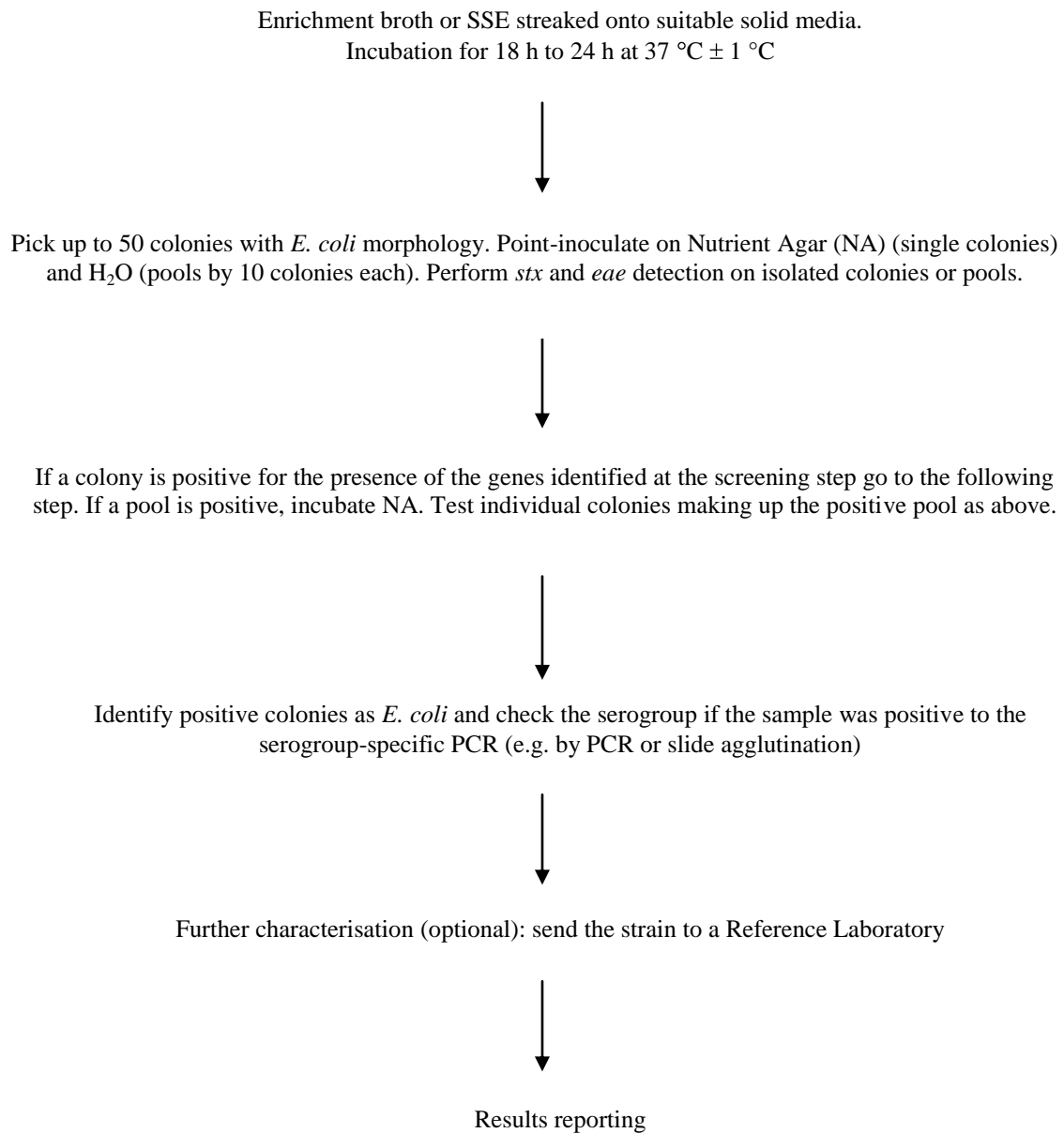
(c): France reported 15 VTEC O104 cases in humans associated to ‘vegetables and juices and other products thereof’ without any additional foodstuff information. Therefore these cases could not be linked to sprouted seeds.



C. FLOW DIAGRAM OF THE SCREENING PROCEDURE OF THE ISO/TS 13136:2012 STANDARD<sup>7</sup>



**D. FLOW DIAGRAM OF THE ISOLATION PROCEDURE OF THE ISO/TS 13136:2012 STANDARD<sup>7</sup>**



(a): If the sample was positive to one of the serogroups-associated genes in the scope of the method, a Serogroup-Specific Enrichment (SSE) may be performed in order to facilitate the isolation.

**E. PRIMER'S SEQUENCE AND THE AMPLIFICATION CONDITIONS FOR *VTX* GENES SUBTYPING**

**Table 1:** Primers developed in this study, except primers for sequencing and detection of *vtx2* (Persson et al., 2007)<sup>(a)</sup>

Gene(s), primer use, and primer	Sequence (5'–3') <sup>(b)</sup>	Position	Amplicon size (bp)	Comments
<b><i>vtx</i> and <i>vtx1</i></b>				
Sequencing				
vtx1-seq-F1	ATGTCATTTCGCTCTGCAATAGGTAC	119–143	1 020	
vtx1-seq-R1	GAAGAAGAGACTGAAGATTCCATCTG	1 113–1 138		
Detection				
vtx1-det-F1	GTACGGGGATGCAGATAAAATCGC	440–462	209	
vtx1-det-R1	AGCAGTCATTACATAAGAACG <b>Y</b> CCACT	622–648		
Subtyping				
vtx1a-F1	CCTTTCCAGGTACAACAGCGGTT	362–384	478	All 6 primers can be used in a triplex PCR for subtyping of <i>vtx/vtx1</i> <sup>(c)</sup>
vtx1a-R2	GGAAACTCATCAGATGCCATTCTGG	815–839		
vtx1c-F1	CCTTTCCCTGGTACAACAGCGGTT	362–384	252	
vtx1c-R1	CAAGTGTGTACGAAATCCCCTCTGA	588–613		
vtx1d-F1	CAGTTAATGCGATTGCTAAGGAGTTTACC	50–78	203	
vtx1d-R2	CTCTTCCTCTGGTTCTAACCCCATGATA	225–252		
<b><i>vtx2</i></b>				
Sequencing and detection				
	F4 GGC <b>A</b> CTGTCTGAAACTGCTCCTGT	606–629	627	For detection, all 4 primers can be used in one reaction; for sequencing, use F4 and R1 for all subtypes except <i>vtx2e</i> and <i>vtx2f</i> , which are sequenced with F4-f and R1-e/f
R1	ATTAAACTGCACTTCAGCAAATCC	1 209–1 232		
F4-f	CGCTGTCTGAGGCATCTCCGCT	606–629	625	
R1-e/f	TAAACTTACCTGGGCAAAGCC	1 209–1 230		
Subtyping				
vtx2a-F2	GCGATACTG <b>R</b> G <b>B</b> ACTGTGGCC	754–774		
vtx2a-R3	CCG <b>K</b> CAACCTTCACTGTAAATGTG	1 079–1 102	349	
vtx2a-R2	GCCACCTTCACTGTGAATGTG	1 079–1 100	347	
vtx2b-F1	AAATATGAAGAAGATATTTGTAGCGGC	968–994	251	
vtx2b-R1	CAGCAAATCCTGAACCTGACG	1 198–1 218		
vtx2c-F1	GAAAGTCACAGTTTTTATATACAACGGGTA	926–955	177	
vtx2c-R2	CCGGCCAC <b>Y</b> TTTACTGTGAATGTA	1 079–1 102		
vtx2d-F1	AAARTCACAGTCTTTATATACAACGGGTG	927–955		
vtx2d-R1	TT <b>Y</b> CCGGCCACTTTTACTGTG	1 085–1 105	179 <sup>(d)</sup>	
vtx2d-O55-R	TCAACCGAGCACTTTGCAGTAG	1 140–1 161	235	
vtx2d-R2	GCCTGATGCACAGGTA <b>C</b> TGGAC	1 184–1 206	280	
vtx2e-F1	CGGAGTATCGGGGAGAGGC	695–713	411	
vtx2e-R2	CTTCTGACACCTT <b>C</b> ACAGTAAAGGT	1 080–1 105		

Gene(s), primer use, and primer	Sequence (5'–3') <sup>(b)</sup>	Position	Amplicon size (bp)	Comments
vtx2f-F1	TGGGCGTCATTCACCTGGTTG	451–475	424	
vtx2f-R1	TAATGGCCGCCCTGTCTCC	856–874		
vtx2g-F1	CACCGGGTAGTTATATTTCTGTGGATATC	203–231	573	
vtx2g-R1	GATGGCAATTCAGAATAACCGCT	771–793		

- (a): PCR conditions are as described in the text below, except annealing temperatures, which were 56 °C for sequencing and detection and 64 °C to 66 °C for the subtyping of *vtx/vtx1* or *vtx2*. Especially, the resolution of *vtx2a*, *vtx2c*, and *vtx2d* may require individual calibration of thermocyclers. A well-defined single colony is inoculated in beef broth and incubated overnight at 37 °C. One hundred microlitres of broth is added to 900 µl of sterile H<sub>2</sub>O, placed in a heating block at 100 °C for 15 min, and centrifuged at 18 000 × *g* for 5 min. Upon transfer to a clean tube, the supernatant is used directly for PCR and stored at –18 °C for further analyses. For PCR, a total volume of 20 µl contains 2.5 µl H<sub>2</sub>O, 10 µl HotStarTaq Master Mix Kit (Qiagen), 1.25 µl of each of two primers (stock solution of primers is 5 µM) and 5 µl supernatant of boiled lysate (stock). The thermocycler conditions are 95 °C for 15 min followed by 35 cycles of 94 °C for 50 s, 56 °C for sequencing and detection, and 64 °C for subtyping for 40 s and 72 °C for 60 s, ending with 72 °C for 3 min. PCR amplicons are stored at 4 °C. The final resolution of *vtx2a*, *vtx2c*, and *vtx2d* may require calibration of individual brands of thermocyclers by testing annealing temperatures from 64 °C to 66 °C on the test panel of reference strains. In our hands, an additional PCR using the *vtx2d* primers was run at an annealing temperature of 66 °C. False-positive *vtx2c* fragments disappeared and true *vtx2d*-positive fragments persisted at this annealing temperature. A total volume (20 µl) for standard PCR contains 2.5 µl H<sub>2</sub>O [if three primers are used (*vtx2a*), the H<sub>2</sub>O volume is reduced to 1.25 µl; if four primers are used (*vtx2d* or detection of all *vtx2* variants), H<sub>2</sub>O is not added]; 10 µl Mastermix (HotStarTaq, Qiagen); 1.25 µl of each of two primers (stock solution of primers is 5 µM) [if three primers are used (*vtx2a*), the H<sub>2</sub>O volume is reduced to 1.25 µl; if four primers are used (*vtx2d* or detection of all *vtx2* variants), H<sub>2</sub>O is not added]; and 5 µl supernatant of boiled lysate (stock).
- (b): Wobble bases are shown in bold.
- (c): For triplex PCR for subtyping of *vtx1*, a total volume of 25 µl contains 12 µl Mastermix (HotStarTaq, Qiagen), 1 µl of each of the four primers for *vtx1c* and *vtx1d* (stock solution of primers is 5 µM), 2 µl of each of two primers for *vtx1a* (stock solution of primers is 5 µM), and 5 µl supernatant of boiled lysate (stock).
- (d): All three reverse primers in the same reaction will result in amplicons of 179 bp with nine *vtx2d* variants, 235 bp with variant *vtx2d*-O55-5905, 280 bp with five *vtx2d* variants, and finally two amplicons of 179 bp and 280 bp with variant *vtx2d*-O73-C165-02.

**F. VIRULENCE CHARACTERISTICS OF REPORTED CONFIRMED VTEC SEROTYPES FROM CASES OF HUMAN INFECTION FROM 2007-2010: CONFIRMED CASES, HOSPITALISED CASES AND HUS CASES**

**Table 1:** Virulence characteristics of reported confirmed VTEC serogroups from cases of human infection from 2007-2010 (based on TESSy data as provided by ECDC)

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
A	HAS (A/B/C)	O157:H7	8	403	131	1		2	545
A	HAS (A/B/C)	O157:H-	3	58	171		2	2	236
B	D	O103:H2	124	1					125
B	HAS (A/B/C)	O26:H11	85	11	4	2			102
B	HAS (A/B/C)	O111:H-	17	3	6			1	27
B	HAS (A/B/C)	O121:H19		22	1				23
B	HAS (A/B/C)	O145:H-	3	20					23
C	D	O113:H21		2		1	5		8
C	D	O5:H-	2		1	2		2	7
C	D	O165:H25		2					2
C	D	O121:H-		2					2
C	HAS (A/B/C)	O104:H21						1	1
C	D	O91:H21					1		1
D	D	O117:H7	1		1	47	1		50
D	D	O146:H21			1	3	4	16	24
D	D	O113:H4				1	7	8	16
D	D	O174:H8						3	3
D	D	O171:H2					1		1
D	D	O172:H-		1					1
E	D	O156:H-	1			3		1	5
E	D	O113:H-		1			1		2
E	D	O84:H-	2						2
E	D	O76:H7	1						1
E	D	O88:H25						1	1
NLK	HAS (A/B/C)	O128:H2			3	4	8	14	29
NLK	HAS (A/B/C)	O145:H28	16	3					19
NLK	HAS (A/B/C)	O76:H19	1			10	1	2	14
NLK	HAS (A/B/C)	O174:H21					10	1	11
NLK	HAS (A/B/C)	O111:H8	5		1				6
NLK	HAS (A/B/C)	O174:H2						5	5
NLK	HAS (A/B/C)	O80:H2		4					4
NLK	HAS (A/B/C)	O105:H18					1	1	2
NLK	HAS (A/B/C)	O91:H10					2		2
NLK	HAS (A/B/C)	O123:H2		2					2
NLK	HAS (A/B/C)	O1:H42					1		1
NLK	HAS (A/B/C)	O121:H2		1					1
NLK	HAS (A/B/C)	O7:H6					1		1

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
NLK	HAS (A/B/C)	O86:H27		1					1
NLK	D	O91:H-				21	1	22	44
NLK	D	O63:H6		25	1				26
NLK	D	O26:H-	11	8					19
NLK	D	O145:H34		17					17
NLK	D	O146:H28					14	2	16
NLK	D	O91:H14				9	1		10
NLK	D	O128:H-	1	3			2	4	10
NLK	D	O156:H7				9		1	10
NLK	D	O146:H-					6	3	9
NLK	D	O125:H6		6					6
NLK	D	O117:H-				6			6
NLK	D	O55:H12				6			6
NLK	D	O78:H-				5		1	6
NLK	D	O181:H49						6	6
NLK	D	O103:H-	3	1	1				5
NLK	D	O2:H6		2			3		5
NLK	D	O177:H-	2	3					5
NLK	D	O6:H-				1		3	4
NLK	D	O166:H28				2	2		4
NLK	D	O43:H2				1	1	2	4
NLK	D	O181:H16				4			4
NLK	D	O55:H7	3						3
NLK	D	O118:H16	3						3
NLK	D	O63:H7			3				3
NLK	D	O178:H7				2		1	3
NLK	D	O176:H-					1	2	3
NLK	D	O156:H25	3						3
NLK	D	O182:H25	3						3
NLK	D	O27:H30					3		3
NLK	D	O132:H34		2					2
NLK	D	O104:H2					2		2
NLK	D	O156:H34	1			1			2
NLK	D	O165:H-		2					2
NLK	D	O88:H-					2		2
NLK	D	O168:H8					2		2
NLK	D	O17:H41					2		2
NLK	D	O112:H8				2			2
NLK	D	O186:H2	2						2
NLK	D	O87:H16					2		2
NLK	D	O26:H7			2				2
NLK	D	O9:H-					2		2

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
NLK	D	O40:H8					2		2
NLK	D	O54:H21					2		2
NLK	D	O136:H20				2			2
NLK	D	O11:H4					1	1	2
NLK	D	O145:H7		2					2
NLK	D	O102:H6					1	1	2
NLK	D	O75:H8					1	1	2
NLK	D	O130:H11				1		1	2
NLK	D	O84:H28	2						2
NLK	D	O113:H6		2					2
NLK	D	O2:H20		1					1
NLK	D	O145:H20		1					1
NLK	D	O20:H45					1		1
NLK	D	O146:H31					1		1
NLK	D	O20:H49					1		1
NLK	D	O21:H21					1		1
NLK	D	O118:H12					1		1
NLK	D	O24:H18				1			1
NLK	D	O112:H2				1			1
NLK	D	O109:H5				1			1
NLK	D	O20:H25	1						1
NLK	D	O166:H15					1		1
NLK	D	O92:H18					1		1
NLK	D	O1:H20				1			1
NLK	D	O14:H28						1	1
NLK	D	O36:H-					1		1
NLK	D	O38:H26				1			1
NLK	D	O15:H-						1	1
NLK	D	O39:H-					1		1
NLK	D	O39:H21					1		1
NLK	D	O12:H-					1		1
NLK	D	O39:H48					1		1
NLK	D	O40:H7						1	1
NLK	D	O91:H7			1				1
NLK	D	O115:H-		1					1
NLK	D	O107:H-					1		1
NLK	D	O177:H45			1				1
NLK	D	O45:H2	1						1
NLK	D	O107:H21					1		1
NLK	D	O48:H7					1		1
NLK	D	O121:H7				1			1
NLK	D	O112:H18				1			1



Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
NLK	D	O5:H16					1		1
NLK	D	O119:H-				1			1
NLK	D	O5:H19						1	1
NLK	D	O114:H41				1			1
NLK	D	O53:H7				1			1
NLK	D	O166:H30				1			1
NLK	D	O168:H28					1		1
NLK	D	O153:H2						1	1
NLK	D	O100:H-					1		1
NLK	D	O153:H25		1					1
NLK	D	O110:H8					1		1
NLK	D	O126:H-		1					1
NLK	D	O2:H29					1		1
NLK	D	O6:H19				1			1
NLK	D	O6:H25			1				1
NLK	D	O169:H18						1	1
NLK	D	O134:H38					1		1
NLK	D	O17:H18					1		1
NLK	D	O91:H26						1	1
NLK	D	O111:H2			1				1
NLK	D	O92:H-				1			1
NLK	D	O65:H2				1			1
NLK	D	O98:H-	1						1
NLK	D	O177:H11			1				1
NLK	D	O70:H-	1						1
NLK	D	O138:H48					1		1
NLK	D	O70:H7					1		1
NLK	D	O139:H-					1		1
NLK	D	O71:H-				1			1
NLK	D	O178:H19					1		1
NLK	D	O75:H-					1		1
NLK	D	O101:H9					1		1
NLK	D	O101:H-					1		1
NLK	D	O111:H4		1					1
NLK	D	O142:H-		1					1
NLK	D	O142:H33		1					1
NLK	D	O77:H18					1		1
NLK	D	O103:H7			1				1
NLK	D	O103:H11	1						1
NLK	D	O79:H14				1			1
NLK	D	O8:H-						1	1
NLK	D	O109:H25	1						1

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
NLK	D	O8:H25			1				1
NLK	D	O186:H-	1						1
NLK	D	O8:H8					1		1
NLK	D	O8:H9					1		1
NLK	D	O104:H-				1			1
NLK	D	O132:H10					1		1
NLK	D	O80:H-		1					1
NLK	D	O132:H21						1	1
NLK	D	O146:H5					1		1
NLK	D	O15:H2		1					1
NLK	D	O84:H1	1						1
NLK	D	O84:H2	1						1
NLK	D	O2:H14					1		1
NLK	D	O117:H11				1			1
NLK	D	O174:H28					1		1
NLK	D	O154:H31				1			1
NLK	D	O89:H-					1		1
NLK	D	O89:H4					1		1
NLK	D	O20:H12			1				1
NLK	D	O89:H8					1		1
NLK	D	O163:H19					1		1
NLK	D	O175:H16					1		1
NLK	D	O20:H4				1			1
NLK	D	O117:H8				1			1
NLK	D	O8:H12					1		1
NLK	D	O102:H-					1		1
NLK	D	O35:H19		1					1
NFT	NFT	O157:HNT	20	3 372	1 228		11		4 631
NFT	NFT	ONT:HNT	82	103	38	53	56	29	361
NFT	NFT	O26:HNT	109	44	24	7	2		186
NFT	NFT	O145:HNT	6	38	1		1		46
NFT	NFT	O103:HNT	40	2		1	1		44
NFT	NFT	O111:HNT	15	9	5	1	2		32
NFT	NFT	ONT:H-	1	3		11	2	13	30
NFT	NFT	O121:HNT	1	20			1		22
NFT	NFT	O128:HNT		4	2	3	9	4	22
NFT	NFT	ONT:H2	1	2			7	2	12
NFT	NFT	O146:HNT		1			4	6	11
NFT	NFT	ONT:H7			2	6	3		11
NFT	NFT	O91:HNT		1		5		4	10
NFT	NFT	O153:HNT		6		1	2		9
NFT	NFT	O5:HNT	7			2			9

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
NFT	NFT	O113:HNT					5	2	7
NFT	NFT	ONT:H18					4	3	7
NFT	NFT	O76:HNT				5		1	6
NFT	NFT	O117:HNT	1			5			6
NFT	NFT	ONT:H19			1	2	3		6
NFT	NFT	O8:HNT		2			4		6
NFT	NFT	O55:HNT		4		1			5
NFT	NFT	O104:HNT		2		2			4
NFT	NFT	ONT:H25	2	2					4
NFT	NFT	O1:HNT	2	1	1				4
NFT	NFT	O123:HNT	2	1	1				4
NFT	NFT	ONT:H21				2		2	4
NFT	NFT	ONT:H28				2	2		4
NFT	NFT	O84:HNT	2				2		4
NFT	NFT	ONT:H10				2	1		3
NFT	NFT	O114:HNT		2	1				3
NFT	NFT	O118:HNT	1	1			1		3
NFT	NFT	ONT:H16				3			3
NFT	NFT	O174:HNT				1	1	1	3
NFT	NFT	O71:HNT	1		2				3
NFT	NFT	ONT:H14	1	1					2
NFT	NFT	ONT:H8					2		2
NFT	NFT	ONT:H4				1	1		2
NFT	NFT	ONT:H9	1				1		2
NFT	NFT	ONT:H30					2		2
NFT	NFT	ONT:H6		1		1			2
NFT	NFT	O126:HNT	1				1		2
NFT	NFT	O80:HNT		2					2
NFT	NFT	O6:HNT				1	1		2
NFT	NFT	O12:HNT		2					2
NFT	NFT	O27:HNT		1			1		2
NFT	NFT	ONON-O157:HNT		1			1		2
NFT	NFT	ONT:H1					1		1
NFT	NFT	ONT:H49					1		1
NFT	NFT	ONT:H29					1		1
NFT	NFT	ONT:H45					1		1
NFT	NFT	ONT:H39				1			1
NFT	NFT	ONT:H12				1			1
NFT	NFT	ONT:H26				1			1
NFT	NFT	ONT:H20				1			1
NFT	NFT	ONT:H5					1		1
NFT	NFT	ONT:H23				1			1

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
NFT	NFT	O23:HNT						1	1
NFT	NFT	O78:HNT				1			1
NFT	NFT	O25:HNT	1						1
NFT	NFT	O127:HNT		1					1
NFT	NFT	O3:HNT				1			1
NFT	NFT	O45:HNT		1					1
NFT	NFT	O98:HNT	1						1
NFT	NFT	O163:HNT					1		1
NFT	NFT	O177:HNT		1					1
NFT	NFT	O7:HNT			1				1
NFT	NFT	O51:HNT		1					1
NFT	NFT	O176:HNT				1			1
NFT	NFT	O2:HNT					1		1
NFT	NFT	O38:HNT				1			1
NFT	NFT	O142:HNT					1		1
NFT	NFT	O166:HNT	1						1
NFT	NFT	O156:HNT	1						1
NFT	NFT	O116:HNT					1		1
NFT	NFT	O150:HNT				1			1
NFT	NFT	O43:HNT					1		1
NFT	NFT	O165:HNT		1					1
NFT	NFT	O148:HNT					1		1
<b>Total</b>			<b>612</b>	<b>4 254</b>	<b>1 642</b>	<b>295</b>	<b>287</b>	<b>188</b>	<b>7 278</b>

(a): NFT = strains that were not fully serotyped. NLK = serotypes that were fully serotyped but were not listed by Karmali et al. (2003). HAS = HUS-associated serotypes. Includes the serotypes that have been associated with reported confirmed HUS cases of human VTEC in EU in 2007-2010.

(b): ONT = the 'O' antigen was untyped/untypeable or reported as unknown. HNT = the 'H' antigen was untyped/untypeable or reported as unknown.

**Table 2:** Virulence characteristics of reported confirmed VTEC serogroups from hospitalised cases of human infection from 2007-2010 (based on TESSy data as provided by ECDC)

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
A	HAS (A/B/C)	O157:H-		12	6		1		19
A	HAS (A/B/C)	O157:H7		2					2
B	D	O103:H2	10						10
B	HAS (A/B/C)	O26:H11	1	3					4
B	HAS (A/B/C)	O145:H-	1	1					2
B	HAS (A/B/C)	O121:H19		1					1
C	HAS (A/B/C)	O104:H21						1	1
D	D	O113:H4					1		1
E	D	O113:H-					1		1
NLK	HAS (A/B/C)	O174:H2						3	3
NLK	HAS (A/B/C)	O1:H42					1		1
NLK	HAS (A/B/C)	O105:H18					1		1
NLK	D	O26:H-	2						2
NLK	D	O63:H6		1					1
NLK	D	O181:H49						1	1
NLK	D	O27:H30					1		1
NFT	NFT	O157:HNT		151	73				224
NFT	NFT	ONT:HNT	2	4	1	2	2	1	12
NFT	NFT	O26:HNT		2	4				6
NFT	NFT	O145:HNT		4					4
NFT	NFT	O103:HNT	3						3
NFT	NFT	O111:HNT		1	1				2
NFT	NFT	O5:HNT	2						2
NFT	NFT	ONT:H19					1		1
NFT	NFT	ONT:H-	1						1
NFT	NFT	ONT:H14		1					1
NFT	NFT	ONT:H4				1			1
NFT	NFT	ONT:H21						1	1
NFT	NFT	ONON-O157:HNT					1		1
NFT	NFT	O150:HNT				1			1
NFT	NFT	O121:HNT		1					1
NFT	NFT	O165:HNT		1					1
<b>Total</b>			<b>22</b>	<b>185</b>	<b>85</b>	<b>4</b>	<b>10</b>	<b>7</b>	<b>313</b>

(a): NFT = strains that were not fully serotyped. NLK = serotypes that were fully serotyped but were not listed by Karmali et al. (2003). HAS = HUS-associated serotypes. Includes the serotypes that have been associated with reported confirmed HUS cases of human VTEC in EU in 2007-2010.

(b): ONT = the 'O' antigen was untyped/untypeable or reported as unknown. HNT = the 'H' antigen was untyped/untypeable or reported as unknown.

**Table 3:** Virulence characteristics of reported confirmed VTEC serogroups from HUS cases of human infection from 2007-2010 (based on TESSy data as provided by ECDC)

Classification based on original Karmali seropathotype approach <sup>(a)</sup>	Classification based on 'modified' Karmali seropathotype approach <sup>(a)</sup>	Serotype <sup>(b)</sup>	<i>eae</i> , <i>vtx1</i>	<i>eae</i> , <i>vtx2</i>	<i>eae</i> , <i>vtx1</i> , <i>vtx2</i>	<i>vtx1</i>	<i>vtx2</i>	<i>vtx1</i> , <i>vtx2</i>	Total
A	HAS (A/B/C)	O157:H7		49	3				52
A	HAS (A/B/C)	O157:H-		20	4		1		25
B	HAS (A/B/C)	O121:H19		8					8
B	HAS (A/B/C)	O26:H11	1	3					4
B	HAS (A/B/C)	O111:H-		1	2				3
B	HAS (A/B/C)	O145:H-		2					2
C	HAS (A/B/C)	O104:H21						1	1
NLK	HAS (A/B/C)	O174:H2						3	3
NLK	HAS (A/B/C)	O145:H28		2					2
NLK	HAS (A/B/C)	O128:H2			1				1
NLK	HAS (A/B/C)	O91:H10					1		1
NLK	HAS (A/B/C)	O174:H21					1		1
NLK	HAS (A/B/C)	O105:H18					1		1
NLK	HAS (A/B/C)	O86:H27		1					1
NLK	HAS (A/B/C)	O123:H2		1					1
NLK	HAS (A/B/C)	O1:H42					1		1
NLK	HAS (A/B/C)	O121:H2		1					1
NLK	HAS (A/B/C)	O80:H2		1					1
NLK	HAS (A/B/C)	O111:H8	1						1
NLK	HAS (A/B/C)	O7:H6					1		1
NLK	HAS (A/B/C)	O76:H19					1		1
NFT	NFT	O157:HNT		125	14		2		141
NFT	NFT	ONT:HNT	1	35	4		10		50
NFT	NFT	O26:HNT	6	24	8				38
NFT	NFT	O145:HNT		4			1		5
NFT	NFT	O111:HNT		3	1				4
NFT	NFT	ONT:H2		2			2		4
NFT	NFT	O128:HNT		1		2			3
NFT	NFT	O114:HNT		2					2
NFT	NFT	O126:HNT	1				1		2
NFT	NFT	O121:HNT		2					2
NFT	NFT	O55:HNT		2					2
NFT	NFT	ONT:H25		1					1
NFT	NFT	ONT:H19					1		1
NFT	NFT	O127:HNT		1					1
NFT	NFT	O1:HNT		1					1
NFT	NFT	O80:HNT		1					1
NFT	NFT	O103:HNT		1					1
<b>Total</b>			<b>10</b>	<b>294</b>	<b>37</b>	<b>2</b>	<b>24</b>	<b>4</b>	<b>371</b>

- (a): NFT = strains that were not fully serotyped. NLK = serotypes that were fully serotyped but were not listed by Karmali et al. (2003). HAS = HUS-associated serotypes. Includes the serotypes that have been associated with reported confirmed HUS cases of human VTEC in EU in 2007-2010.
- (b): ONT = the 'O' antigen was untyped/untypeable or reported as unknown. HNT = the 'H' antigen was untyped/untypeable or reported as unknown.



**GLOSSARY, ABBREVIATIONS AND DEFINITIONS**

<i>aaiC</i>	Chromosomally-encoded gene encoding secreted protein of enteroaggregative <i>Escherichia coli</i> (EAEC)
<i>aggR</i>	Plasmid-encoded regulator gene
AAF	Aggregative adherence fimbriae
A/E lesions	Attaching/Effacing lesions
<i>astA</i>	Gene responsible for production of heat-stable enterotoxin (EAST1)
CDC	Centers for Disease Control and Prevention (USA)
CEN	European Committee of Standardisation
CFA	Colonisation factor antigens
CT-SMAC	Sorbitol-MacConkey medium
<i>bfp</i>	Bundle forming pili gene
DAEC	Diffuse adherent <i>E. coli</i>
DALY	Disability Adjusted Life Years
<i>eae</i>	Intimin-coding gene
EAEC	Enteroaggregative <i>E. coli</i>
EAF	Enteropathogenic <i>E. coli</i> adherence factor
EAST1	Enteroaggregative heat-stable toxin
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EHEC	Enterohaemorrhagic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EPEC	Enteropathogenic <i>E. coli</i>
ETEC	Enterotoxigenic <i>E. coli</i>
EU	European Union
EU-RL-VTEC	European Union Reference Laboratory for VTEC
ExPEC	Enteric/diarrhoeogenic or extra-intestinal
FW&E	Food, water and environmental
HAS	Haemolytic uraemic syndrome (HUS)-associated serotype(s)

<i>hly</i>	Gene encoding production of haemolysin
HC	Haemorrhagic colitis
HUS	Haemolytic uraemic syndrome
IMS	Immuno-magnetic separation
Ipa	Invasion plasmid antigens
ISO	International Organization for Standardization
LEE	The genetic locus of enterocyte effacement
LPS	Lipopolysaccharide
LT	Heat-labile enterotoxin
MLVA	Multi-locus variable number of tandem repeat analysis
PAI	Pathogenicity island
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
RTE	Ready-to-eat
Saa	STEC autoagglutinating adhesion
Serogroup	Classification of <i>E. coli</i> strains based on identification of ‘O’ (lipopolysaccharide) antigen – e.g. O157, O104
Serotype	Classification of <i>E. coli</i> strains based on identification of ‘O’ (lipopolysaccharide) and ‘H’ (flagella) antigen – e.g. O157:H7; O104:H4
Seropathotype	Empirical classification scheme for VTEC based on disease severity and serotype
ST	Heat-stable enterotoxin
STEC	Shiga toxin-producing <i>E. coli</i> , also known as VTEC
TESSy	The European Surveillance System
UPEC	Uropathogenic <i>E. coli</i>
<i>vtx1</i>	Verocytotoxin 1 gene
<i>vtx2</i>	Verocytotoxin 2 gene
Vtx	Verocytotoxin, also known as Shiga toxin
VTEC	Verocytotoxin-producing <i>E. coli</i> , also known as verotoxigenic <i>E. coli</i> , verocytotoxigenic <i>E. coli</i> , verotoxin-producing <i>E. coli</i> and Shiga toxin-producing <i>E. coli</i> (STEC)
WGS	Whole genome sequencing