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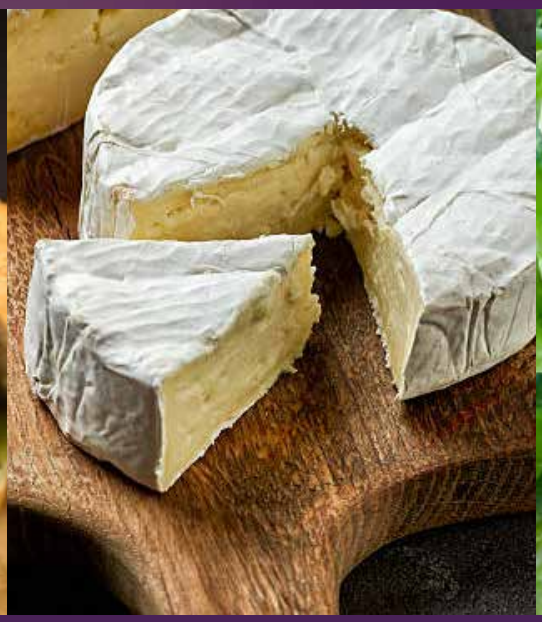
AUTHORITY OF IRELAND

Report of the Scientific Committee
of the Food Safety Authority of Ireland

2019

Advice on Shiga toxin-producing *Escherichia coli* (STEC) detection in food


Shiga toxin-producing *Escherichia coli* (STEC) are synonymous with verocytotoxigenic *Escherichia coli* (VTEC). Similarly, *stx* genes are synonymous with *vtx* genes. The terms STEC and *stx* have been used throughout this report.

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Advice on Shiga toxin-producing *Escherichia coli* (STEC) detection in food

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CONTENTS

ABBREVIATIONS

AA
aggregative adherence

ANSES
French Agency for Food, Environmental and Occupational Health and Safety

ATCC
American Type Culture Collection

aEPEC
atypical enteropathogenic *E. coli*

BFP
bundle-forming pili

BPW
Buffered Peptone Water

CDC
(US) Centers for Disease Control and Prevention

CFU
colony-forming unit

CIDR
Computerised Infectious Disease Reporting

CT-SMAC
Sorbitol MacConkey Agar with Cefixime and Tellurite

DAEC
diffusely adherent *E. coli*

DAFM
Department of Agriculture, Food and the Marine

DNA
deoxyribonucleic acid

EAF
EPEC adherence factor

EAEC
enteroaggregative *E. coli*

EAHEC
enteroaggregative-haemorrhagic *E. coli*

EAST1
EAEC heat-stable enterotoxin 1

EC
European Commission

ECDC
European Centre for Disease Prevention and Control

EEA
European Economic Area

EFSA
European Food Safety Authority

EHEC
enterohaemorrhagic *E. coli*

EIEC
enteroinvasive *E. coli*

EPEC
enteropathogenic *E. coli*

ETEC
enterotoxigenic *E. coli*

EU-FORS
European Union Foodborne Outbreak Reporting System

ExPEC
extraintestinal pathogenic *E. coli*

FAO
Food and Agriculture Organization of the United Nations

FBO
food business operator

FDA
Food and Drug Administration

FSA
Food Standards Agency (UK)

FSAI
Food Safety Authority of Ireland

FSIS
(USDA) Food Safety and Inspection Service

g
gram(s)

GRAS
generally recognised as safe

H_2O_2
hydrogen peroxide

HACCP
hazard analysis and critical control point

HC
haemorrhagic colitis

HPSC
Health Protection Surveillance Centre

HlyE
Haemolysin E

HSE
Health Service Executive

HUS
haemolytic uraemic syndrome

IAL
invasion-associated locus

IMS
immunomagnetic separation

Ipa
invasion plasmid antigens

ISO
International Organization for Standardization

LEE
locus of enterocyte effacement

LT
thermo-labile toxin

MLST
multilocus sequence typing

mM
millimolar

MSs
Member States

NaCl
Sodium chloride

QPS
qualified presumption of safety

PCR
polymerase chain reaction

PFGE
pulsed-field gel electrophoresis

PHE
Public Health England

RASFF
Rapid Alert System for Food and Feed

rRNA
ribosomal ribonucleic acid

RTE
ready-to-eat

ShET
Shigella enterotoxin 1

SNP
single nucleotide polymorphism

spp.
species

ST
thermo-stable toxin

STEC
Shiga toxin-producing *E. coli*

stx
Shiga toxin-encoding genes

Stx
Shiga toxin

TBX
Tryptone Bile X-Glucuronide

tEPEC
typical enteropathogenic *E. coli*

TESSy
The European Surveillance System

UK
United Kingdom

UHT
ultra-heat treatment

USA
United States of America

USDA
United States Department of Agriculture

VTEC
verocytotoxigenic *E. coli*

VTEC NRL
National VTEC Reference Laboratory

WGS
whole genome sequencing

WHO
World Health Organization

EXECUTIVE SUMMARY

The evolving picture of human Shiga toxin-producing *Escherichia coli* (STEC) illness and changes in the methodology for STEC detection in human clinical samples and in food has resulted in a lack of agreement across Europe on the risk posed and the appropriate risk-based action to be taken when STEC is detected in food. In 2014, the European Commission (EC) attempted to introduce a harmonised approach to assess and manage the risk of STEC in food, but European Union (EU) Member States (MSs) were unable to reach a common agreement and the EC suspended this work in 2016. A number of individual EU MSs have made their own risk assessments and policy decisions based on human epidemiology data and consumer practices relevant to their country, and some of these are summarised in this report.

Since 2008 – and with the exception of 2011, when Germany reported the highest rate due to a large *E. coli* O104:H4 outbreak – Ireland has had the highest reported STEC notification rate in Europe. In 2016, Ireland's notification rate was 15.6 cases per 100,000 population.

This report has been produced in response to a Request for Advice to the Food Safety Authority of Ireland (FSAI) Scientific Committee in December 2016 regarding STEC detection in food, which is included in **Appendix 1**. As part of this process, in September 2017, the FSAI convened a group of experts from five EU MSs, who discussed with the STEC Working Group their risk assessment and risk management strategies. Their answers to the questions in the Request for Advice have been included in Section 5 of this report. Current Irish epidemiological data on STEC have been reviewed to assess the risk if STEC is detected in food in Ireland, and the Scientific Committee has concluded the following:

1. Should foods be categorised with regard to risk from VTEC/STEC and if yes, how?

When STEC is detected (i.e. culture isolation of an *E. coli* containing Shiga toxin-encoding (*stx*) gene(s))[1] in a food, the risk of illness is dependent on the type of food, its likely final preparation prior to consumption and the vulnerability of the consumer to illness. It is thus concluded that ready-to-eat (RTE) and non-RTE foods have different risk profiles with regard to STEC:

- **RTE food**[2] includes food that is intended to be consumed less than thoroughly cooked, i.e. following a treatment that will not/is unlikely to remove the risk associated with STEC.
- **Non-RTE food** includes food that is intended to be consumed following a treatment that will remove the risk of STEC. This category includes carcases and whole cuts of meat. It also includes minced meat intended to be thoroughly cooked. This is in line with the FSAI recommendation to thoroughly cook beef burgers to a core temperature of no less than 75 °C or an equivalent time-temperature combination (e.g. to a core temperature of 70 °C for at least two minutes) (FSAI, 2006, 2018a, 2018b).

[1] Public health risk cannot be assessed based on detection of *stx* gene(s) by molecular methods only (i.e. a positive polymerase chain reaction (PCR) result/presumptive positive), except in those scenarios where there is additional information that indicates that there is a public health risk or non-compliance.

[2] Commission Regulation (EC) No 2073/2005 defines 'RTE food' as "food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern."

2. What is the risk associated with the detection of STEC in foods (category based on the answer to Question 1) depending on the presence/absence of virulence genes (*eae*/*aaiC* and *aggR*) and/or the serogroup?

There are significant challenges in the risk assessment and management of STEC in that the profile of strains causing human illness has continued to change since it first emerged as a cause of human illness. This has included changes in understanding the role of both the serogroup and the presence/absence of particular genes as indicators of STEC virulence potential. *E. coli* O157:H7 was the first serogroup implicated in STEC human infections (in the 1980s). In the 2000s, further serogroups (*E. coli* O26, O103, O111 and O145) were identified as being most commonly linked to human infection and, along with O157, became known as the 'top five' STEC serogroups. In 2011, the serogroup O104 was added to this group following a European outbreak linked to sprouted fenugreek seeds, making these serogroups the 'top six'. A review of Irish and international epidemiological data has shown that the profile of STEC strains associated with human illness has evolved in recent years and now includes many serogroups outside the traditional 'top six'. In Ireland in 2004, 85% of all notifications were linked to STEC O157, whereas data from 2012 to 2016 show that only 28% of notifications were linked to O157, with 21% of symptomatic cases linked to approximately 70 diverse serogroups outside of the other 'top five'.

In STEC strains, the presence of the *eae* gene (a gene encoding for intimin, a protein which facilitates intimate attachment to the host intestinal epithelial cells) has historically been used as a predictor of human illness potential, but recent international and Irish data have shown that this is changing. Between 2012 and 2016, among culture-confirmed STEC notifications in Ireland, 17.8% were *eae* negative, and among culture-confirmed STEC-associated haemolytic uraemic syndrome (HUS) cases, 6.8% were *eae* negative. The 2011 *E. coli* O104:H4 outbreak strain was a hybrid enteroaggregative-haemorrhagic *E. coli* carrying the *aaiC* and *aggR* genes and the *stx*2a gene (a subtype of the *stx*2 gene), but there is a lack of data on the presence/absence of these enteroaggregative *E. coli* genes (*aaiC* and *aggR*) in Irish clinical and food-derived isolates.

It is concluded that, at the present time, there is no scientific evidence to differentiate the potential risk of illness from STEC based on (i) the serogroup/serotype or (ii) the presence/absence of the *eae*/*aaiC* and *aggR* genes. Consequently, any STEC cultured from a food constitutes a potential risk of illness, although the risk posed is different depending on the food category, as stated in the answer to Question 1. This position may be revised in the future, based on new scientific evidence.

3. What is the risk associated with the detection of EPEC in food when the EPEC belongs to:

a. The serogroups currently most commonly associated with severe illness (i.e. referred to as the EU 'top six' – *E. coli* O157, O26, O111, O103, O145 and O104:H4), or

b. Other serogroups?

It is a possibility that when testing for STEC, an enteropathogenic *E. coli* (EPEC) may be detected. An EPEC is an *E. coli* strain possessing the *eae* gene (a gene encoding for intimin, a protein which facilitates intimate attachment to the host intestinal epithelial cells) but lacking the *stx* gene(s) characteristic of STEC. Some EU MSs have taken action following the detection of EPEC in certain foodstuffs (details are available on the EC's Rapid Alert System for Food and Feed (RASFF) Portal). The detection of EPEC and the associated recall actions taken by some EU MSs have been in the context of the ISO/TS 13136 test method for STEC, whereby an *eae*-positive but *stx*-negative *E. coli* (EPEC) was confirmed in a food sample that was originally screened as *stx* positive by PCR (presumptive STEC detection).

The possibility that the EPEC isolates are derivatives of STEC that have lost their Shiga toxin-encoding (Stx-encoding) phage (containing *stx* gene(s)) cannot be excluded in this scenario. It also raises the question of whether there is the potential for an EPEC strain to acquire an Stx-encoding phage during storage of the food prior to consumption.

Based on current scientific evidence, it is concluded that, although plausible, the loss and acquisition of an Stx-encoding phage are rare events under typical conditions of chilled food storage. The conclusion is that the detection of EPEC in food is not an indicator for the detection of STEC. This position may be revised in the future, based on new scientific evidence.

4. What is the risk associated with *Hafnia* strains, such as *Hafnia alvei* and *Hafnia paralvei*, deliberately added to some dairy products as ripening cultures and which may be *stx* positive?

The genus *Hafnia* belongs to the Enterobacteriaceae family, as does STEC, and is a group of commensal (generally recognised as harmless) bacteria which can be found in food. *Hafnia* may also be deliberately added as a starter culture during the process of making cheese.

It has been reported that some *Hafnia* spp. have been isolated from foods that were PCR positive for the *stx* gene, and that a *Hafnia* strain had cytotoxigenic potential similar to that of STEC, but there is, at present, no evidence to indicate that *stx*-positive *Hafnia* strains can cause human illness. *Hafnia* has only very rarely been implicated as a cause of opportunistic infection in humans. Therefore, there is currently no evidence to conclude that the presence of a *Hafnia* spp. poses a risk to human health.

5. In a batch of food (category based on the answer to Question 1), what action should be taken based on a presumptive positive PCR STEC result in the context of the previous and/or subsequent batch (or batches produced close in time) being confirmed culture positive?

It has been concluded that the public health risk cannot be assessed based on detection of *stx* gene(s) by molecular methods only (i.e. a positive PCR result/presumptive positive).

However, where there is additional information that indicates a public health risk (e.g. batches of the same product from which STEC has been cultured), a presumptive positive STEC result (positive PCR only) may be taken as indicative of a risk. In those cases, the detection of *stx* gene(s) by PCR only may be taken as contributing evidence to support an intervention.

The risk management action(s) to be taken will be determined by the competent authority on the basis of an individual risk assessment. This risk assessment should examine additional information that might indicate a public health risk (e.g. cases of illness or other potentially relevant epidemiological information) or non-compliance (e.g. ineffective food safety management systems). Factors such as the origin of the raw material, the nature of the food item and its intended use, and the degree of separation between the batches should also be taken into consideration when assessing the risk. This applies to both RTE and non-RTE foods.

GENERAL RECOMMENDATIONS

- Scientific knowledge will continue to deepen our understanding of the human clinical epidemiology and the virulence characteristics and serotypes of STEC circulating in the agri-food chain. Whole genome sequencing (WGS) technologies are now starting to generate new scientific data on the presence and absence of a wide range of virulence genes and may in the future facilitate the identification of genetic markers in STEC which more accurately predict human virulence potential. It is therefore recommended that the advice provided in this report, which is based on current scientific knowledge and current Irish epidemiological information, be revisited periodically, taking account of any new data.
- In the context of managing the risk for food categorised as 'non-RTE food', in particular minced meat, it is recommended that periodic national education campaigns be run for both consumers and food business operators (FBOs) to raise and maintain awareness of the risk of eating or serving undercooked minced meat.

BACKGROUND

Shiga toxin-producing *Escherichia coli* (STEC), also known as verocytotoxigenic *Escherichia coli* (VTEC), is defined by the presence of one or both Shiga toxin genes: *stx*1 and *stx*2. Human infection with STEC can be asymptomatic or cause a spectrum of illnesses ranging from mild, non-bloody diarrhoea through to bloody diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome (HUS), and death.

STEC is a normal commensal in the gastrointestinal tract of ruminants, including cattle, sheep, goats and other farmed animals. STEC is potentially transmitted through contaminated water, contact with livestock or contaminated environments, or contaminated food. The infective dose is very low (possibly as low as 10 cells ingested) and person-to-person transmission is common among close contacts. The earliest food-reported vehicle associated with an STEC outbreak was beef burgers, but since then, a variety of foods have been linked with human illness.

In 2016, the European Union (EU) human STEC notification rate, based on symptomatic cases only, was 1.82 cases per 100,000 population, whereas Ireland's notification rate was 15.6 cases per 100,000 population, the highest reported rate in Europe. There is variation in surveillance systems at the EU Member State (MS) level, however, which makes comparison between MSs difficult.

There are significant challenges in the risk assessment and management of STEC. The profile of strains has continued to change since it first emerged as a cause of human illness. *E. coli* O157:H7 was the first serogroup implicated in STEC human infections (in the 1980s). In the 2000s, further serogroups (*E. coli* O26, O103, O111 and O145) were identified as being most commonly linked to human infection and, along with O157, became known as the 'top five' STEC serogroups. In 2011, *E. coli* O104 was added to this group following a European sprouted seed outbreak, making these serogroups the 'top six'. Since 2013, a wider diversity of STEC serogroups has been linked to human illness.

In STEC strains, the presence of the *eae* gene (a gene encoding for intimin, a protein which facilitates intimate attachment to the host intestinal epithelial cells) has historically been used as a predictor of human illness potential; however, the 2011 *E. coli* O104 outbreak strain was a hybrid enteroaggregative-haemorrhagic *E. coli* carrying the *aaiC* and *aggR* genes and the *stx*2a gene. As STEC has continued to evolve from a public health perspective, the methodology to detect and identify STEC in both human clinical samples and food samples has also changed in order to enable detection of clinically relevant strains. Methods used to test both human clinical samples and foods for STEC have changed from a culture-based method, specifically designed for the detection of *E. coli* serogroup O157 (*stx*+ and *stx*–) in the 1990s (ISO 16654:2001), to a polymerase chain reaction (PCR) method combined with culture (ISO/TS 13136:2012) in 2012 for the 'top six' serogroups. The ISO/TS 13136:2012 method involves screening by PCR for certain deoxyribonucleic acid (DNA) markers (presumptive result), followed by confirmation that these markers are present in a cultured STEC isolate (confirmed result).

There is still no standardised method to detect other STEC serogroups. This has resulted in historical data on the types of STEC strains in circulation in food (and in food-producing animals) being biased towards the methods which were then available, with the vast majority of data relating to the serogroup O157, limited data on the 'top six' serogroups and very significant knowledge gaps on the prevalence and diversity of other types of STEC in food. It is well recognised that many samples test positive by PCR screening for the *stx* gene(s), but *E. coli* carrying the *stx* gene(s) is not subsequently cultured. This failure to confirm a positive presumptive STEC result may be due to samples potentially containing a mix of dead and live bacteria, phages, or free DNA, as well as virulence or O-serogroup determinants that can cause positive PCR screening reactions being present in other bacterial species. The sensitivity of the PCR screening method could be a contributing factor, meaning lower limits of detection for STEC cells than the culture method. These discrepancies between PCR and culture-based detection methods require further research.

In the EU, there is only one legal microbiological criterion for STEC in food (Commission Regulation (EU) No 209/2013 amending Regulation (EC) No 2073/2005), which covers the 'top six' serogroups (O157, O26, O103, O111, O145, and O104:H4) in sprouts (excluding sprouts that have received a treatment effective to eliminate *Salmonella* spp. and STEC). Commission Regulation (EU) No 209/2013 (Recital 12) recognises, however, that it cannot be excluded that other STEC serogroups may be pathogenic to humans. Regulation (EC) No 852/2004 requires food business operators (FBOs) to develop, implement and maintain a food safety management system based on the principles of hazard analysis and critical control point (HACCP). As part of this requirement, FBOs may decide to test for STEC in other foods (in particular raw beef, cheese made with raw milk, and fresh horticulture produce). In addition, FBOs may be asked by their customers to test food for STEC or may be required to do so when exporting to a country outside the EU. Finally, authorities may choose to test for STEC as a result of a suspicion of a public health risk or as part of a national survey aimed at assessing the microbiological safety of a specific food. Article 14 of Regulation (EC) No 178/2002 prohibits food from being placed on the market if it is unsafe; therefore, where STEC is detected in a food for which there is no legal microbiological criterion, the risk must be assessed to determine whether the food is safe.

The evolving picture of human STEC illness and the changes in methodology for STEC detection in food have resulted in a lack of agreement across Europe on the risk posed and the appropriate risk-based action to be taken when STEC is detected in food. In April 2013, the European Food Safety Authority (EFSA) published a scientific opinion entitled Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment (EFSA Panel on Biological Hazards, 2013). This opinion acknowledged that it was not possible at the time to fully define human pathogenic STEC or to identify factors for STEC that absolutely predict its potential to cause human disease. It proposed a molecular approach for the categorisation of STEC according to the potential risk of illness.

In 2014, the European Commission (EC) attempted to introduce a harmonised approach to assessing and managing the risk of STEC in food, but MSs were unable to reach a common agreement and the EC suspended this work in 2016. In 2018, a global report by the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Member Countries reviewed specific aspects of STEC in food related to risk management and concluded that where countries identify STEC as a food safety risk, control measures should be based on the health risks assessed within a country, targeting identified high-risk foods and the STEC of highest health risk (FAO and WHO, 2018). A number of individual EU MSs have made their own risk assessments and policy decisions based on risk assessments of human epidemiology data and consumer practices relevant to their country, and some of these are summarised in the main report. This report has been produced in order to establish the risk in Ireland associated with the consumption of foods in which STEC has been detected.

1. STEC AND HUMAN ILLNESS

1.1 Pathogenic *Escherichia coli*

Escherichia coli are facultative Gram-negative rods within the Enterobacteriaceae family. Although most *E. coli* are harmless commensal organisms that form part of the natural gastrointestinal flora of humans and animals, there are also pathogenic strains which can cause a variety of illnesses in humans and animals. Based on their pathogenesis, pathogenic *E. coli* strains have been grouped into two broad pathotypes: extraintestinal pathogenic *E. coli* (ExPEC), and intestinal pathogenic *E. coli* or diarrheagenic *E. coli*. Within the intestinal pathogenic *E. coli*, different pathotypes have been described, and each group has different virulence traits and mechanisms of pathogenicity (Table 1). However, pathogenic *E. coli* constitutes a genetically heterogeneous group of bacteria, with recent studies providing evidence that some strains of *E. coli* only share between roughly 20% and 40% of their genome (Lukjancenko *et al.*, 2010; Messerer *et al.*, 2017). The diversity within the *E. coli* species and the overlap in gene content between this and related species suggests a continuum rather than sharp species borders in this group of Enterobacteriaceae (Lukjancenko *et al.*, 2010; Tenaillon *et al.*, 2010; Meier-Kolthoff *et al.*, 2014), making it a dynamic and complex classification (Meier-Kolthoff *et al.*, 2014).

Table 1 Major intestinal pathogenic *E. coli* pathotypes and virulence-associated factors

E. coli pathotype	Virulence characteristics	Marker gene
STEC or VTEC or enterohaemorrhagic *E. coli* (EHEC)	Presence of Shiga toxin 1 (Stx1) and/or 2 (Stx2). Phage encoded. Other virulence factors may be commonly present, such as *eae*-intimin as a marker of the pathogenic island locus of enterocyte effacement (LEE) and plasmid-encoded *hly* gene-producing haemolysin.	*stx*1, *stx*2 +/- *eaeA* +/- *hly*
Typical enteropathogenic *E. coli* (tEPEC)	Presence of both intimin (as a marker of the pathogenic island LEE) and the bundle-forming pili (BFP) contained in the EPEC adherence factor (EAF) plasmid.	*eaeA, bfpA* +/- *hly*
Atypical enteropathogenic *E. coli* (aEPEC)	Presence of intimin (as a marker of the LEE).	*eae*A
Enterotoxigenic *E. coli* (ETEC)	Presence of thermo-labile (LT) and/or thermo-stable (ST) toxins and Cytolysin A (Cly A).	*lt, st* +/- *hly*
Enteroinvasive *E. coli* (EIEC)	Presence of the invasion-associated locus (IAL) of the invasion plasmid antigens (Ipa).	*ial* *ipaH*
Enteroaggregative *E. coli* (EAEC)	Presence of plasmid-encoded *aggR* master regulon. Most genes associated with the aggregative adherence (AA) and EAEC virulence are controlled by this regulon. Toxins: EAEC heat-stable enterotoxin 1 (EAST1), *Shigella* enterotoxin 1 (ShET) and Haemolysin E (HlyE)	*aggR*
Diffusely adherent *E. coli* (DAEC)	Presence of surface afimbrial adhesins as AfaE-I and AfaE-III, which are encoded on the Afa/dr/daa operon or fimbrial (Dr) adhesins.	*afaC*

Source: Adapted from Croxen *et al.* (2013)

1.2 STEC: toxin production

STEC is currently the pathotype which has the most links to serious human illness and large outbreaks. STEC pathotypes are the only *E. coli* pathotypes which are notifiable when detected in humans in Ireland (S.I. No 707/2003). It should be noted that while the term STEC is used throughout this report, the term VTEC (verocytotoxigenic *E. coli*) is the formal term used in legislation for the purposes of Irish human disease notification.

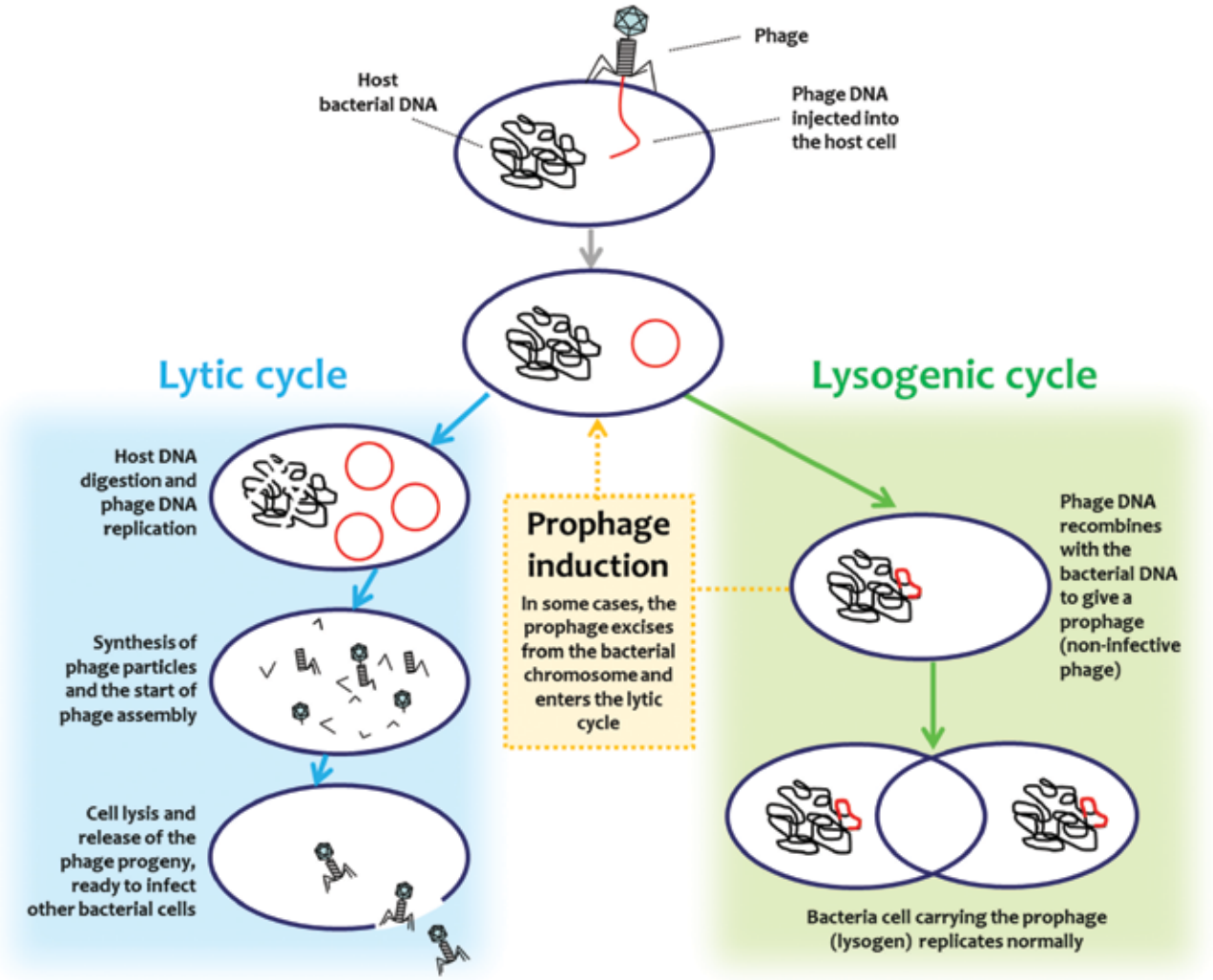
All STEC strains are able to produce Shiga toxins (Stx), their main virulence factor, and a single STEC strain may carry one or more Shiga toxin-encoding genes (*stx*) in its genome. Stx from *E. coli* are classified into two major types – Stx1 and Stx2 – and each group comprises several subtypes (Stx1a, Stx1c, Stx1d, Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g). Stx subtyping is not only useful for STEC characterisation, but is also valuable for diagnosis, as some types and subtypes of Stx have been epidemiologically associated with different clinical outcomes after STEC infection.

The *stx* genes are generally carried by Stx-encoding bacteriophages (bacteria-specific viruses) which can play a role in the transfer of the *stx* genes to other bacteria. These Stx-encoding phages have a phage cycle regulation and can be in a lysogenic or lytic state (Figure 1). In the lysogenic state, the DNA of the phage is integrated into the host bacterial cell chromosome and the expression of the *stx* gene is inhibited. In this lysogenic state, phages are referred to as 'prophages' and the host bacterial cells as 'lysogens'. The phage lysogenic state is very stable; however, a switch from the lysogenic state to a lytic state can be induced. This induction can be triggered by stress conditions, such as exposure to DNA-damaging agents or certain antibiotics.

Toxin production and the subsequent release of toxin is coupled to the induction of the phage to enter the lytic cycle. An additional result of the induction process is that the bacterial host cells lyse and release free phage particles that can infect other bacteria, resulting in the emergence of new pathogenic strains (Gamage *et al.*, 2004; Rode *et al.*, 2011; Tozzoli *et al.*, 2014; Chan and Ng, 2016). This type of evolutionary process likely resulted in the emergence of the hybrid *E. coli* O104:H4 pathotype which caused a large outbreak in Germany in 2011 (Frank *et al.*, 2011), where an EAEC acquired the Stx-encoding phage, resulting in an enteroaggregative-haemorrhagic *E. coli* (EAHEC) that carries EAEC- and STEC-associated virulence genes.

It is difficult to detect and isolate free Stx-encoding phages, but some studies have described their occurrence in cattle faeces, river water, and sewage (Muniesa *et al.*, 2004; Dumke *et al.*, 2006; Oot *et al.*, 2007; McDonald *et al.*, 2010), demonstrating the circulation of these phages in the environment. The possibility for transmission of Stx-encoding phages in various food matrices is further discussed in Section 3.3.

Figure 1 Lytic and lysogenic cycle pathways used by Stx-encoding phages



Source: Adapted from Henry *et al.* (2012)

1.3 STEC: public health concern

STEC are of particular public health concern due to the risk of serious health complications and the potential for outbreaks. The incubation period ranges from three to eight days. Infection with STEC can cause a spectrum of illness, ranging from mild, non-bloody diarrhoea to bloody diarrhoea, haemorrhagic colitis, HUS, and death. It has been reported that the incidence of HUS in outbreaks of STEC infection has typically been between about 7% and 10% (Lynn *et al.*, 2005; Tarr *et al.*, 2005; Radosavljevic *et al.*, 2015), with children under five years old and the elderly being the most susceptible. HUS is characterised by acute kidney failure, bleeding, and neurological symptoms. Stx-related HUS is the most common cause of acute renal failure in childhood and constitutes at least 80% of all HUS cases in children (Lynn *et al.*, 2005).

Symptom severity is determined by several factors, including the type of Stx produced and other virulence characteristics of the bacteria (Ostroff *et al.*, 1989; Beutin *et al.*, 1998; Eklund *et al.*, 2002; Friedrich *et al.*, 2002; Ethelberg *et al.*, 2004; Brooks *et al.*, 2005). The patient's age and immune status also plays an important role. For

example, children under the age of five years are at a higher risk of developing clinical disease when infected, and infants are at increased risk of death from dehydration and toxaemia.

The infective dose is very low, and person-to-person transmission is common among close contacts. As a faecal-oral pathogen, STEC is potentially transmitted through contaminated food, contaminated water, and contact with livestock or contaminated environments. Internationally, a wide range of transmission routes and vehicles have been implicated in STEC outbreaks. The earliest reported food vehicle associated with an STEC outbreak was beef burgers (Griffin and Tauxe, 1991), but since then a variety of foods have been linked with human illness, including fresh produce, raw milk products, and cookie dough (CDC, 2018).

1.4 STEC notification trends

The European Centre for Disease Prevention and Control (ECDC) and the EFSA compile data for the EU/European Economic Area (EEA) on trends in STEC, among other infectious diseases. Surveillance is governed by the EU case definition, which includes only notification of symptomatic STEC cases. Data are reported annually by MSs for the European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks, which is published jointly by the ECDC and the EFSA. There is variation in surveillance systems at the MS level, which may make comparison difficult between MSs. Moreover, some MSs do not provide details (or only provide a limited range) of the serotypes causing STEC infections. For example, there are a few MSs where only STEC-associated HUS cases are reported, and therefore their incidence rates should be considered an underestimate of disease caused by STEC. While STEC diagnosis by culture and serodiagnosis methods is still practised widely across the EU/EEA, diagnosis by PCR with/without achieving a subsequent confirmatory culture (strain isolation) is increasing in Ireland and in other MSs (see note in **Box 1**).

Box 1: *STEC notification to ECDC surveillance system*

The notification of STEC infections is mandatory in all but six EU MSs (Belgium, France, Italy, Luxembourg, Spain and the United Kingdom). Portugal reported STEC data for the first time in 2015. The surveillance systems for STEC infections have full national coverage in all the MSs except two (Belgium and France), although not all MSs report in the same way. While some countries report on all STEC infections regardless of symptoms, STEC surveillance in France is centred on paediatric HUS surveillance only, and in Italy it is primarily based on the national registry of HUS. Diagnosis of human STEC infections is generally done by culture methods from stool samples and indirect diagnosis by the detection of antibodies against the O-lipopolysaccharides of *E. coli* in serum in cases of HUS. Diagnosis by direct detection of Stx or the *stx* gene(s) by PCR with and without subsequent strain isolation is increasing.

While the Irish STEC case definition is more sensitive than the EU case definition (see note in **Box 2**), reporting of Irish notification data to the EU surveillance system is performed using the EU case definition, and all comparisons with EU data in this report are made under the EU case definition.

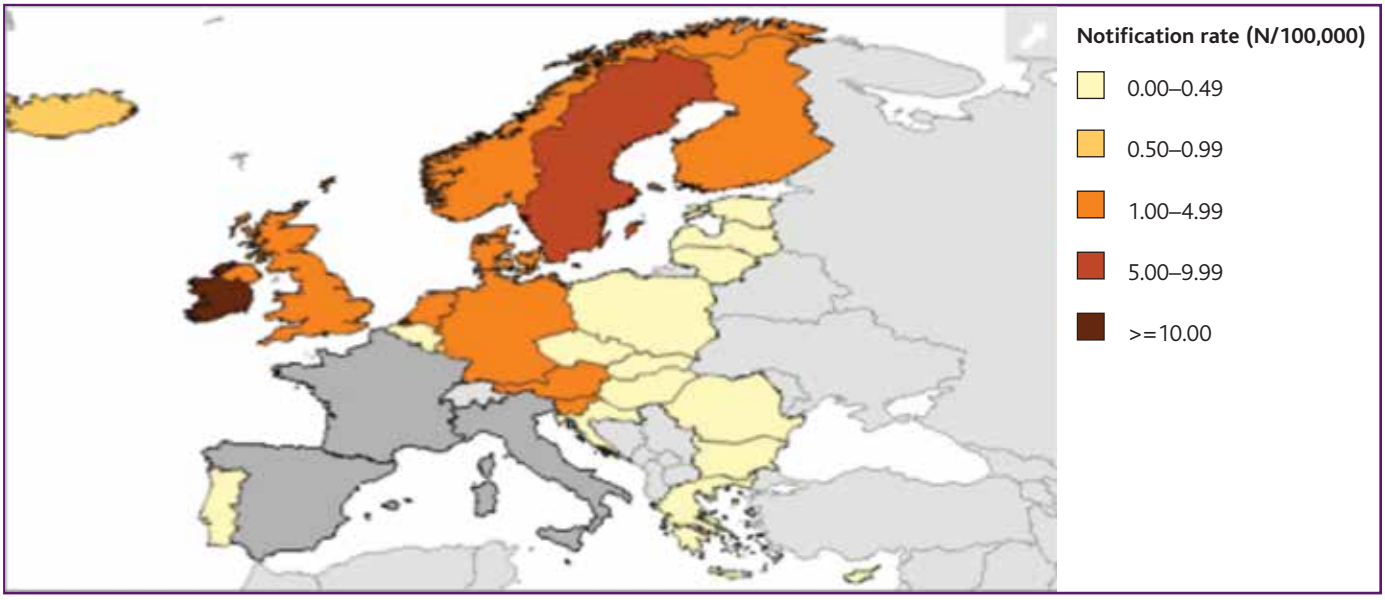
Box 2: *The Irish case definition for surveillance of STEC*

The Irish case definition for surveillance of STEC allows for the reporting of symptomatic and asymptomatic infections. However, when Ireland's data are compared in the EU context, the EU case definition is used and asymptomatic notifications are not included.

In 2016, the EU notification rate was 1.82 cases per 100,000 population, which was an 8.3% increase compared with 2015 (1.68 cases per 100,000 population). Over the five-year period from 2012 to 2016, six MSs (Finland, France, Ireland, Malta, Romania and Spain) reported significantly increasing trends, and three MSs (Luxembourg, the Netherlands and Slovakia) had decreasing trends (EFSA and ECDC, 2016).

Since 2008, Ireland has had the highest reported STEC notification rate in Europe, with the exception of 2011, when Germany reported the highest rate due to a large *E. coli* O104:H4 outbreak linked with fenugreek seeds (EFSA and ECDC, 2016). In 2016, the notification rate for the Republic of Ireland was 15.6 cases per 100,000 population; the next highest rates were reported in Sweden, the Netherlands and Denmark (6.48, 3.92, and 3.68 cases per 100,000 population, respectively) (EFSA and ECDC, 2016) (Figure 2).

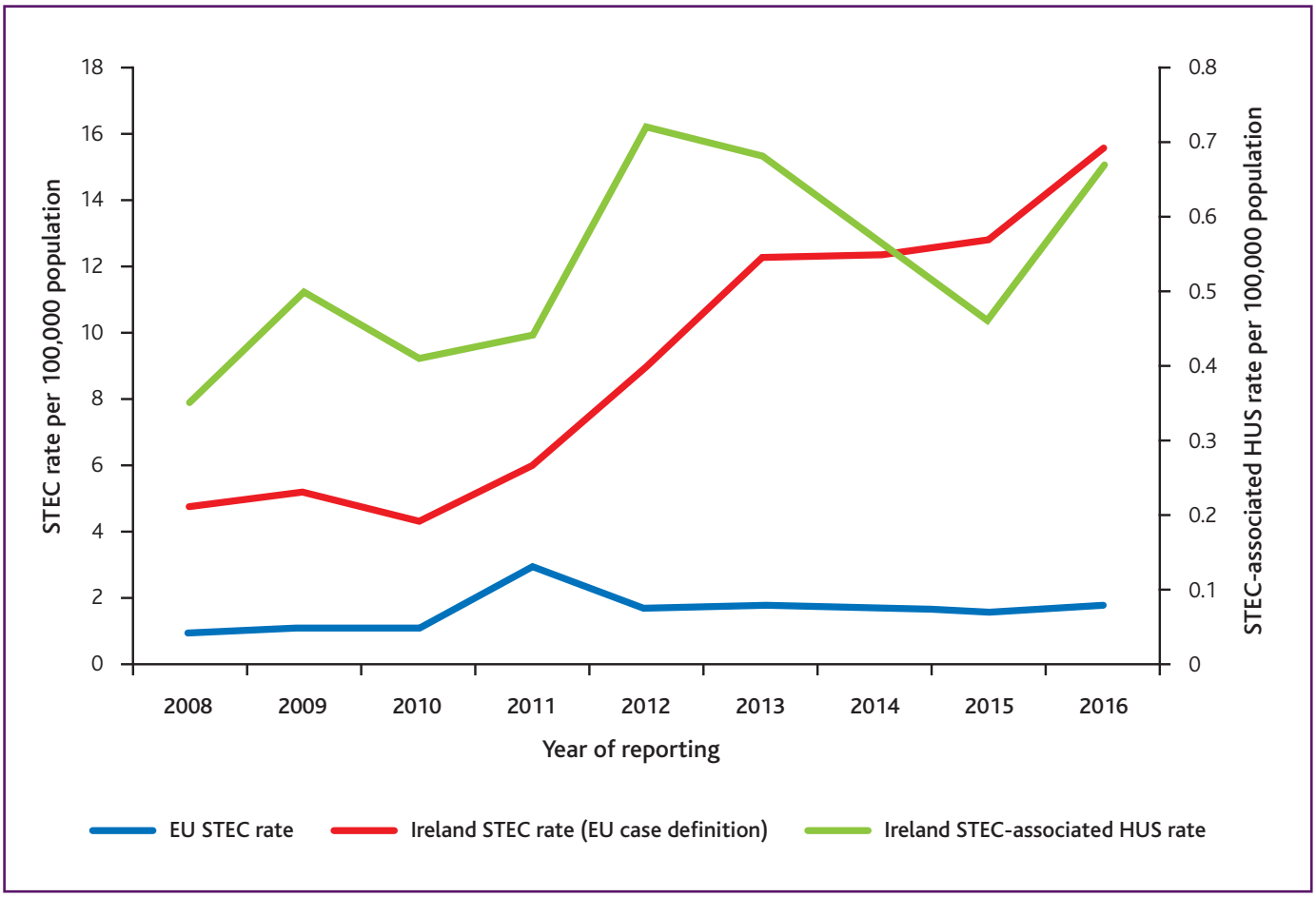
Figure 2 Map of Europe illustrating notification rates for confirmed STEC cases by country (EU/EEA), 2016



Map generated using the ECDC Surveillance Atlas of Infectious Diseases. Note: EU countries shown in grey indicate that incidence is not calculated, as national coverage in those countries is incomplete.

STEC notification rates in Ireland have seen a dramatic increase since 2012 (Figure 3); a variety of factors may have contributed to this, including greater environmental contamination of private water supplies due to high rainfall in 2012 (HPSC, 2012) and the introduction since 2012 of PCR testing for STEC screening in the majority of clinical laboratories.

Figure 3 STEC and STEC-associated HUS notification rates in Ireland compared with the EU STEC notification rate, 2008–2016



Data source for STEC cases: ECDC Surveillance Atlas of Infectious Diseases, based on EU case definition. Data source for STEC-associated HUS cases in Ireland: Computerised Infectious Disease Reporting (CIDR).

1.5 Illness severity

Over the five-year period from 2012 to 2016, there were a total of 3,531 STEC notifications in Ireland (Table 2). Of these, 82.4% (n=2,910) were symptomatic and 16.5% (n=582) were asymptomatic. Among symptomatic cases, 1 in 20 developed HUS (about 5%), which is a little lower than previously reported – i.e. an international average of between 7% and 10% (Lynn *et al.*, 2005; Tarr *et al.*, 2005; Radosavljevic *et al.*, 2015) – and a further one in three developed bloody diarrhoea without progression to HUS (about 33%) (Table 2). Of the symptomatic cases, 40.3% were hospitalised (n=1,173) (data not shown).

In the 13-year period from 2004 to 2016, five deaths were attributed to STEC: one in 2009, two in 2013, one in 2014 and one in 2015 (HPSC, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013a, 2013b, 2014, 2016, 2017), representing an average STEC case fatality rate of 0.1% (n=4,997). Two of these fatal cases were HUS cases, equating to a case fatality among STEC-associated HUS cases of 0.75% (2/266; see Table 5) in that period.

Table 2 Number of STEC notifications by clinical presentation, Ireland, 2012–2016*

Clinical presentation		Total number of notifications	Percentage based on the number of symptomatic cases (n=2,910)
Symptomatic (total cases=2,910)	HUS	145	4.98%
	Bloody diarrhoea (no HUS)	974	33.47%
	Diarrhoea	1,608	55.26%
	Not specified	183	6.29%
Asymptomatic		582	–
Unknown		39	–
Total		**3,531**	–

*Includes all STEC notifications reported under Irish STEC case definitions (see Box 2). Data source: CIDR.

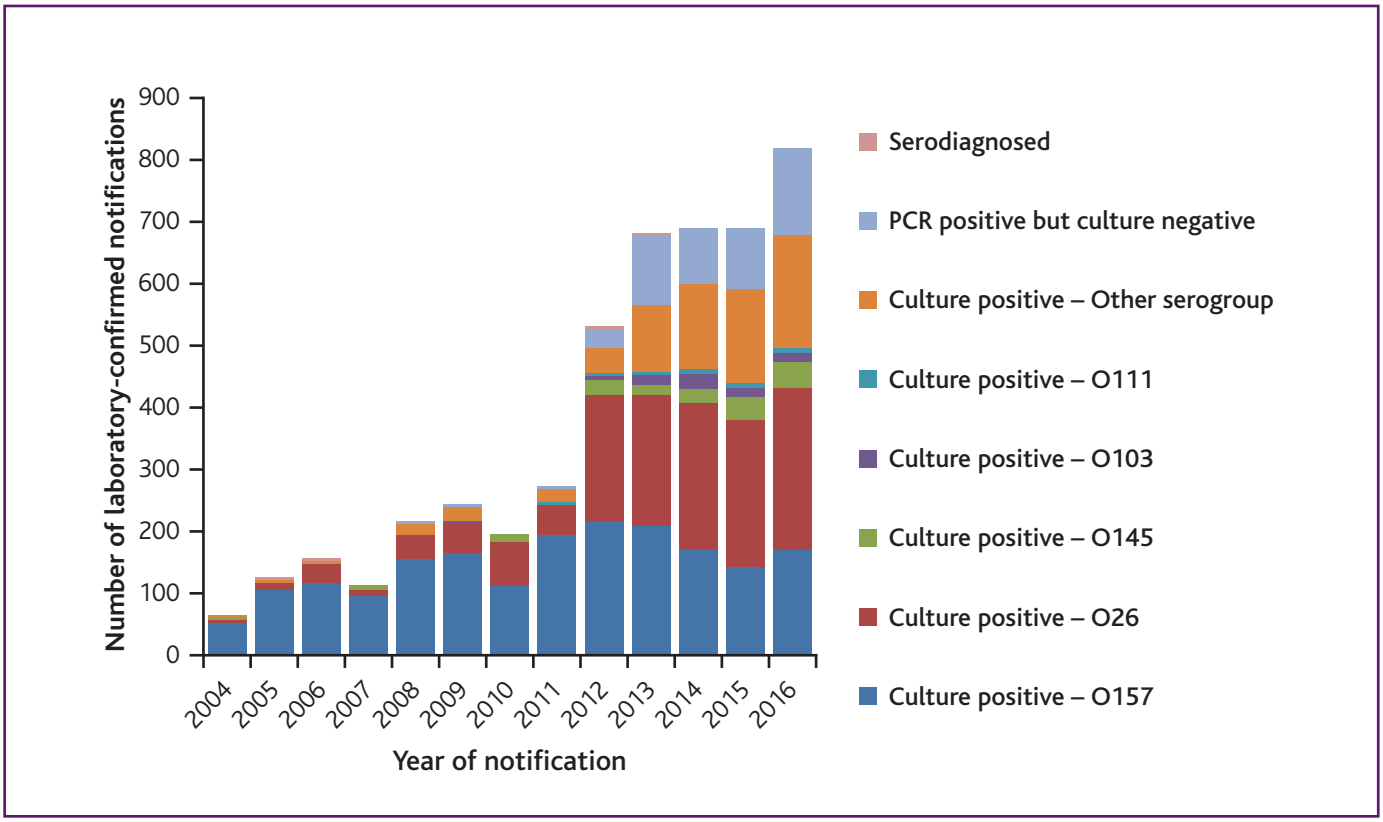
1.6 Human disease and STEC virulence

The increase in STEC notifications in Ireland since 2012 was accompanied by a change in serogroup distribution among reported culture-confirmed notifications[3] (Figure 4). This has been strongly influenced by the gradual introduction, across primary hospital laboratories, of multiplex PCR as the first choice for STEC detection followed by STEC culture for all PCR-positive specimens. This serogroup-independent approach has improved the understanding of the relative clinical importance of different STEC serogroups.

Figure 4 shows the distribution of notified STEC cases by method of laboratory confirmation during 2004–2016. Among all laboratory-confirmed notifications (n=3,408) in the five-year period from 2012 to 2016, 86% were reported on the basis of culture confirmation (n=2,931), 13.9% on the basis of being PCR positive but culture negative (n=474), and 0.1% on the basis of serodiagnosis (n=4). Among the culture-confirmed notifications in this period, 31.4% corresponded to the STEC O157 serogroup (n=920). STEC serogroups outside of the other 'top five' were detected in 21.1% of culture-confirmed STEC notifications (n=617) (**Appendix 2**). This differs substantially from the understanding prior to 2012. In 2004, 85% of all Irish STEC notifications were linked with STEC O157 (data not shown), compared with 28% in the period from 2012 to 2016.

[3] Note that laboratory-confirmed notifications include those that are (i) culture confirmed, (ii) confirmed by PCR but culture negative, and (iii) confirmed by serodiagnosis.

Figure 4 Number of laboratory-confirmed STEC notifications by criteria for notification (and serogroup for culture-confirmed cases) in Ireland, 2004–2016



Data source: CIDR.

Appendix 2 shows the diversity of serogroups among symptomatic and asymptomatic culture-confirmed STEC notifications in Ireland from 2012 to 2016 (around 75 serogroups were identified in this five-year period). This diversity has become increasingly evident since PCR was introduced in order to screen for *stx*1 and *stx*2 genes in many primary laboratories across Ireland. The association between the less common STEC serogroups and human illness may not be as evident from historical Irish data when diagnostic methods were more focused on serogroup O157 or from countries whose human STEC data are based on laboratory practice which does not test for all possible STEC serogroups.

As the overwhelming majority of asymptomatic STEC notifications are identified as a result of screening high-risk contacts during outbreak investigations, the range of serogroups associated with asymptomatic STEC notifications mirrors, to a large extent, the serogroups associated with symptomatic STEC notifications. A prevalence study of the general population would be required to determine whether there are STEC strains routinely being carried asymptomatically that are not usually linked with human illness.

Stx type is recognised as one of the key determinants of disease severity among STEC cases. Table 3 shows the distribution of *stx* genes among STEC notifications in general and among HUS-associated STEC notifications in Ireland in the period from 2012 to 2016. Among culture-confirmed STEC notifications, strains containing the *stx*2 gene alone were associated with ~38% of notifications. Strains containing both *stx*1 and *stx*2 genes or containing only the *stx*1 gene were associated with ~35% or ~27% of notifications, respectively. Among culture-confirmed STEC-associated HUS notifications, a higher proportion (~63%) were associated with strains containing the *stx*2 gene alone, while ~2% were associated with strains containing the *stx*1 gene alone. A similar pattern was detected among PCR-positive but culture-negative notifications (both for all notifications and for STEC-associated HUS notifications) (Table 3). Among culture-confirmed STEC notifications, 17.8% were *eae* negative; among culture-confirmed STEC-associated HUS cases, 6.8% were *eae* negative (Table 4).

Table 3 Number (and percentage) of laboratory-confirmed STEC notifications and STEC-associated HUS notifications by *stx* gene type, Ireland, 2012–2016*

Criteria for reporting	*stx* type	Number of STEC notifications (%)[a]	Number of STEC-associated HUS notifications (%)[a,b]
Culture confirmed	*stx*1	788 (27.0%)	2 (1.9%)
	*stx*1 + *stx*2	1,012 (34.6%)	38 (35.5%)
	*stx*2	1,122 (38.4%)	67 (62.6%)
	Not reported	9	0
	Total	**2,931**	**107**
Mixed infection[c]	Mixed infections	56	4
PCR positive but culture negative	*stx*1	167 (36.1%)	2 (14.3%)
	*stx*1 + *stx*2	121 (26.1%)	2 (14.3%)
	*stx*2	175 (37.8%)	10 (71.4%)
	Not reported	10	0
	Total	**473**	**14**

* Includes all STEC notifications reported under Irish STEC case definitions (see Box 2).

[a] Percentages have been calculated taking into account only the notifications where the *stx* type was reported.

[b] Any case for which HUS was not specifically recorded as 'Yes' was assumed to be a 'No'.

[c] Mixed infections are reported separately, as several had different *stx* gene complements for the different strains. Mixed infections include cases that were culture confirmed with two or more strains, or culture confirmed with one strain and an *Stx* PCR result which was inconsistent with the strain isolated, suggesting that more than one strain may have been present.

Data source: CIDR.

Table 4 Number (and percentage) of culture-confirmed STEC notifications and STEC-associated HUS notifications by *eae* status, Ireland, 2012–2016*

eae status	Number of STEC notifications (%)[a]	Number of STEC-associated HUS notifications (%)[a,b]
Positive	1,816 (82.2%)	68 (93.2%)
Negative	394 (17.8%)	5 (6.8%)
Not reported	721	34

*Includes all STEC notifications reported under Irish STEC case definitions (see Box 2).

[a] Percentages have been calculated taking into account only the notifications where the *eae* status was reported.

[b] Any case for which HUS was not specifically recorded as 'Yes' was assumed to be a 'No'.

Data source: CIDR and National VTEC Reference Laboratory (data not published).

Table 5 shows that in Ireland, O157 was the most commonly identified serogroup among HUS cases during the 13-year period between 2004 and 2016 (n=120; 45.1%), with O26 the second most common serogroup (n=60; 22.6%). The majority of HUS cases were associated with strains which contained an *stx*2 gene, either alone (n=164; 61.7%) or in combination with an *stx*1 gene (n=57; 21.4%). From these results, it is not possible to determine whether the strains contained only one or multiple gene variants for *stx*2. Subtyping of *stx* genes was not routinely performed in Ireland before the introduction of whole genome sequencing (WGS) in late 2016. These data show that among culture-confirmed HUS notifications associated with a single strain, 17 out of 213 (8%) were infected with serogroups outside the 'top five'. Additionally, for nine HUS cases (4%), there was only evidence of detection of a strain carrying *stx*1 alone (six culture-confirmed and three positive by PCR only).

Table 5 Number of STEC-associated HUS notifications by serogroup, criteria for reporting, and *stx* type, Ireland, 2004–2016

Criteria for reporting	Serogroup	*stx*1	*stx*1 + *stx*2	*stx*2	Combinations	NR	Total
Culture positive	O157*	–	8	112	–	–	120
	O26*	3	43	14	–	–	60
	Ungroupable	–	1	7	–	–	8
	O145*	–	1	9	–	–	10
	O103*	1	–	3	–	–	4
	O55	–	–	2	–	–	2
	O111*	–	2	–	–	–	2
	O113	–	–	1	–	–	1
	O5	1	–	–	–	–	1
	O78	–	–	1	–	–	1
	O2	–	–	1	–	–	1
	O177	–	–	1	–	–	1
	O182	1	–	–	–	–	1
	O148	–	–	1	–	–	1
Mixed infection		–	–	–	6	–	6
Serodiagnosis	O157*	–	–	–	–	5	5
	O26*	–	–	–	–	1	1
PCR positive only		3	2	12	–	–	17
Clinical HUS		–	–	–	–	16	16
Epi-linked cases§		–	–	–	–	8	8
Total		**9**	**57**	**164**	**6**	**30**	**266**

NR = not reported

* Belongs to the 'top five'.

§ Notified HUS cases meeting the clinical criteria for VTEC and with an epidemiological link – see VTEC case definition at http://www.hpsc.ie/notifiablediseases/casedefinitions/

Data source: CIDR.

STEC Stx subtyping was not routinely performed at the National VTEC Reference Laboratory (VTEC NRL) before 2016; however, a study by Carroll *et al*. (2015) on HUS caused by STEC strains in Ireland from January 2012 to March 2013 was carried out at the VTEC NRL at the Health Service Executive Dublin Mid-Leinster Public Health Laboratory, Cherry Orchard Hospital, which looked at 31 HUS cases and the *stx* subtypes associated with these cases (Table 6). The majority (n=19; 61.3%) of STEC-associated HUS cases were infected with a non-O157 STEC strain, and five cases (16.1%) were related to a non-'top six' serogroup. This is evidence that non-O157 STEC serogroups are as important as STEC O157 as a human pathogen and in terms of public health significance. Although the majority of STEC HUS cases harboured the *stx*2 gene (n=29; 93.5%), the presence of *stx*1-only HUS cases (n=2; 6.5%) shows that such strains cannot be overlooked as a potential cause of severe illness.

Table 6 *stx* subtypes associated with 31 HUS cases, Ireland, 2012–2013

Serogroup	*stx* subtypes									
	1a	1a2a	1a2a2c	1a2a2d	2a	2a2b2c	2a2c	2a2c2d	2a2d	Total
O26	2	4	-	3	1	–	–	–	2	12
O157	–	–	1	–	3	–	7	1	–	12
O145	–	–	–	–	–	–	–	–	2	2
O55	–	–	–	–	2	–	–	–	1	3
O130	–	–	–	–	1	–	–	–	–	1
O91	–	–	–	–	–	1	–	–	–	1
Total	**2**	**4**	**1**	**3**	**7**	**1**	**7**	**1**	**5**	**31**

1.7 Source of STEC human infection

In Ireland, person-to-person spread is the most common transmission route reported in STEC outbreaks (particularly in childcare facilities); contaminated drinking water is generally the second most commonly suspected mode of transmission (Garvey *et al*., 2016). Exposure to water from contaminated untreated or poorly treated private water supplies has historically been recognised as a strong risk factor for STEC infection in Ireland (O'Sullivan *et al*., 2008). Cases linked to food, including raw milk products and undercooked beef burgers, have also been reported in recent years.

Under the enhanced surveillance system in place for STEC notifications, information regarding possible transmission routes is routinely collected on all notifications. However, the strength of evidence of the transmission routes reported in the case of outbreaks is usually much stronger than for sporadic cases.

1.8 Foodborne STEC outbreaks

Internationally, a wide range of transmission routes and vehicles have been implicated in STEC outbreaks. The earliest identified food vehicle associated with STEC infection was undercooked beef burgers; since then, additional foods have been linked with human illness, including fresh produce, milk products and, recently, cookie dough and flour.

In the United States of America (USA) in 1982, two outbreaks of bloody diarrhoea linked with ground beef patties were found to be due to *E. coli* O157 (Griffin and Tauxe, 1991). Meat continued to be a source of STEC infections worldwide. Adams *et al*. (2016) reviewed foodborne STEC O157 infections in England and Wales from 1983 to 2012. Food was reported to have contributed to 101 outbreaks, including 38 attributed to eating contaminated meat; 16 attributed to eating undercooked meat, such as burgers at barbecues; and 22 to cross-contamination of cooked meats. In September 2005, an outbreak of *E. coli* O157 in South Wales with 157 cases was caused by cooked meats (Salmon and Collective outbreak control team, 2005).

Several STEC outbreaks internationally have been linked to sprouted seeds and other fresh produce. This includes one of the largest outbreaks of STEC in Europe, when a 2011 STEC O104:H4 outbreak resulted in more than 3,800 cases of illness and 54 deaths in Germany (Frank *et al*., 2011). The outbreak was traced to consumption of fenugreek seeds from Egypt (EFSA, 2011). In general, contamination of fresh produce with STEC is likely to occur during the pre-harvest period from contact with STEC-contaminated faeces and/or soil amendments, infected workers and food handlers, the use of contaminated water for irrigation of food crops and washing of fruit and vegetables, or contaminated equipment used in harvesting. Contamination during transport and storage may also occur.

From 2000 to 2010, a global study identified 24 outbreaks of STEC infection associated with dairy products (Farrokh *et al*., 2013). Those diagnosed with clinical symptoms of STEC infection went on to develop HUS in 19 of the outbreaks. Twelve of these outbreaks were linked to the consumption of raw milk and five were linked to consumption of raw milk cheese. The STEC serogroups causing the outbreaks were primarily O157 (present in 19 outbreaks), as well as O26 (4 outbreaks), O121 (2 outbreaks), O145 (2 outbreaks), O80 (2 outbreaks) and O84 (1 outbreak). Six of the outbreaks were caused by multiple STEC serogroups; for example, one linked to a raw milk brie cheese (O26 and O80) and another linked to a pasteurised milk cheese (O121, O26 and O84).

In a review of STEC O157 infections between 1983 and 2012 in England and Wales, Adams *et al*. (2016) reported three outbreaks in 1999 caused by milk pasteurisation failures, one of which affected 88 people (Goh *et al*., 2002). Outbreaks caused by post-pasteurisation contamination of milk occurred in 2000 and 2002, and two outbreaks in 2000 were associated with drinking raw milk. An English outbreak of STEC O157 *stx2* in 2014 resulting in nine cases, two of which developed HUS, was linked to the consumption of raw cow's milk; WGS was used to link cases of illness with the suspected farm (Butcher *et al*., 2016). In 2016, a raw milk blue cheese was implicated as the most probable cause of a Scottish outbreak of STEC which caused illness in 22 people, 11 of whom were admitted to hospital and one of whom died (FSS, 2016).

An examination of the foodborne outbreaks listed on the US Centers for Disease Control and Prevention (CDC) website also indicates some less frequently identified food vehicles (CDC, 2018). In one outbreak in 2017 in the USA, 32 people became infected with STEC O157 across 12 states. Twelve people were hospitalised and nine people developed HUS. Infection was attributed to a specific brand of soy nut butter. In 2009, pre-packaged raw cookie dough was linked to an outbreak of STEC O157, in which 72 people from 30 states were infected; 34 people were hospitalised and 10 developed HUS. This was the first time that raw cookie dough had been linked with STEC infection. In 2016, an outbreak involving STEC O121 and O26 in the USA was linked to raw flour from a particular mill. The outbreak strains infected 63 people, 17 of whom were hospitalised, one with HUS (FDA, 2017). A subsequent outbreak of STEC O121 in Canada between November 2016 and April 2017 was also linked to flour (Public Health Agency of Canada, 2017). In total, there were 30 cases of STEC O121 and eight individuals were hospitalised.

The EFSA collates data annually on foodborne outbreaks under Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents. The evaluation of the strength of evidence implicating a suspected food vehicle in foodborne outbreaks as being strong or weak is based on an assessment of all available types of evidence. This may include microbiological, epidemiological, descriptive environmental and information on tracing back the investigated foodstuffs, according to the European Union Foodborne Outbreak Reporting System (EU-FORS) guidance and the latest published manual for reporting on foodborne outbreaks produced by the EFSA (EFSA, 2018). Table 7 shows the relative importance of different food vehicles among foodborne STEC outbreaks with strong evidence reported to the EFSA in the period from 2013 to 2016 (EFSA and ECDC, 2015a, 2015b, 2016, 2017). During this time period, 14.8% (n=30) of STEC outbreaks reported to the EU as being foodborne had strong evidence.

Table 7 Number of STEC outbreaks in the EU with strong evidence, by suspected vehicle, 2013–2016

Foods implicated	2013	2014	2015	2016	Total	Percentage of strong-evidence outbreaks where each food was implicated
Vegetables and juices, and other products thereof	3	2	–	2	**7**	23.3%
Cheese	2	–	1	2	**5**	16.7%
Bovine meat and products thereof	4	–	–	–	**4**	13.3%
Milk	–	3	–	–	**3**	10.0%
Chicken burgers and beef burgers	–	–	1	–	**1**	3.3%
Various meat products	–	–	1	–	**1**	3.3%
Fish and fishery products	1	–	–	–	**1**	3.3%
Herbs and spices	1	–	–	–	**1**	3.3%
Mixed-leaf lettuce and raw minced lamb	–	–	1	–	**1**	3.3%
Not specified	1	–	–	5	**6**	20.0%
Total number of foodborne STEC outbreaks with strong evidence	**12**	**5**	**4**	**9**	**30**	–
Total number of foodborne STEC outbreaks reported	73	38	50	42	**203**	–

Data source: EFSA and ECDC (2015a, 2015b, 2016, 2017).

1.9 STEC outbreaks in Ireland

Since 2004, all outbreaks[4] of infectious disease in Ireland are notifiable under S.I. No. 707/2003. This includes both family and general outbreaks. The system is very sensitive, particularly for the detection of STEC family outbreaks, as there is an active public health investigation of notified STEC cases, which frequently results in the discovery of additional STEC cases either in household contacts or close contacts. Garvey *et al*. (2016) reviewed Irish STEC outbreak data for the period from 2004 to 2012; outbreaks where person-to-person spread was reported as the sole transmission route accounted for more than half of all outbreaks (123/219 outbreaks; 56.2%) and outbreak cases, most notably in childcare facilities. The next most significant transmission route was waterborne spread from untreated or poorly treated private water supplies (55/219; 25.1%). Food was reported as a suspected transmission route for ~10% of these STEC outbreaks (21/219), ranging in size from one to seven people ill (median two people ill). The majority of outbreaks reported as foodborne were associated with private homes (18/21; 85.7%). No microbiological or analytical epidemiological evidence was reported implicating specific food items in any of these outbreaks. Suspect foods based on descriptive epidemiological evidence were reported for only four household outbreaks (minced beef for two outbreaks, and goat and lamb meat for one outbreak each), while a meal eaten out was suspected for one small travel-associated outbreak.

[4] The Health Protection Surveillance Centre (HPSC) defines an outbreak of infection or foodborne illness as "two or more linked cases of the same illness, or the situation where the observed number of cases exceeds the expected number, or a single case of disease caused by a significant pathogen (e.g. diphtheria or viral haemorrhagic fever). Outbreaks may be confined to some of the members of one family or may be more widespread and involve cases either locally, nationally or internationally."

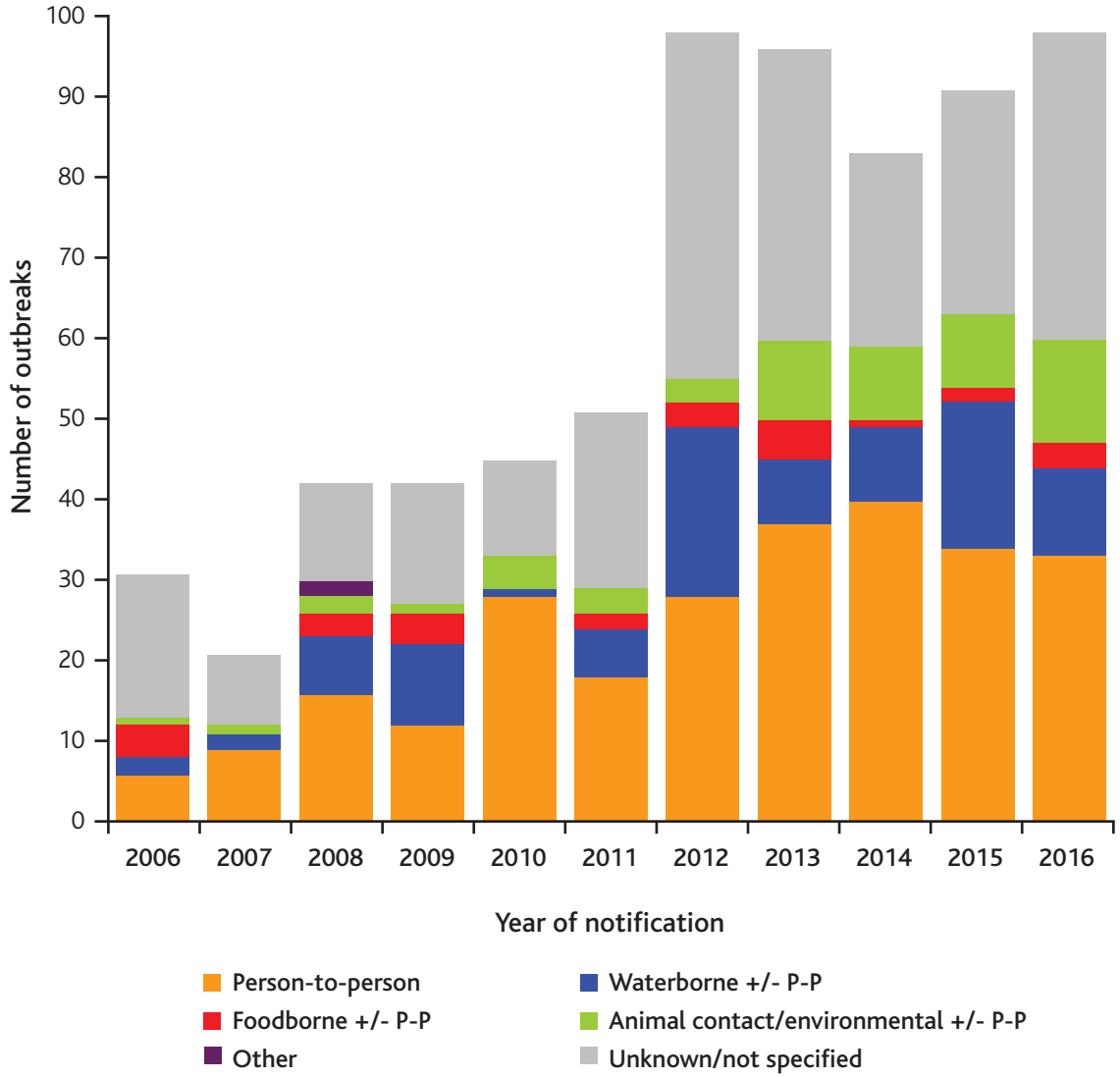
In addition to the 21 outbreaks reported as suspected to be foodborne in that time period (2004 to 2012), there were at least four general STEC outbreaks reported to be associated with commercial premises with unknown transmission routes, and it is possible that some or all of these were foodborne, although other transmission routes could not be ruled out by the outbreak investigation teams (Garvey *et al*., 2016). There is likely under-ascertainment of foodborne outbreaks because evidence that an outbreak is foodborne can be difficult to establish. Not finding a link between a specific food and illness can happen for several reasons; **Box 3** lists some of these reasons. When no association can be established, it does not mean that the illness or outbreak was not foodborne, just that the source could not be determined.

Box 3: *Reasons why evidence implicating food vehicles during outbreak investigations can be difficult to obtain (adapted from CDC, 2015)*

- Food items with a short shelf life are often no longer available by the time an outbreak is known. If the suspected food item is available, the pathogen may be difficult to detect in that food due to other organisms overgrowing the pathogen as the food started to spoil, or there may be no established test to detect the pathogen in the suspected food.
- Microorganisms have an uneven distribution in food matrices and the pathogen may have only been in the food portion that was consumed. A sample taken from a portion that was not contaminated may have a negative result and thus microbiological evidence to confirm a suspected food vehicle is unavailable, but this does not mean the food was not a possible source of illness.
- An outbreak may be reported so long after it occurred that a full investigation is not possible (e.g. for pathogens or viruses with particularly long incubation times prior to onset of illness).
- An initial investigation may not have led to a specific food hypothesis, or an analytical study may not find a specific food exposure, because the number of illnesses was small and the study consequently lacked statistical power; because multiple food items were potentially contaminated; because people ill during the outbreak did not remember correctly all the foods eaten in the time period prior to getting ill (recall bias); or because the food was a stealth food. Stealth foods are those that people eat but are unlikely to remember, such as garnishes, condiments, or ingredients that are part of a food item, such as the filling in a snack cracker.

Figure 5 shows the number of notified STEC outbreaks in Ireland between 2006 and 2016, which includes the period (2004–2012) studied by Garvey *et al*. (2016). The picture has remained similar since 2012, with few foodborne outbreaks reported. Between 2013 and 2016, food was reported as contributing to transmission for 11 STEC outbreaks (representing 4.5% of those with a reported transmission route). Of the outbreaks reported as suspected to be foodborne in the earlier study period of 2004 to 2012, 0/21 (0%) had strong evidence to support food as the transmission route. Three of 11 (27.3%) outbreaks related to food during the period from 2013 to 2016 had strong evidence to support food as the transmission route (Table 8).

Figure 5 Number of STEC outbreaks by suspected transmission route and year, Ireland, 2006–2016



Note: In this figure, reported transmission routes were grouped for simplicity. Any outbreak where food contributed was reported as foodborne, any outbreak where water contributed was reported as waterborne, and any other outbreak where animal contact contributed was reported as animal contact. Person-to-person (P-P) outbreaks include only those outbreaks reported as being due only to person-to-person transmission.

+/- indicates with or without.

Data source: CIDR.

Table 8 STEC outbreaks notified in Ireland where there was strong evidence implicating a particular food item

Year	Organism	Number ill	Food vehicle	Evidence
2016	STEC O157 (*stx*1 and *stx*2)	8 (11 outbreak-related cases: 8 microbiologically confirmed and 3 probable)	Undercooked beef burgers	Epidemiological and microbiological evidence. WGS confirmed that all 8 case isolates and the food (raw minced meat) isolate were within a 5-SNP cluster.
2015	STEC O26 (*stx*1)	2	Unpasteurised milk cheese	Epidemiological and microbiological evidence. *E. coli* O26 *stx*1 with a PFGE profile closely related to a strain isolated from one outbreak case was detected in the unpasteurised milk cheese.
2013	STEC O157 (*stx*2)	2	Unpasteurised milk cheese	Epidemiological and microbiological evidence. An isolate from one cheese sample was indistinguishable by PFGE analysis from an isolate from one of the outbreak cases.

WGS – whole genome sequencing; SNP – single nucleotide polymorphism; PFGE – pulsed-field gel electrophoresis.

2. STEC METHODS OF ANALYSIS

The chosen method of microbiological analysis is dependent on:

1. The types of samples to which the method is applied, e.g. food, feed, water, or clinical sample
2. The method's purpose as detection, enumeration, isolation, typing, etc.
3. The method's scope as capable of detecting all or one subset of strains, e.g. O157:H7 serotype, several STEC O-groups or all STEC
4. The methodological approach, e.g. exploitation of biochemical, serological or genetic characteristics of the organisms; phage typing; WGS; and
5. Whether it is a screening (presumptive) or confirmatory method.

The analysis of food samples presents a particular challenge, as pathogens are generally unevenly distributed and present in low numbers in the midst of a variety of complex food matrices. This section focuses on methods for food samples.

There are standard methods recognised internationally or nationally that are considered a benchmark and comprise the unequivocal cultured confirmation of isolates, and there are alternative methods which are rapid but that do not aim to isolate the microorganism (although most rapid methods can be followed by isolation and confirmation using standard methods). The decision to use one method or another can be guided by:

1. National rules, trade requirements, type of control, and whether it is a food industry sample or an official control sample. For example, testing of sprouts in the EU for STEC must follow the most recent edition of the ISO/TS 13136 method or an alternative method that has been validated in accordance with the requirements of Commission Regulation (EC) No 2073/2005, as amended, and raw beef for the US market must follow the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) method MLG 5.09. Official control actions are preferably taken on food of unequivocally robust confirmed isolation results, while the food industry might opt to apply alternative methods or proprietary methods for its routine checks in order to ensure a safe supply of food.
2. The capability and capacity of laboratories. This can be a limiting factor when deciding what method to use, as the isolation steps for STEC methods require a biological containment setting that might not be present in the available laboratories.
3. Whether strain typing is required. Single-colony isolation is desirable in order to apply typing methods to further characterise the STEC strains (serotyping, phage typing, virulotyping,[5] PFGE, WGS), which permits epidemiological studies, comparison with other STEC strains, and full assessment of the strains' risk profile.

STEC detection in foodstuffs has typically focused on *E. coli* O157 alone or on the subset of serotypes (sometimes called 'non-O157 STECs') associated with serious illness and major outbreaks: the so-called 'top five' and, since the large outbreak of STEC O104 in 2011, the 'top six' or 'big six'. However, as highlighted in Section 1 of this document, there is an increasing awareness of the diversity of STEC serotypes associated with human illness, both in the EU and worldwide. It is prudent to note that the less concrete the target, the more difficult and arduous the analysis becomes.

[5] Virulotyping is a type of genotyping technique that identifies specific virulence genes within the pathogen's genome.

2.1 Detection method for *E. coli* O157 in food

There is currently an International Organization for Standardization (ISO) horizontal method for the detection of *E. coli* serogroup O157 in food and animal feeding stuffs, ISO 16654:2001, which is recommended for monitoring food and feed in the EU and elsewhere. It was reviewed and re-endorsed in 2012, with one amendment in 2017. This ISO method relies on the bacterium's phenotypic characteristics, such as growth in selective conditions and its biochemical and serological properties. A similar approach is applied by other methods considered as national standards, e.g. MLG 5.09, used by the USDA FSIS; MFHPB-10, used in Canada; or method No 164, used by the Scandinavian NMKL.

In brief, the first stage of ISO 16654:2001 involves enrichment in a selective medium, modified tryptone soy broth with novobiocin (or acriflavine for milk and dairy products). This medium is a minimally selective broth that gives a somewhat limited differential specificity favouring isolation of *E. coli* O157, as opposed to other Gram-negative bacteria. For the isolation of stressed *E. coli* O157, pre-enrichment in a non-selective broth is necessary, e.g. Buffered Peptone Water (BPW). An incubation temperature of 41–42 °C further enhances selectivity.

The second stage is an immunomagnetic separation (IMS) procedure which is carried out at six hours of incubation and again, if necessary, at 12 to 18 hours of incubation using commercially available immunomagnetic beads coated with antibodies specific to *E. coli* O157. IMS increases sensitivity by concentrating *E. coli* O157 relative to background microflora, which may overgrow or mimic *E. coli* O157 cells on selective agars.

The third stage is plating out the magnetic beads onto two selective agar media. The most widely used plating medium for the isolation of typical non-sorbitol-fermenting strains of *E. coli* O157 is Sorbitol MacConkey Agar with Cefixime and Tellurite (CT-SMAC). The plates are then incubated for 18 to 24 hours at 37 °C and examined for typical colonies. Colonies of typical non-sorbitol-fermenting strains of *E. coli* O157 are colourless on CT-SMAC. As some strains are sensitive to tellurite and/or are sorbitol-fermenting, the use of a second isolation agar, such as one of the newer chromogenic media, is recommended.

The final stage is the confirmation of the isolated colonies. Up to five typical colonies from each agar plate are streaked onto a non-selective agar plate, usually nutrient agar, and further tested for indole formation to confirm that the isolates are *E. coli*. Confirmation of *E. coli* O157 can be done serologically using a slide agglutination test with antiserum specific to *E. coli* O157. It is important to emphasise that this method does not distinguish between pathogenic and non-pathogenic *E. coli* O157. Further characterisation such as detection of pathogenic characteristics and typing should be done at a reference laboratory. This requires using a PCR method to detect the genes necessary for toxin production (*stx*1 and *stx*2) in the *E. coli* O157 isolates.

2.2 PCR-based detection of STEC O157 and non-O157 in food

Since there is a huge variation of biochemical and phenotypic characteristics among STEC strains, the PCR-based methods for detection of non-O157 strains are primarily based on the single common feature that characterises all STEC strains, i.e. the presence of virulence genes (*stx*1 and/or *stx*2) or the Shiga toxin encoded by those genes (Stx1 and/or Stx2). The most common approach is to incorporate a screening step based on PCR detection of the virulence and the O-serogroup genes after enrichment of the sample in broth. Such a screening method does not result in an isolate unless the enriched broth is spread on agar plates. In addition to the advantages of obtaining isolates for epidemiological studies and unequivocal confirmation, the isolation-based methods are particularly important for STEC detection in food samples in order to allow confirmation at colony level of the virulence genes in the isolated *E. coli* strain. This is because a sample can potentially contain a mix of dead and live bacteria, phages, and free DNA, and the virulence determinants and O-serogroup determinants that cause the positive PCR screening reactions can be harboured in separate organisms.

Internationally recognised methods for STEC detection have been published since 2012. There is an ISO horizontal method, ISO/TS 13136:2012 (which is currently under review), for the detection of STEC (any serotype) and the determination of O157, O111, O26, O103, O145 and O104:H4 serogroups. This is the prescribed method for the testing of STEC in sprouts under the EU regulation on microbiological criteria for foodstuffs (Commission Regulation (EC) No 2073/2005). The USDA FSIS prescribes the use of the standard method, MLG 5B.05, to detect and isolate its 'top six' major non-O157 STEC serogroups (O26, O45, O103, O111, O121 and O145) in meat products, carcases, and environmental sponges (the method has also been extended for use on raw ground beef mixed with raw pork and/or raw poultry products), which is to be used in combination with the MLG 5.09 method for O157:H7.

The first step in ISO/TS 13136:2012 is an enrichment of the sample (Figure 6). Both the ISO and the USDA FSIS methods proceed afterwards with real-time PCR-based screening procedures of DNA extracts from the enriched buffer. PCRs for *stx* and *eae* virulence genes and for the top serogroups O-antigen genes are usually carried out in sequential steps.

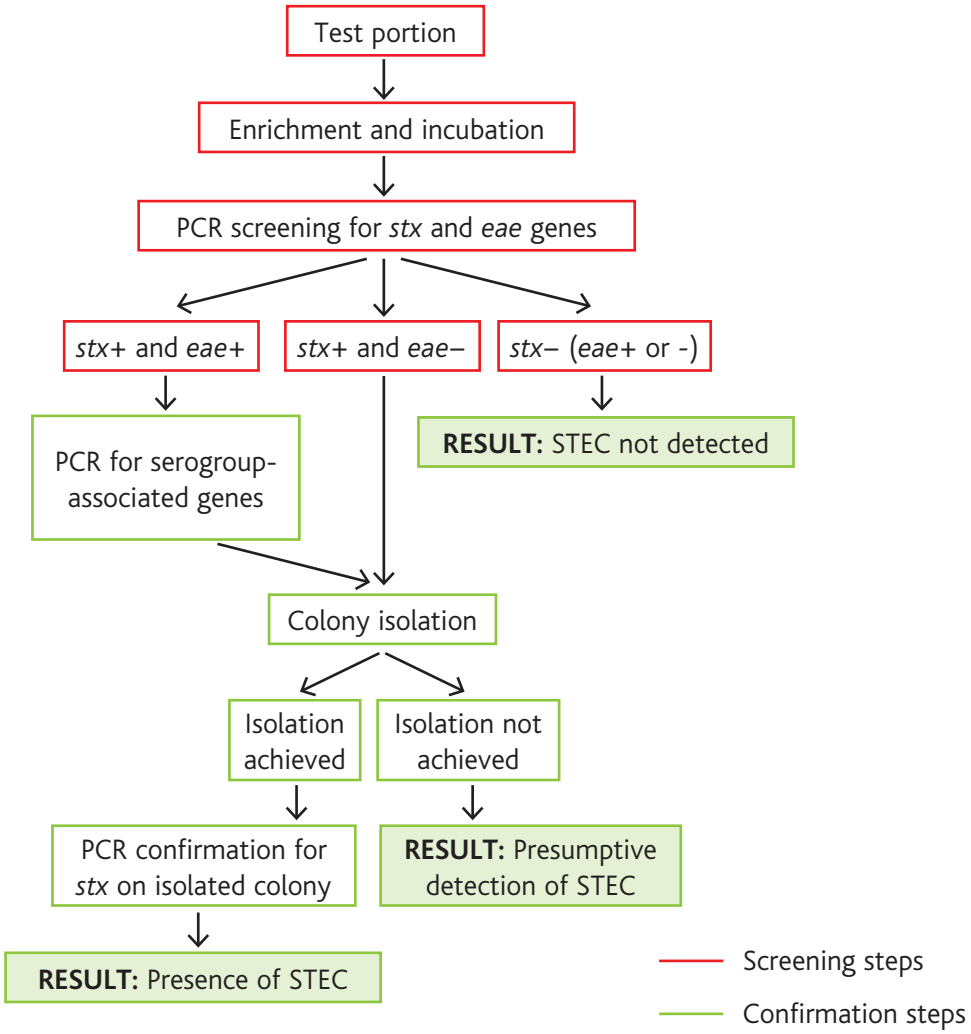
After a negative PCR reaction for the *stx* gene(s), the procedure is stopped and the result is reported as 'STEC not detected' in the test portion of the sample analysed. If the PCR reaction is positive for the *stx* gene(s), the laboratory will normally proceed with additional PCRs for *eae* and the serogroup and with cultural isolation. The isolation of presumptive STEC may be attempted regardless of the *eae* and O-serogroup PCR screening result.

Culture isolation of presumptive STEC from the *stx*-positive screening enrichments proceeds by plating onto selective agar plates, which might be preceded by IMS with serogroup-specific antibodies in those samples where the O-serogroup has previously been identified by PCR. Knowledge of the O-serogroup can also be exploited to inform the choice of selective media. In addition to the serogroup O157 selective media mentioned in Section 2.1, a few media that are selective for other *E. coli* serogroups have been developed, such as a modified CT-SMAC medium for *E. coli* serogroup O26 that uses rhamnose instead of sorbitol, as have several other *E. coli* chromogenic media. *E. coli* media, such as MacConkey Agar or Tryptone Bile X-Glucuronide (TBX), are used at this stage to allow the growth of a wide range of *E. coli* strains.

Following incubation, the plates are examined in order to select colonies for confirmation. This step requires considerable dexterity and patience, as each type of colony (both typical and atypical) seen on the plate – or up to 50 colonies, according to the ISO standard – has to be checked before ruling out the presence of STEC in the sample. The ISO method directs that PCRs for *stx* gene(s), the *eae* gene and/or serogroup genes, as required, are carried out in pools of 10 colonies and in individual colonies. The USDA FSIS method recommends the use of latex agglutination to select positive colonies to the O-serogroup of interest, which must later be confirmed to contain *stx* gene(s) or produce the Shiga toxin.

In any method, the confirmation of the target markers in a pure culture derived from one single colony is necessary in order to report the sample as positive for the presence of an STEC strain (Figure 6).

Figure 6 Simplified ISO/TS 13136:2012 flow diagram



2.3 Discrepancies between PCR screening and culture-confirmed results for STEC in food

Isolation by testing individual colonies or pools of up to 10 colonies is laborious, time-consuming and often unsuccessful. This is why cultural methods for the enrichment, isolation and confirmation of STEC are still evolving.

The failure to confirm the presence of STEC in a sample after a PCR-positive *stx* screening could be attributed to the presence in the sample of free phages, dead cells, or non-culturable but viable STEC strains. It could also be interpreted as meaning that the PCR screen reactions were caused by the presence of the virulence determinants or O-serogroup determinants in separate organisms. The inconsistency in the results could also be attributed to a false negative confirmation caused by the outgrowing of STEC by other *E. coli* during the enrichment step.

The possibility of using alternative PCR targets that are more linked to human pathogens, e.g. the intimin subvariants, has been proposed to reduce the number of samples screening positive using the O-serogroups PCR. The introduction of a step to remove dead DNA is also incorporated in some commercial kits, and this is aimed towards reducing the rate of presumptive positive samples.

As of summer 2017, a revision of the ISO/TS 13136:2012 method is under way, which is trying to address the following:

- Problems with the enrichment medium. It is known that most non-O157 *E. coli* show inhibition when grown in the presence of supplements such as novobiocin or acriflavine. This is why the use of a low-nutrient, non-selective enrichment medium such as BPW is the preferred option to resuscitate very or slightly stressed *E. coli* cells.
- Lack of inclusion of some *stx* gene subtypes. In particular, *stx2f* has been reported as not being detected by ISO primers.
- Possible addition of enteroaggregative *E. coli* (EAEC) virulence genes to the screening PCR step. Although EAEC is primarily non-zoonotic in origin, and transmission mainly occurs by person-to-person spread, the contamination of foods by asymptomatic carriers can also occur.
- Inclusion of a protocol for spent irrigation water from sprouting seeds.
- Improvement in isolation rate by introducing a step to eliminate background flora:
    - Acid treatment: treatment with a low pH (2.0) solution for one hour applied independently from IMS or as a post-IMS treatment.
    - Dilution of the enrichment medium before plating.

2.4 Alternative methods for the detection and isolation of STEC

A number of rapid methods for the detection and isolation of *E. coli* O157 are available as commercial test kits and systems. Most are aimed at the food industry and are designed for testing minced beef and sprouts, but can also be applied to other foods. Many of the commercial products currently available for *E. coli* O157 are based on immunoassay technology. Results are available in 20 to 26 hours following an 18- to 24-hour enrichment step. Fully automated immunoassay systems are also available. Adding an immunoconcentration step to the method can reduce incubation times and improve sensitivity. The concentrated sample can then be tested using an immunoassay or other detection method.

Several manufacturers have developed immunoassay-based technology to produce very simple-to-use immunochromatographic lateral flow assays. These are usually supplied with an enrichment broth and typically claim to provide a clear visual presence or absence result in 10 to 15 minutes after an enrichment step of only eight hours. A significant number of commercial test kits and systems detecting STEC O157 using PCR technology have been launched since 2004, many of which include real-time detection. Using proprietary media and/or IMS, the preliminary enrichment step can be reduced to as little as six to eight hours. Results of the PCR assay are then available within a further four hours.

Several PCR-based kits designed to detect the top US serogroups or the top EU serogroups in meat products or in sprouts are now available. These include the Pall Corporation GeneDisc® Plates – Food Pathogen Detection system; the Hunter® system from InstantLabs Inc.; the iQ Check by Bio-Rad; the BIOTECON Foodproof® test kit, which includes serogroup O104; and the EHEC GENE-UP PCR Kit by bioMérieux, which combines *stx*, *eae* and the 'top six' serogroups in one kit.

2.5 Whole genome sequencing

Currently, the most commonly used strategies to differentiate bacterial strains and to characterise them are molecular-based subtyping such as PFGE, multilocus sequence typing (MLST), and other PCR-based subtyping methods. These methods provide DNA sequence data or banding patterns, known as molecular fingerprints, for each bacterium being studied. WGS is now being implemented as the next-generation subtyping tool for microbial tracking by many international laboratories – including the USDA, the US Food and Drug Administration (FDA), the US Centers for Disease Control and Prevention (CDC), and Public Health England (PHE), among others – and has now been introduced in Ireland by the Department of Agriculture, Food and the Marine (DAFM) laboratories and by the National VTEC Reference Laboratory (VTEC NRL) of the Health Service Executive (HSE). WGS reveals the complete DNA composition of a bacterium, enabling a better understanding of those variations contained in the bacterial genome, both within and between species, allowing for the differentiation between organisms with a precision that other technologies cannot match. WGS also provides data on the presence and absence of a wide range of virulence genes encoding toxins, adherence and invasion mechanisms. As this technology advances and is more widely applied, it will support both routine surveillance and outbreak investigations, as well as risk management actions, although it will also create challenges in assessing the potential risk to human health from atypical strains. For sucessful implementation and standardisation of WGS, it will be critical to establish bioinformatics pipelines that are capable of assembling, annotating and interpreting the large datasets generated. A coordinated international approach (Franz *et al*., 2014; Oulas *et al*., 2015) and databases will be required. Efforts towards this include the 100K Foodborne Pathogen Genome Project (Weimer, 2017), the GenomeTrakr Network (FDA), the Global Microbial Identifier, Advanced Molecular Detection (CDC), and the joint EFSA/ECDC molecular typing database, which will include WGS in the near future.

3. STEC OCCURRENCE IN FOOD

As STEC has continued to evolve from a public health stance, the methodology to detect and identify STEC in food has also changed in order to enable detection of clinically relevant strains. Methods changed from a culture method specifically designed for *E. coli* O157 (toxigenic and non-toxigenic) (ISO 16654:2001) in the 1990s to a PCR method combined with culture in 2012 (ISO/TS 13136:2012) for detection of STEC belonging to the 'top six' serogroups. There is still no standardised method to detect other STEC serogroups outside the 'top six' in food. This has resulted in historical data on the types of strains of STEC in circulation in animals, the environment, and food being biased towards methods which were then available, with the vast majority of data relating to *E. coli* O157, limited data relating to the 'top six' serogroups, and very significant knowledge gaps on the prevalence and diversity of other types of STEC in food.

3.1 STEC occurrence in ready-to-eat foods

Ready-to-eat (RTE) foods are defined by Commission Regulation (EC) No 2073/2005, as amended, as "food intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern." Fresh produce that is labelled with an instruction to wash before consumption, but is consumed without cooking, is considered RTE. Although washing can reduce microbial contamination on the plant surface, it is not effective in eliminating microorganisms of concern or reducing them to an acceptable level because some pathogens can adhere strongly to the surface or become internalised within the plant tissue.

RTE foods more commonly associated with STEC contamination include raw or undercooked ground beef, raw drinking milk and raw milk dairy products, fresh produce, and sprouted seeds. Water intentionally incorporated into a food during its manufacture, preparation or treatment is included under the definition of 'food' in Article 2 of Regulation (EC) No 178/2002. In 2016, the FSAI published a leaflet describing best practice for sourcing water for the irrigation of fresh produce (FSAI, 2016). Drinking water can also be considered an RTE food, as it is intended for direct human consumption without the need for cooking or other processing. In Ireland, untreated or poorly treated private drinking water supplies have been repeatedly highlighted as a concern in relation to STEC infection (Garvey *et al*., 2016), and among STEC cases reported in Ireland in 2015, 33% reported exposure to private well water (HPSC, 2016).

Appendix 4 outlines data on the prevalence of STEC in RTE fresh produce in selected Irish and international studies. In the wake of the 2011 *E. coli* O104:H4 outbreak linked to sprouted seeds from Egypt, the EFSA adopted a scientific opinion on the risk posed by STEC and other pathogenic bacteria in seeds and sprouted seeds (EFSA, 2011). It concluded that the contamination of dry seeds with bacterial pathogens is the most likely initial source of the sprout-associated outbreak and that, due to the high humidity and the favourable temperature during sprouting, bacterial pathogens present on dry seeds can multiply during sprouting and result in a public health risk (EFSA, 2011). In order to mitigate the identified risks, it was considered necessary to introduce additional requirements for sprouted seeds. This resulted in four Commission Regulations to cover the import (Commission Regulation (EU) No 211/2013), traceability (Commission Implementing Regulation (EU) No 208/2013), microbiological criteria (Commission Regulation (EU) No 209/2013), and approval of establishments producing sprouts (Commission Regulation (EU) No 210/2013).

It is well recognised that STEC may be present in the faeces of dairy animals and, as a result, milk can become contaminated with these bacteria. Studies on STEC in raw milk in Ireland have generally focused on the examination of bulk tank milk filters, and in those studies, the presence of STEC O157 in filters ranged from 0% to 3.1% and the presence of STEC O26 ranged from 0% to 6.3%. The isolates recovered had varying virulence profiles (see **Appendix 5**).

A number of studies (**Appendix 6**) have investigated how *stx*-negative *E. coli* and STEC, if present in raw milk, would survive during the manufacture and ripening of various cheese varieties. In general, the studies show growth of STEC during manufacture and a gradual decline during ripening, but in most cases STEC was still detectable at the end of the ripening period. Results from international studies show that presumptive detection rates of STEC in different cheese types using PCR screening are much higher than detection rates of *stx*-positive *E. coli* by culture in the same samples (**Appendix 7**).

In the *European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015*, data on STEC in food were reported by 20 MSs, Iceland, and Switzerland, for a total of 20,886 samples (EFSA and ECDC, 2016). A large spectrum of STEC serogroups was reported in food, with STEC O157 being the most frequent (45%, 271/602 positive samples for STEC). It is important to note that results from different investigations may not be directly comparable when comparing STEC data across European countries due to the differences in sampling strategies and analytical methods (PCR or culture) applied. Table 9 summarises data on STEC in selected RTE foods from the 2015 report by the EFSA and the ECDC.

Table 9 Prevalence of STEC in selected RTE food categories[a]

Food category	Number of single samples tested	Number of STEC-positive samples (%)
Milk (other than raw milk) and dairy products	2,718[b]	73 (2.7%)
Raw milk	1,472	25 (1.7%)
Fruit and vegetables	1,330	2 (0.15%)
Sprouted seeds	925	2 (0.22%)

[a] Data source: Adapted from the *European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2015*.

[b] The samples were mainly collected from cheese (84.5%), followed by milk (10.8%) and other types of dairy products (4.7%).

3.2 STEC occurrence in non-RTE foods

Food that the producer, manufacturer or packer intends to be given a treatment before consumption, which would lower the risk from STEC, is considered non-RTE. Cooking food so that it reaches a core temperature of a minimum of 75 °C (or equivalent temperature and time combinations, e.g. 70 °C for two minutes) is considered to be thorough cooking, as it is effective in eliminating microorganisms of concern or reducing them to an acceptable level (FSAI, 2006, 2018a, 2018b). This type of cooking is an effective intervention for reducing and eliminating pathogenic microorganisms such as STEC in meat. If whole, intact red meat such as steak or a joint is contaminated with STEC, the pathogen will be on the surface of the meat, whereas the inside of meat cuts is generally considered to be pathogen-free. Therefore, cooking for such intact meats only needs to inactivate pathogens on the meat surface. However, when beef cuts are processed into minced beef, or when the whole meat is tenderised using blades/needles or when injected, it can lead to transfer of pathogens from the surface into the centre of the product (Corliss *et al*., 2015), and so the cooking needs to inactivate pathogens which may be on the inside of the meat. The heat resistance of STEC will be affected by factors such as intraspecific differences and food characteristics, including formulation (e.g. levels of fat and salt), pH, and water activity. Nevertheless, the application of a thermal treatment so that the food reaches a core temperature of a minimum of 75 °C (equivalent to 70 °C for two minutes) is scientifically validated to produce a safe product. This is captured in a recent report by the FSAI Scientific Committee where equivalent time-temperature combinations for thoroughly cooking beef burgers are presented (FSAI, 2018b); in the case of other foods, equivalent time-temperature combinations are available in FSAI Guidance Note No. 20 (FSAI, 2006).

Differences in culinary beef cooking and consumption habits across geographical regions may also have an impact on the risk posed by STEC contamination in beef products. An Ipsos MRBI survey commissioned by *safe*food (Corcoran and Porter, 2017) and conducted in December 2016 and January 2017 found that, in the Republic of Ireland (n=504 respondents), 327 people questioned expressed a preference for well-done burgers when dining out (64.9%), 67 for medium-well (13.3%), 26 for medium (5.2%), 16 for medium-rare (3.2%) and 2 for rare (0.4%). Sixty-six respondents (13%) said that they did not eat burgers. Within the Dublin region (n=147), 84 (57.2%) expressed a preference for well-done, 26 (17.7%) for medium-well, 10 (6.8%) for medium, nine (6.1%) for medium-rare and one (0.69%) for rare. Overall, 86 respondents thought that rare burgers were safe to eat (17.1%). There appears to be a growing trend towards increasing consumption of rare or undercooked beef burgers (Mintel Group Ltd., 2016). A recent study in the United Kingdom (UK) (FSA, 2015) found that while the majority of UK consumers (68%) did not eat rare burgers, 11% did so at least once a month. In 2016, there was an outbreak of STEC O157 associated with a restaurant in Ireland serving undercooked burgers (HPSC, 2017). Despite the increasing trend for the serving of undercooked beef burgers in restaurants, major gaps have been identified in food server knowledge and risk communication both verbally by restaurant staff and via consumer advisory messages on menus which would allow consumers to make informed food safety decisions (Thomas *et al*., 2016). In Ireland, placing a disclaimer notice on a menu which advises of the dangers of consuming undercooked minced meat does not exempt FBOs from their obligation under food law to only serve safe food (FSAI, 2018a). In this context it is recommended that education campaigns are run periodically at national level for both consumers and FBOs in order to raise awareness of the risk of eating or serving undercooked minced beef.

It is acknowledged that the UK has a different approach. In May 2016, the UK Food Standards Agency (FSA) published *The safe production of beef burgers in catering establishments: advice for food business operators and LA officers* (FSA, 2016). That document states that the serving of burgers which are not thoroughly cooked is only acceptable when there are controls in place which involve: (i) steps throughout the supply chain to minimise and/or reduce the risk of contamination of meat used to make burgers, (ii) a process or processes which achieve a minimum reduction of 4 $\log_{10}$ of the harmful bacteria initially present in food (equivalent to killing 99.99% of bacteria), and (iii) messages that inform consumers of the potential risks from burgers that are not thoroughly cooked. The document also states that "burgers that are less than thoroughly cooked should not be served to children and there should be information available to other potentially vulnerable people about the risks before they order a burger to ensure they can make an informed choice."

There is no legal requirement to test raw meat for STEC in the EU, although some FBOs may still test for STEC in order to meet customer requirements or to meet microbiological criteria in countries to which Irish food businesses are exporting. One example of this arises from Ireland's approval from the US authorities to export Irish beef to the US market since January 2015. Irish beef must comply with STEC testing requirements and procedures as agreed by the USDA and the DAFM in order to ensure access to the US market. The USDA FSIS considers raw, non-intact beef products (e.g. minced or diced beef) or the components of these products to be adulterated if found to have *E. coli* O157:H7, or any of the following six non-O157 STEC serogroups: O26, O45, O103, O111, O121 and O145 (Federal Register, 2012). The USDA FSIS began verification testing for these non-O157 STEC serogroups in domestic and imported beef manufacturing trimmings from cattle slaughtered on or after 4 June 2012. In order to meet these requirements, testing of Irish beef for export to the US market began in 2016, presenting challenges to the competent authorities in Ireland on how to manage non-compliances if and when they arise.

A number of research studies conducted in Ireland on carcases and raw meat (beef, lamb and pig) at abattoirs and retail outlets indicate that STEC O157 and other serogroups may be present at a low prevalence rate (0.9% to 3.0%), and these isolates had a wide diversity in terms of the presence and absence of virulence genes detected (see **Appendix 8**).

In 2014, three STEC-positive samples were reported (two in bovine raw meat products and one in raw minced beef meat) from a total of 309 meat samples tested under the official control testing in Ireland (FSAI, 2014). The serotype for those three positive samples was not determined. In 2016, official control testing on raw beef meat preparations from supervised DAFM establishments yielded three positive STEC isolates (O26, O157 and O145) out of 175 samples tested. Other official control testing samples consist of carcase swabs and trims of meat intended for grinding collected at approved USDA plants.

Table 10 summarises data on STEC in selected non-RTE meat foods. The data were extracted from the *European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015* (EFSA and ECDC, 2016).

Table 10 Prevalence of STEC in selected non-RTE food categories

Food category	Number of samples tested	STEC-positive samples (%)
Fresh ovine and caprine meat	532	65 (12.2%)
Other ruminant meat (deer)	31	3 (9.7%)
Fresh bovine meat	2,560	41 (1.6%)
Fresh meat from other animals[a]	355	4 (1.1%)

[a] Including meat from horse, rabbit, pig, wild boar and poultry.

Data source: Adapted from the *European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2015* (EFSA and ECDC, 2016).

3.3 Isolation of enteropathogenic *E. coli* when testing for STEC in food

Data in **Appendices 2, 4, 5, 8, 9** and **10** reflect the variety of serogroups and virulence profiles within *E. coli* isolates from clinical samples and from a range of food and food-producing animals, which poses a substantial challenge in assessing the risk posed by such strains from a public health perspective.

As highlighted in Section 2, methods applied to assess the occurrence of *E. coli* and STEC in food have traditionally been serogroup focused, originally on O157 and subsequently on other top serogroups (O26, O103, O111 and O145), so the historical information available is biased towards these serogroups. In more recent years, a move to PCR-based screening methods for genes for *stx*, *eae* and selected serogroups (the so-called 'top five' or 'top six') has resulted in the subsequent isolation of: (i) some *E. coli* cultures which are *stx* positive but do not belong to the targeted serogroups, and (ii) enteropathogenic *E. coli* (EPEC) (*E. coli* with *eae* but no *stx* gene(s)) which may be of the targeted serogroups. This latter scenario arises following ISO/TS 13136 for the detection of STEC in food whereby an *eae*-positive but *stx*-negative *E. coli* (i.e. EPEC) is confirmed in a food sample that was originally screened as *stx* positive by PCR (presumptive STEC detection). In fact, EPEC strains have been the subject of RASFF alerts by some MSs in Europe (see **Appendix 3**).

This raises the question of whether the EPEC isolates are derivatives of STEC that have lost their Stx-encoding phage (containing the *stx* gene(s)) and also raises the question of whether there is potential for an EPEC strain to acquire the phage during storage of the food prior to consumption. As discussed in Section 1.2, the phage can convert between a lytic and lysogenic state, and in the lytic state the phage is free and could potentially transfer into another bacterium, resulting in the emergence of a pathogenic STEC strain or, for example, the conversion of an EPEC to an STEC. However, from current scientific evidence summarised below, it is concluded that this scenario is more likely in an evolutionary context and, although plausible, the acquisition of an Stx-encoding phage is a rare event under typical food conditions.

In summarising the likelihood for an STEC to lose an Stx-encoding phage, some culture-based studies have shown that STEC O26 may lose Stx-encoding phages both spontaneously and in the presence of an inducing agent (mitomycin C) (Bielaszewska *et al*., 2007; Bonanno *et al*., 2016). The loss of *stx* genes and potential for genomic rearrangement for the conversion of STEC into EPEC has also been documented *in vivo* in patients with haemolytic uraemic syndrome (HUS) (Mellmann *et al*., 2005; Bielaszewska *et al*., 2007). However, studies in this regard in food matrices are very limited, as are studies on whether stress conditions to which STEC cells are exposed during either food production or during isolation/detection methodology could induce the phage into the lytic cycle with the release of free phages.

A study by Bonanno *et al*. (2017) tested whether phage induction and release could occur after exposure to the selective agents used in the analytical enrichment and detection procedure for STEC detection in food (ISO/TS 13136), including novobiocin, acriflavine, cefixime-tellurite, and bile salts. The authors observed no significant effect on phage induction when these chemical agents were tested separately. Although a combination of these parameters and the effect of incubation temperatures was not studied, the authors concluded that the lack of phage induction suggests that EPEC isolates from *stx*-positive food samples are unlikely to have been derived from STEC by a loss of their phage during the enrichment or isolation procedures.

In addition, Fang *et al*. (2017) evaluated the effect of stressors related to food preservation – including heat, pressure, oxidative (hydrogen peroxide) and acid (lactic acid and hydrochloric acid (HCl)) stress – on the induction of the Stx2-encoding prophage and expression of *stx*2 in STEC O104:H4. It was demonstrated that neither pressure (200 megapascal for seven minutes) nor heat (50 °C for three hours) induced the prophage. The expression of *stx*2 was, however, induced by pH 2.5 (broth acidified with HCl) and pH 3.5 (broth acidified with lactic acid), but low pH did not result in the release of infectious phage particles. Similarly, Bonanno *et al*. (2017) evaluated the influence of different physicochemical parameters related to the cheese-making process on inducing Stx-encoding phages in STEC O26:H11 into the lytic cycle. The authors demonstrated that oxidative stress (H_2O_2 at 3 mM) and, to a lesser extent, salt stress (NaCl at <3%) had the ability to induce Stx-encoding phages. When tested in real cheese-making conditions – where cheeses were produced using milk inoculated with an *stx*1- or *stx*2-positive STEC O26:H11 strain – induction of the phage was observed through the detection of free Stx1 and Stx2 phages in 3/48 and 7/48 samples, respectively, collected at various time points during cheese production (6 hours, 1 day, 8 days, and 28 days). Further research to understand the influence of food-processing-related stressors on Stx-prophage induction is needed in order to facilitate the control of STEC in food systems by minimising prophage induction during food production and storage.

It is noted that a free *stx* phage in itself does not pose a human health risk, as a phage requires the transcriptional and translational machinery of a bacterial cell in order to replicate and reproduce Stx (Martínez-Castillo *et al*., 2013; Krüger and Lucchesi, 2015). Even assuming that the STEC had been induced into the lytic cycle in the food, the phage replication rate would be expected to be very low at chilled storage temperatures (Rode *et al*., 2011; Picozzi *et al*., 2012; Martínez-Castillo and Muniesa, 2014).

However, the presence of such free phage particles raises the possibility that these phages could infect (lysogenise) other *E. coli* strains (e.g. EPEC) and convert them into STEC. To date, there are very few studies on the potential for Stx-encoding phage transfer between *E. coli* in food matrices. Imamovic *et al*. (2009) observed that phage-mediated transfer of the *stx*2 gene to non-toxigenic *E. coli* occurred in water, ultra-high-temperature-processed (UHT) whole milk, and minced beef or salad diluted 1:4 (weight/volume) in Ringer's solution. Nonetheless, the authors indicated that the optimal conditions in which the experiments were carried out (incubation time and temperature) may not be encountered outside the laboratory and that the number of donor and recipient *E. coli* strains needed for phage-mediated transfer and lysogenisation were too high to be expected in a food sample. The authors concluded that although phage-mediated transfer can take place under the appropriate conditions, it is unlikely to occur in food, and thus it does not seem to be a high-risk method of *stx* gene(s) transmission.

In parallel, lysogenisation did not occur either in pH 3.7 orange juice, which was attributed to the acidic pH limiting the growth of (the recipient) *E. coli* strains, or when the food samples were kept at 4 °C, due to the rigidity of cell membranes at low temperatures preventing phage infection (Imamovic *et al*., 2009). These results were further confirmed by Picozzi *et al*. (2012) using UHT milk. More recently, a study by Nyambe *et al*. (2017) investigated whether lysogenisation of different *E. coli* pathotypes with Stx2-encoding phages would occur in laboratory broth, milk, and ground beef mix, but this only occurred when the number of donor and recipient *E. coli* cells was $>10^3$ CFU/mL (or g), followed by an incubation for 18 hours at 37 °C (optimum donor and recipient strain growth temperature). The authors concluded that under typical conditions of food storage, Stx2-encoding phage transfer into other *E. coli* would be a rare event.

A recent opinion from the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) has also concluded that the loss and uptake of *stx* gene(s) is a rare event (ANSES, 2017). Thus, the detection of an EPEC in a food is not an indicator of risk for an STEC to emerge from Stx-encoding phage uptake in the food.

However, it is noted that the presence of a free Stx-encoding phage in a food could result in an *stx*-positive PCR screening for STEC, but public health risk cannot be assessed based on detection of *stx* gene(s) by molecular methods only (i.e. a positive PCR result/presumptive positive). This scenario would therefore not impact on the culture-confirmed STEC result, unless there were additional information indicating a public health risk or non-compliance.

3.4 Potential presence of *stx* gene(s) in non-*E. coli* bacteria in food

The genus *Hafnia* belongs to the Enterobacteriaceae family. The latest version of the *Taxonomic Outline of Bacteria and Archaea* (Garrity *et al*., 2007) indicates that the genus only contains one species, *Hafnia alvei*; however, based on DNA relatedness and 16S ribosomal ribonucleic acid (rRNA) gene sequencing studies, Huys *et al*. (2010) proposed the inclusion of a new species, *Hafnia paralvei*. *Hafnia* spp. are a group of commensal bacteria, part of the intestinal flora of humans and animals, which have been reported as opportunistic bacteria in humans that may cause a range of infections associated with underlying illnesses or predisposing factors, such as immunocompromised patients (Janda and Abbott, 2006; Padilla *et al*., 2015). At present, there is very little epidemiological, clinical and laboratory data to support *Hafnia* as a cause of human gastroenteritis.

Hafnia spp. are frequently detected in milk or smear cheeses, and these species have been shown to contribute to the flavour properties of the cheeses (Irlinger *et al*., 2012). They can be naturally present in raw milk used to manufacture cheese or they can be used as starter cultures, particularly *H. alvei,* which is deliberately added as part of the cheese-making process after pasteurisation in order to reintroduce a flora of organisms normally found in raw milk. This results in flavour profiles in soft cheeses made from pasteurised milk that are typical of cheeses made with raw milk (Australian Specialist Cheesemakers' Association, 2016). While most *Hafnia* spp. are considered to have the status of 'generally recognised as safe' (GRAS) due to a documented history of use in fermented foods (Bourdichon *et al*., 2012), they have not been assigned a qualified presumption of safety (QPS) status by the EFSA.

While it is plausible that an Enterobacteriaceae spp. such as *Hafnia* could take up free *stx* phages, there is no evidence to support this, and further research with donor and recipient strains would be needed, as well as further investigation of whether a phage that is incorporated into a *Hafnia* strain would be maintained in a lysogenic or lytic cycle in the cell. Some studies dating back to the late 1990s have reported that *Hafnia* isolates from food were *stx* positive by PCR screening on initial testing, but were subsequently found to be negative on a repeat test performed after three months' storage at −80 °C (Lindberg *et al*., 1998). A further study from 1999 reported that four out of five Shiga-like toxin-producing isolates from raw milk cheese samples were identified as *H. alvei* using biochemical identification (API 20E, bioMérieux) and complementary tests (indole production, Klieger test, ß-glucuronidase activity) (Vivegnis *et al*., 1999). However, considering the advancements in molecular typing since 1999, it is reasonable to think that current techniques may now identify those strains as another species in light of the considerable controversy in the literature regarding the enteropathogenicity of *Hafnia* spp. based on many reported

misidentifications of *Escherichia albertii* strains as *H. alvei* (Abbott *et al*., 2003; Huys *et al*., 2003; Janda and Abbott, 2006). Nonetheless, where starter cultures of *Hafnia* spp. are to be deliberately added to an RTE food, for example a fermented dairy or meat product, it would be good practice to initially screen for the presence of *stx* gene(s) and ensure their absence in order to prevent a presumptive detection for STEC (positive PCR screening) when following ISO/TS 13136. In terms of any scientific evidence for a link between *stx*-positive *Hafnia* and human illness, a study by Abbott *et al*. (2011), which investigated strains of *Hafnia* spp. of clinical origin (n=32) by screening for cytotoxic activity on Vero cells, observed a characteristic cytopathic effect similar to that of STEC in 63% of the *H. alvei* and *H. paralvei* strains tested. However, the study did not indicate whether those strains contained the *stx* genes(s) or not. A case of HUS attributed to a *H. alvei* strain active on Vero cells was reported, but the toxin appeared to be immunologically unrelated to Stx1 and Stx2 (Crandall *et al*., 2006). A recent study by Wang *et al*. (2016) reported that *H. alvei* ATCC 29926 was negative for the *stx* gene(s). Therefore, it is concluded that there is, at present, no evidence to indicate that *stx*-positive *Hafnia* strains can cause human illness or pose a risk to human health.

3.5 STEC in food-producing animals

It is well recognised that STEC may be carried in the gastrointestinal tracts of food-producing animals and shed in faeces, thus presenting a source of contamination for the food chain, in particular for meat, dairy and horticultural foods. Since the 1990s, a number of studies have been conducted in Ireland on the shedding of *E. coli* (*stx*–) and STEC by food-producing animals and contamination levels on the animal hide and fleece, which are recognised as the key source of pathogen contamination on animals entering abattoirs. These studies are summarised in **Appendices 9** and **10**. The majority of the earlier studies focused on detecting the O157 serotype only. This research may have been limited solely to the identification of STEC O157:H7, as it was the serotype most commonly associated with outbreaks of STEC infection at the time, and due to the restricted capability of the laboratory methods used to detect non-O157 STEC. A greater proportion of non-O157 STEC serotypes are reported in more recent Irish studies, due to the increased use of methodologies which can detect any STEC regardless of the serotype.

The results of the earlier Irish studies which solely investigated the prevalence of STEC O157 solely indicate that the average rate of shedding of *E. coli* O157 in cattle was 3.5% (ranging from 0.66% to 7.6%). More recent studies which focused on other serogroups (e.g. O26, O103, O111 and O145) indicated that these serogroups are less frequent, and in these studies the prevalence ranged from not detected to 1.5%. A smaller number of Irish studies investigated the prevalence of all STEC serogroups in food-producing animals and found a wide variety of different serogroups present. Only two studies in Ireland have focused on the shedding of STEC by ovine animals, and both were focused on O157, which was present in 5.8% of samples in one study (Prendergast *et al*., 2011) and absent in the other (Lenahan *et al*., 2007). One study showed low-level shedding of O157 by pigs (0.63%) (Lenahan *et al*., 2009).

Among all serogroups from all animal hosts, a wide diversity in the presence of virulence-related genes was noted, with many isolates missing the typical virulence gene *eae*. The presence of serogroups without the *stx* gene(s) has also been documented. In 2003, McEvoy *et al*. reported that all 56 isolates recovered from four carcases that tested positive for *E. coli* O157:H7 (out of 36 carcases) were *stx* negative but *eae* positive. From 250 faecal, rumen and carcase samples examined a few months later, 18 *E. coli* O157:H7 isolates were recovered, and only one of them was *stx* negative (5.6%, n=1/18) (McEvoy *et al*., 2003). A study performed by Prendergast *et al*. (2011) in beef and sheep slaughter plants in Ireland found that 9 out of 65 *E. coli* O157 isolates were EPEC (i.e. *stx* negative but *eae* positive) (13.8%). Similarly, Lynch *et al*. (2012) isolated 67 serovar-positive *E. coli* (O26, O103, O111, O145 or O157) from rectal faecal swabs, milk filter, and bulk tank milk samples. From those, only 10 rectal faecal swabs were regarded as pathogenic, including four EPEC and six STEC. Murphy *et al*. (2016) reported three *stx*-negative O157 isolates (13%) from the 23 *E. coli* isolates that were confirmed as O157 out of 529 recto-anal swabs taken from two Irish dairy herds. Two Irish studies looked at the concentration of STEC shed by cattle, and reported that some beef and

dairy cattle are shedding O157 and O26 at exceptionally high levels (>10,000 CFU g^{-1}) (these animals are also called super-shedders), and such animals pose a significant risk to the food chain (McCabe *et al*., 2016; Murphy *et al*., 2016) (**Appendix 10**).

In the *European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015*, data on STEC in animals were provided by 11 MSs, 10 of which followed the EFSA technical specifications for the monitoring and reporting of STEC and adapted the standard methods of ISO/TS 13136:2012, ISO 16654:2001, NMKL 164:2005 and DIN 10167:2004-3 to test animal samples. In Ireland, STEC is not notifiable when detected in animals.

The presence of STEC was reported in 6.8% (n=467) of the 6,881 animal samples tested in 2015 by 11 MSs (EFSA and ECDC, 2016). The highest prevalence was reported in sheep and goats (18.5%), followed by cattle and pigs (8.3% each). The information on the STEC serogroups was only provided for 210 strains out of the total 467 positive samples; STEC O157 was the most common serogroup (69%). As for the non-O157 serogroups identified in STEC from animals, O2 was the most commonly reported, identified in more than 50% of the non-O157 isolates, followed by O1 (24.6% of the non-O157 isolates).

More specifically, in cattle (4,084 sample units of cattle tested including 3,321 animals, five herds and 758 slaughter batches from seven MSs), a total of five STEC serogroups were reported among the STEC-positive samples (O1, O2, O103, O121 and O157). STEC O157 was the most frequently reported serogroup, followed by serogroup O2. Only four MSs reported the results of STEC testing in sheep and goats; 19.8% (n=41/207) of sheep samples and 7.7% (n=2/26) of goat samples were positive for STEC. The highest prevalence of STEC was reported by Spain and was found in the caecal contents of sheep (53.7%, n=29/54). Only two MSs (Germany and Italy) reported results of STEC testing in pigs. Germany found that 10.1% (n=48/475) of pigs were positive for STEC; however, no information on the serogroup of the isolated strains was reported. In Italy, all 102 pigs tested were negative for STEC.

4. RISK ASSESSMENTS AND ACTIONS ON DETECTION OF STEC IN FOOD IN CERTAIN COUNTRIES

Following the 2011 *E. coli* O104:H4 outbreak linked to sprouted seeds, the EFSA published a Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment (EFSA BIOHAZ, 2013). This opinion acknowledged that it was not possible, at the time, to fully define human pathogenic STEC or identify factors for STEC that absolutely predict the potential to cause human disease. A molecular approach utilising genes encoding virulence characteristics additional to the presence of *stx* genes was proposed for the categorisation of the potential risk for consumers' health (Table 11). These 'risks' have been categorised as group I (high potential risk) through to group III (unknown risk).

Table 11 Proposed[a] molecular approach for the categorisation of VTEC (*vtx* present)

Group	Genes[b]	Serogroups	Potential risk[c]	
			Diarrhoea	HUS/HC[d]
I	*eae* positive or (*aaiC* and *aggR*) positive	O157, O26, O103, O145, O111, O104	High	High
II	*eae* positive or (*aaiC* and *aggR*) positive	Any other	High	Unknown
III	*eae* negative and (*aaiC* plus *aggR*) 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 The European Surveillance System (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.

Data source: Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment (EFSA BIOHAZ, 2013)

STEC strains falling under group I should be regarded as representing a high risk. For STEC that would fall under group II, there is still uncertainty about whether or not they are able to cause HUS, due to as yet unknown additional virulence mechanisms. For STEC that would fall under group III, there is uncertainty about whether or not they are able to cause disease and it is not possible 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 STEC strains according to risk. The EFSA scientific opinion highlights that this approach will need to be periodically revised in light of new epidemiological information and 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.

The classification of 'unknown' risk has caused a major challenge for regulators and FBOs in deciding on action to take when an STEC of an unusual serogroup or virulotype is recovered from food, particularly where it sits outside the 'top six' serogroups and does not have *eae* or *aaiC* and *aggR* genes.

In 2014, the EC attempted to introduce a harmonised approach to assessing and managing the risk of STEC based on the ESFA opinion. However, MSs failed to agree and the EC suspended this work in 2016. A number of individual EU MSs have now made their own risk assessments and policy decisions based on human epidemiology data relevant to their country.

Below is a summary of the STEC risk assessment and management approaches being taken in five MSs (Denmark, France, Germany, the Netherlands and the UK). This summary is based on a meeting between the STEC Working Group and experts from these MSs in September 2017.

4.1 United Kingdom

The UK Food Standards Agency (FSA) has a working position which has been agreed with Government and stakeholders but has not yet been officially finalised and published.

It is based on confirmed presence of STEC (i.e. one or more of the *stx* genes are detected in an isolate of an *E. coli* strain) followed by application of different risk management interventions based on food profile. It deems that public health risk cannot be assessed based on detection of *stx* genes by molecular methods alone (a positive PCR result/presumptive positive) with the exception of when there is additional information that would support further action e.g. where there is an indication of public health risk (e.g. cases of illness, epidemiological information) or non-compliance (e.g. ineffective food safety management systems), the detection of *stx* genes may be taken as contributing evidence to support an intervention.

The two profiles of food are:

- **RTE foods** and foods that are lightly cooked or not thoroughly cooked before consumption (e.g. burgers served 'pink').[6] The action required when the presence of STEC is confirmed (i.e. *stx*1 and/or *stx*2 detected in an isolate of an *E. coli* strain) is withdrawal and/or recall (under Article 14 of EC Regulation 178/2002 on the General Principles of Food Law), investigation of the source of the STEC contamination and a review of HACCP-based procedures. For example, in the case of detection of STEC in raw milk cheese, a review of the HACCP-based food safety management system would be required which might include sampling of various stages of the process to identify the source of contamination.

  If food is not yet at retail it could be diverted to an approved establishment for further processing to eliminate or reduce the risk to an acceptable level.

- **Foods that will be processed or cooked before consumption** so the risk from STEC is reduced to an acceptable level or eliminated. When the presence of STEC of a serogroup frequently associated with severe disease ('the top six') AND containing *eae* or *aggR*/*aaiC* is confirmed, this is considered on a case by case basis. A withdrawal or recall will not generally be required but there is flexibility to do so if additional information indicates this is necessary to protect public health. The FBO should provide assurances that the affected food will be processed appropriately prior to consumption. Labelling or documentation providing cooking/handling instructions can be presented as evidence here. If evidence cannot be provided a withdrawal/recall may be needed.

  Investigation of the source of the STEC contamination and a review of the HACCP-based food safety management system would be required. It would be expected that a business would make reasonable efforts to improve the hygiene of their process to prevent STEC contamination in future but it is accepted that elimination of STEC is not realistic or necessary in all cases as it is reasonable to expect food handlers at later stages in the chain to apply hygiene controls that will manage the risk.

[6] The FSA has issued advice to caterers wishing to serve burgers less than thoroughly cooked which outlines options for caterers to use in order to demonstrate that they have controlled the risks using measures other than thorough cooking (FSA, 2016).

4.2 The Netherlands

A Dutch policy document on STEC was approved in 2013 by the Food and Consumer Product Safety Authority and the Ministry of Public Health. Risk is classified based on virulence genes, serotype and risk profile of the food.

Foods are classified into 2 groups of risk (high and low) by evaluating the final preparation step of food by the consumer or food handler:

- **High-risk profile foods:** "Foods reasonably expected for direct human consumption without the need for cooking or other processing effective to eliminate STEC or reduce it to an acceptable level (e.g. RTE food, rare or undercooked beef)". In such foods, any living (viable) STEC (*stx*1+ and/or *stx*2+) isolated in 25 g of food make the food unsafe and action (i.e. withdrawal and/or recall and corrective measures) is required.
- **Low-risk profile foods:** "Foods reasonably expected for human consumption after cooking or other processing effective to eliminate STEC or reduce to an acceptable level". In such foods any living (viable) STEC (*stx*1+ and/or *stx*2+) that is positive for *eae* or *aaiC*/*aggR* and belongs to an epidemiologically relevant serogroup (currently O157, O26, O103, O145, O111, O104, O45, O121, O174) isolated in 25 g of food make the food unsafe and require action (i.e. withdrawal and/or recall and corrective measures).

4.3 France

In France, human surveillance of STEC is focused on HUS cases in children <15 years, so the epidemiological data differ significantly from other EU MSs. The French data (based on HUS cases in children <15 years) show that the O157 serogroup proportion is decreasing (from 34% in 2011 to 17% in 2015). An O80 serogroup emerged in France in 2010, and in 2015 it represented the third most frequent serogroup. The vast majority of strains isolated in the HUS cases exhibited the virulence characteristics (*stx*1 and/or *stx*2, *eae*) with the virulence profiles of the strains of human and food origin noted to be similar.

In national instructions currently in force, the hazard considered is any isolated *E. coli* strain which possesses *stx*1 and/or *stx*2 and *eae* gene(s) and belongs to one of the following serotypes: O157:H7, O26:H11, O145:H28, O103:H2 or O111:H8. These highly pathogenic strains should be taken into account by FBOs in their HACCP plan (cheese and beef sector in particular). Self-inspections should be implemented in order to verify the effectiveness of the control measures. In the minced beef sector, the minimum sampling rate is:

- In frozen minced beef, systematic screening for serotype O157:H7 in each batch, and at least one analysis per week for the other four serotypes.
- In chilled minced beef, screening for serotype O157:H7 in a batch at least once a week.

Moreover, the competent authority organises yearly surveillance plans (beef trimmings, ground meat and raw milk cheeses). If a highly pathogenic STEC is detected, the action is to withdraw/recall and conduct additional analysis of adjacent batches.

Following a request from the French Agency for Food, Environmental and Occupational Health and Safety (ANSES), reviewed the definition of the highly pathogenic STEC in light of the recent French and European epidemiological data and evaluated the efficacy of different sampling plans in the minced beef sector. In its opinion of 18 May 2017, ANSES concluded that:

- Any strain of *E. coli* isolated in humans or in food should be regarded as an EHEC if it has the virulence genes *stx*1 and/or *stx*2 and *eae* or other gene(s) encoding a system of adhesion to the human digestive tract.
- The list of the serotypes to be screened for as a priority in food remains valid: O157:H7, O26:H11, O103:H2, O145:H28 and O111:H8. This list may be revised on the basis of new epidemiological data, in particular the results of the investigations under way concerning the source of the O80:H2 serotype.

- The modelling carried out shows that the application of a microbiological criteria (n=1, m=absence in 25 g) would lead to a significant risk reduction. To achieve the levels of performance calculated in this opinion, the microbiological criteria must include the five major serotypes and be applied to all batches.

4.4 Denmark

Demark is assessing the risk from STEC in food based on determination of (i) hazard/pathogenicity of the isolate and (ii) risk associated with the food.

Foods are placed in two risk profiles:

- **RTE food or food consumed without prior sufficient heat treatment.** For such foods, the hazard is an isolated *E. coli* strain which is *vtx*+ or *vtx*+/*eae*+ or *vtx*+/*aaiC*+ and *aggR*+. If detected, action includes withdrawal/recall of products. Raw minced meat is included in this risk profile due to a recent consumer trend for eating it undercooked.
- **Food subjected to heat treatment or otherwise sufficiently treated prior to consumption.** For these foods the risk evaluation is based on level of contamination, presence of virulence genes and intended use of product. Corrective action is required if considered unsafe according to Article 14 (EC Regulation 178/2002). The level of contamination is very often based on results from five tested sample units from a single batch. In these instances the results are considered to be: low contamination if one sample unit out of five is positive; or high contamination if two or more sample units out of five are positive. In terms of food profile (food item and intended use), beef cuts in retail could be considered intermediate risk while carcases at slaughter and frozen cuts intended to be eaten after heat treatment usually are considered low risk.

As an example, Denmark carried out a screening project in 2017 where meat samples were taken in cutting facilities: If one sample was positive (out of five), there was no further action. If >1 sample(s) were positive, action was considered (based on the presence of virulence genes and the intended use of the product).

4.5 Germany

The German ALTS (Food Hygiene and Food of Animal Origin working group), a federal and governmental state panel of experts, has reviewed the risk of VTEC/STEC in food and made the following conclusions:

- For the evaluation of VTEC/STEC in food the detection of verotoxin or the verotoxin gene, and the isolation of the bacterium are both required.
- Virulence markers such as the *eae* gene are often associated with severe illness, but *eae*-negative *E. coli* strains are not unusual within the causative agents responsible for diarrhoea. Therefore, subject to new findings regarding virulence factors, all VTEC have to be considered as potentially pathogenic causing human illness.
- The evaluation of risk "to be detrimental to human health" resulting from this knowledge, and therefore resulting in withdrawal and/or recall and corrective measures, should be limited to ready-to-eat food. Additional follow-up investigation must be performed at the producer level.

Besides the categorisation of food regarding the presence of STEC/VTEC in different risk profiles (RTE and non-RTE), the purpose of use and preparation as well as labelling and local consumption habits have to be considered.

5. ANSWERS TO THE REQUEST FOR ADVICE QUESTIONS

These are the responses by the STEC Working Group to the questions posed in the request for advice from the FSAI Scientific Committee (**Appendix 1**).

1. Should foods be categorised with regard to risk from VTEC/STEC and if yes, how?

When STEC is detected (i.e. culture isolation of an *E. coli* containing *stx* gene(s))[7] in a food, the risk of illness is dependent on the type of food, its likely final preparation prior to consumption and the vulnerability of the consumer to illness. It is thus concluded that RTE and non-RTE foods have different risk profiles with regard to STEC:

- **RTE food**[8] includes food that is intended to be consumed less than thoroughly cooked, i.e. following a treatment that will not/is unlikely to remove the risk associated with STEC.
- **Non-RTE food** includes food that is intended to be consumed following a treatment that will remove the risk of STEC. This category includes carcases and whole cuts of meat. It also includes minced meat intended to be thoroughly cooked. This is in line with the FSAI recommendation to thoroughly cook beef burgers to a core temperature of no less than 75 °C or an equivalent time-temperature combination (e.g. to a core temperature of 70 °C for at least two minutes) (FSAI, 2006, 2018a, 2018b).

2. What is the risk associated with the detection of STEC in foods (category based on the answer to Question 1) depending on the presence/absence of virulence genes (*eae*/*aaiC* and *aggR*) and/or the serogroup?

There are significant challenges in the risk assessment and management of STEC in that the profile of strains causing human illness has continued to change since it first emerged as a cause of human illness. This has included changes in understanding the role of both the serogroup and the presence/absence of particular genes as indicators of STEC virulence potential. *E. coli* O157:H7 was the first serogroup implicated in STEC human infections (in the 1980s). In the 2000s, further serogroups (*E. coli* O26, O103, O111 and O145) were identified as being most commonly linked to human infection, and along with O157, became known as the 'top five' STEC serogroups. In 2011, the serogroup O104 was added to this group following a European outbreak linked to sprouted fenugreek seeds, making these serogroups the 'top six'. A review of Irish and international epidemiological data has shown that the profile of STEC strains associated with human illness has evolved in recent years and now includes many serogroups outside the traditional 'top six'. In Ireland in 2004, 85% of all notifications were linked to STEC O157, whereas data from 2012 to 2016 show that only 28% of notifications were linked to O157, with 21% of symptomatic cases linked to approximately 70 diverse serogroups outside of the other 'top five'.

In STEC strains, the presence of the *eae* gene (a gene encoding for intimin, a protein which facilitates intimate attachment to the host intestinal epithelial cells) has historically been used as a predictor of human illness potential, but recent international and Irish data have shown that this is changing. Between 2012 and 2016, among culture-confirmed STEC notifications in Ireland, 17.8% were *eae* negative; and among culture-confirmed STEC-associated haemolytic uraemic syndrome (HUS) cases, 6.8% were *eae* negative. The 2011 *E. coli* O104:H4 outbreak strain was a hybrid enteroaggregative-haemorrhagic *E. coli* carrying the *aaiC* and *aggR* genes and the *stx*2a gene (a subtype of the *stx*2 gene), but there is a lack of data on the presence/absence of these enteroaggregative *E. coli* genes (*aaiC* and *aggR*) in Irish clinical and food-derived isolates.

[7] Public health risk cannot be assessed based on detection of *stx* gene(s) by molecular methods only (i.e. a positive PCR result/presumptive positive), except in those scenarios where there is additional information that indicates there is a public health risk or non-compliance.

[8] Commission Regulation (EC) No 2073/2005 defines 'RTE food' as "food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organisms of concern."

It is concluded that, at the present time, there is no scientific evidence to differentiate the potential risk of illness from STEC based on (i) the serogroup/serotype or (ii) the presence/absence of the *eae*/*aaiC* and *aggR* genes. Consequently, any STEC cultured from a food constitutes a potential risk of illness, although the risk posed is different depending on the food category as stated in the answer to Question 1. This position may be revised in the future, based on new scientific evidence.

3. What is the risk associated with the detection of EPEC in food when the EPEC belongs to:

a. The serogroups currently most commonly associated with severe illness (i.e. referred to as the EU 'top six' – *E. coli* O157, O26, O111, O103, O145 and O104:H4), or

b. Other serogroups?

It is a possibility that when testing for STEC, an enteropathogenic *E. coli* (EPEC) may be detected. An EPEC is an *E. coli* strain possessing the *eae* gene (a gene encoding for intimin, a protein which facilitates intimate attachment to the host intestinal epithelial cells) but lacking the Shiga toxin-producing gene(s) (*stx*) characteristic of STEC. Some EU MSs have taken action following the detection of EPEC in certain foodstuffs (details available on the EC RASFF Portal). The detection of EPEC and the associated recall actions taken by some MSs have been in the context of the ISO/TS 13136 test method for STEC, whereby an *eae*-positive but *stx*-negative *E. coli* (EPEC) was confirmed in a food sample that was originally screened as *stx* positive by PCR (presumptive STEC detection). The possibility that the EPEC isolates are derivatives of STEC that have lost their Stx-encoding phage (containing *stx* gene(s)) cannot be excluded in this scenario. It also raises the question of whether there is potential for an EPEC strain to acquire an Stx-encoding phage during storage of the food prior to consumption.

Based on current scientific evidence, it is concluded that, although plausible, the loss and acquisition of an Stx-encoding phage are rare events under typical conditions of chilled food storage. The conclusion is that the detection of EPEC in food is not an indicator for the detection of STEC. This position may be revised in the future, based on new scientific evidence.

4. What is the risk associated with *Hafnia* strains such as *Hafnia alvei* and *Hafnia paralvei*, deliberately added to some dairy products as ripening cultures and which may be *stx* positive?

The genus *Hafnia* belongs to the Enterobacteriaceae family, as does STEC, and is a group of commensal (generally recognised as harmless) bacteria which can be found in food. *Hafnia* may also be deliberately added as a starter culture during the process of making cheese.

It has been reported that some *Hafnia* spp. have been isolated from food that were PCR positive for the *stx* gene, and that a *Hafnia* strain had cytotoxigenic potential similar to that of STEC, but there is, at present, no evidence to indicate that *stx*-positive *Hafnia* strains can cause human illness. *Hafnia* have only very rarely been implicated as a cause of opportunistic infection in humans. Therefore, there is currently no evidence to conclude that the presence of a *Hafnia* spp. poses a risk to human health.

5. In a batch of food (category based on the answer to Question 1), what action should be taken based on a presumptive positive PCR STEC result in the context of the previous and/or subsequent batch (or batches produced close in time) being confirmed culture positive?

It has been concluded that the public health risk cannot be assessed based on detection of *stx* gene(s) by molecular methods only (i.e. a positive PCR result/presumptive positive).

However, where there is additional information that indicates a public health risk (e.g. batches of the same product from which STEC has been cultured) a presumptive positive STEC (positive PCR only) may be taken as indicative of a risk. In those cases, the detection of *stx* gene(s) by PCR only may be taken as contributing evidence to support an intervention.

The risk management action(s) to be taken will be determined by the competent authority on the basis of an individual risk assessment. This risk assessment should examine additional information that might indicate a public health risk (e.g. cases of illness, or other potentially relevant epidemiological information) or non-compliance (e.g. ineffective food safety management systems). Factors such as the origin of the raw material, the nature of the food item and its intended use, and the degree of separation between the batches should also be taken into consideration when assessing the risk. This applies to both RTE and non-RTE foods.

5.1 General recommendations

- Scientific knowledge will continue to deepen our understanding of the human clinical epidemiology and the virulence characteristics and serotypes of STEC circulating in the agri-food chain. WGS technologies are now starting to generate new scientific data on the presence and absence of a wide range of virulence genes, and may in the future facilitate the identification of genetic markers in STEC which more accurately predict human virulence potential. It is therefore recommended that the advice provided in this report, which is based on current scientific knowledge and current Irish epidemiological information, be revisited periodically, taking account of any new data.
- In the context of managing the risk for food categorised as 'non-RTE food', in particular minced meat, it is recommended that periodic national education campaigns be run for both consumers and FBOs to raise and maintain awareness of the risk of eating or serving undercooked minced meat.

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7. ANNEX I

APPENDIX 1 Request for Advice from the FSAI Scientific Committee

Topic title: Advice on STEC detection in food

Date requested: 30 September 2016

Date accepted: 2 December 2016

Target deadline for advice: One year from date of acceptance a draft will be submitted to the Scientific Committee.

Form of advice required: A report which addresses the questions posed

Background/context

EU context

There is a lack of agreement across Europe on the appropriate risk-based action to be taken when Shiga toxin-producing *E. coli* (STEC), also called verocytotoxigenic *E. coli* (VTEC), is detected in food using a PCR-based method (ISO/TS 13136:2012). This method involves screening for certain DNA markers (giving a presumptive result) followed by confirmation that these markers are presented in a cultured *E. coli* isolate (the confirmed result).

Currently, there is only one legal criterion set for STEC in Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, and this is the prescribed method for the testing of STEC in sprouts. Private and official control testing for STEC in other foods (in particular raw beef and cheese made with raw milk) is, however, common in some EU countries.

The legal microbiological criterion for STEC in sprouts was introduced by Regulation (EU) No 209/2013 as a direct result of the large outbreak in 2011 of *E. coli* O104:H4, which was linked to sprouted fenugreek seeds and affected 3,950 people, causing 53 deaths. The criterion is set for the five serogroups identified at the time as being those most commonly associated with severe illness (namely O157, O26, O103, O111 and O145) as well as the serogroup (O104:H4) that caused the outbreak in 2011 (a previously uncommon serogroup with an unusual combination of virulence factors of STEC/VTEC and enteroaggregative *E. coli* (EAEC)). Regulation (EU) No 209/2013 (Recital 12) recognises, however, that it cannot be excluded that other STEC serogroups may be pathogenic to humans. These STEC may cause less severe forms of disease such as diarrhoea and/or bloody diarrhoea, or more severe illness such as haemolytic uraemic syndrome (HUS) in which the red blood cells are destroyed and the kidneys fail.

In April 2013, the European Food Safety Authority (EFSA) published a Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. This opinion acknowledged that it was not possible at the time to fully define human pathogenic VTEC or identify factors for VTEC that absolutely predict the potential to cause human disease. It proposed a molecular approach for the categorisation of VTEC according to potential risk of illness (Table 1). In 2014, the European Commission (EC) attempted to introduce a harmonised approach to assessing and managing the risk of VTEC/STEC, based on the EFSA opinion. Member States, however, failed to agree and the EC suspended this work in 2016.

Table 1 Excerpt from EFSA Panel on Biological Hazards (BIOHAZ); Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. *EFSA Journal*, 11(4): 3138.

Table 14 Proposed[a] molecular approach for the categorisation of VTEC (*vtx* present)

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

Irish context

In Ireland, raw milk cheese producers, the beef sector and sprouts producers have been most affected by the lack of scientific certainty regarding the pathogenicity of STEC.

The FSAI has taken the position that the confirmed detection (i.e. confirmed by culture) of an *E. coli* carrying a toxin gene (*stx*) in a ready-to-eat (RTE) food is a risk, irrespective of the serogroup or the presence of additional virulence factors (e.g. *eae*). Irish raw milk cheese producers have argued that this is not proportionate to the risk and is unsustainable for their sector. The Farmhouse and Artisan Cheese & Dairy Producers' European network (FACEnetwork) has asked MSs to consider using a higher level of certainty regarding the STEC hazard characterisation, such as the presence of *stx* with either *eae* or *aggR*/*aaiC* in an isolated *E. coli*, or other combinations of markers or virulence factors. FACEnetwork has also highlighted the fact that members of the Enterobacteriaceae such as *Hafnia alvei*, which may be *stx* positive, are deliberately added to some dairy products as ripening cultures.

The FSAI has in a few incidents assessed a batch of RTE food as unsafe, based on a presumptive PCR result. In these incidents, the decision was taken in the context of either a batch previous to the PCR-positive batch or subsequent to it being culture confirmed for the same or a different STEC serogroup.

In the case of raw beef, the FSAI had in the past not considered the detection of STEC a hazard, provided that the product carried an instruction to cook before consumption. This was because traditionally Ireland was a country where minced beef and beef burgers were thoroughly cooked. In recent years, however, consumer practices and consumer preferences when eating out appear to be changing. In 2016, Ireland had an outbreak of STEC O157 associated with a premises serving undercooked burgers. Against this background, in 2016 the FSAI took a more precautionary view regarding the detection of 'higher risk' STEC in minced beef or beef known to be destined for mincing. 'Higher risk' STEC is defined as *E. coli* confirmed to have an *stx* gene and either the *eae* or the *aaiC* and *aggR* genes. It was decided not to confine this definition to the serotypes most commonly associated with severe illness, as these serotypes have been shown to change (ECDC, 2014). An Irish study (Kennedy *et al*., 2005) found that 87%

of Irish consumers cook hamburgers well done, 12% cook them medium and 1% cook them rare. A more recent UK study (FSA, 2015) found that while the majority of UK consumers (68%) did not eat rare burgers, 11% did so at least once a month.

In 2016, one MS initiated food recalls in cheese and raw meat based on the detection of *eae*-positive but *stx*-negative *E. coli*, i.e. enteropathogenic *E. coli* (EPEC) (see RASFF Portal). The risk assessment from this MS is that studies have shown that *stx* genes can be easily acquired or lost by *E. coli*, so the *eae*-positive *E. coli* can either acquire *stx* genes and become pathogenic or derive from an *stx*-positive, *eae*-positive pathogenic clone. In our experience, this MS has taken this approach with the *E. coli* serogroups most commonly associated with most severe disease (i.e. *E. coli* O157, O26, O111, O103, O145 and O104:H4) and which are the specific focus of the method for detection of STEC (ISO/TS 13136:2012).*

In conclusion, the FSAI is seeking the Scientific Committee's view on the risk associated with the consumption of foods in which STEC has been detected.

Questions for the Scientific Committee

1. Should foods be categorised with regard to risk from VTEC/STEC and if yes, how?
2. What is the risk associated with the detection of STEC in foods (category based on the answer to Question 1) depending on the presence/absence of virulence genes (*eae*/*aaiC* and *aggR*) and/or the serogroup?
3. What is the risk associated with the detection of EPEC in food when the EPEC belongs to:
    a. The serogroups currently most commonly associated with severe illness (i.e. referred to as the EU 'top six' – *E. coli* O157, O26, O111, O103, O145 and O104:H4), or
    b. Other serogroups?
4. What is the risk associated with *Hafnia* strains, such as *Hafnia alvei* and *Hafnia paralvei*, deliberately added to some dairy products as ripening cultures and which may be *stx* positive?
5. In a batch of food (category based on the answer to Question 1), what action should be taken based on a presumptive positive PCR STEC result in the context of the previous and/or subsequent batch (or batches produced close in time) being confirmed culture positive?

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* Taking into account the most recent adaptation of this method by the European Union reference laboratory for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC), for the detection of STEC O104:H4.

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APPENDIX 2 Culture-confirmed STEC notifications by symptom status, Ireland, 2012–2016

Serogroup	Symptomatic	Asymptomatic	Unknown	Total
O26	895	231	9	1,135
O157	784	125	11	920
O145	125	25	1	151
O103	62	7	1	70
O146	46	10	4	60
O111	34	4	–	38
O91	25	10	1	36
O182	29	5	–	34
O5	25	7	–	32
O84	19	3	–	22
O113	11	5	–	16
O177	12	3	–	15
O55	9	5	–	14
O78	11	1	–	12
O76	9	2	–	11
O128ab	10	1	–	11
O-rough	8	2	–	10
O117	7	2	–	9
O8	7	1	–	8
O130	5	–	1	6
O108	4	2	–	6
O2	4	–	1	5
O98	4	1	–	5
O165	4	–	1	5
O118	4	–	–	4
O153	4	–	–	4
O128ac	4	–	–	4
O150	3	–	–	3
O105ac	2	1	–	3
O178	3	–	–	3
O183	1	1	1	3
O149	3	–	–	3
O156	1	2	–	3
O174	2	–	1	3
OE11362-78	3	–	–	3

Serogroup	Symptomatic	Asymptomatic	Unknown	Total
O75	3	–	–	3
O136	3	–	–	3
O128ad	1	2	–	3
O112ab	2	–	–	2
O123	2	–	–	2
O185	2	–	–	2
O126	1	–	1	2
O105c	2	–	–	2
O71	2	–	–	2
O101	2	–	–	2
O74	2	–	–	2
O105	1	1	–	2
O166	2	–	–	2
OE7477-77	1	1	–	2
O107	1	1	–	2
O181	2	–	–	2
O163	2	–	–	2
O169-0183	1	1	–	2
O87	–	–	1	1
O134	1	–	–	1
O186	1	–	–	1
O38	1	–	–	1
O80	–	–	1	1
O44	1	–	–	1
O90	1	–	–	1
O104	1	–	–	1
O148	1	–	–	1
O175	1	–	–	1
O159	1	–	–	1
O7	1	–	–	1
O112	1	–	–	1
O176	1	–	–	1
O9	1	–	–	1
O73	1	–	–	1
O121	1	–	–	1
O138	1	–	–	1
O18ab	1	–	–	1

Serogroup	Symptomatic	Asymptomatic	Unknown	Total
O140	1	–	–	1
O22	1	–	–	1
O180	1	–	–	1
O28ab	1	–	–	1
O141	1	–	–	1
Ungroupable	161	40	–	201
Total	**2,394**	**502**	**35**	**2,931**

APPENDIX 3A RASFF alerts notified to the EC in 2016 and 2017 (until end of September 2017) relating to the detection of STEC (n=82) in food. Tables exported from RASFF Portal.

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Dietetic foods, food supplements, fortified foods	16/03/2017	2017.0337	Alert	Official control on the market	Germany	*Bacillus cereus* (62000 CFU/g) and shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in barley grass powder from Germany	Informing recipients	Distribution to other member countries	Serious
Dietetic foods, food supplements, fortified foods	18/10/2016	2016.1429	Alert	Official control on the market	Germany	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in organic herbal food supplement from Germany, with raw material from India	Recall from consumers	Distribution to other member countries	Serious
Dietetic foods, food supplements, fortified foods	09/09/2016	2016.1255	Alert	Company's own check	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in organic barley grass tablets from Germany	Recall from consumers	Distribution to other member countries	Serious
Dietetic foods, food supplements, fortified foods	02/08/2016	2016.1034	Alert	Official control on the market	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+; 0145 /25g) in organic wheatgrass tablets from Germany	Recall from consumers	Distribution to other member countries	Serious
Fruits and vegetables	22/09/2017	2017.1492	Information for attention	Official control on the market	Finland	Shigatoxin-producing *Escherichia coli* (*stx*2+) in lamb's lettuce (*Valerianella locusta*) from Italy	Informing recipients	Product (presumably) no longer on the market	Serious
Fruits and vegetables	03/08/2017	2017.1155	Information for attention	Company's own check	Germany	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in beetroot sprouts from the Netherlands	Return to consignor	Product (presumably) no longer on the market	Undecided
Fruits and vegetables	07/09/2016	2016.1241	Information for attention	Food poisoning	Finland	Foodborne outbreak suspected to be caused by shigatoxin-producing *Escherichia coli* (*stx*+, *eae*+, 3100 CFU/g) in rucola from Denmark, via Sweden	No stock left	Product (presumably) no longer on the market	Serious
Fruits and vegetables	28/04/2016	2016.0526	Information for attention	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in fresh bean sprouts (tauge) from the Netherlands	Informing authorities	Product (presumably) no longer on the market	Serious
Herbs and spices	22/11/2016	2016.1600	Information for attention	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in fresh basil from Thailand	Informing recipients	Product (presumably) no longer on the market	Serious

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Herbs and spices	18/11/2016	2016.1586	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+) and *Salmonella* (presence /25g) in fresh mint from Laos, via the Netherlands	Withdrawal from the market	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	14/09/2017	2017.1442	Information for attention	Border control – consignment released	Portugal	Shigatoxin-producing *Escherichia coli* (*stx*2+; *eae*+; O26) in frozen beef from Uruguay	Destruction	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	07/09/2017	2017.BPX	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1+) in frozen beef from Brazil	Import not authorised	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	01/09/2017	2017.BPC	Border rejection	Border control – consignment detained	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+) in frozen beef meat from Brazil	Import not authorised	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	30/08/2017	2017.1309	Information for attention	Border control – consignment released	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ *eae*– /25g) in frozen boneless beef from Brazil	Unknown	Distribution restricted to notifying country	Undecided
Meat and meat products (other than poultry)	30/08/2017	2017.1310	Information for follow-up	Border control – consignment released	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ *eae*– /25g) in frozen boneless beef from Brazil	Unknown	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	28/08/2017	2017.BOI	Border rejection	Border control – consignment detained	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in frozen beef from Brazil	Import not authorised	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	11/08/2017	2017.BML	Border rejection	Border control – consignment detained	Germany	Shigatoxin-producing *Escherichia coli* (041:H14; *stx*1+ /25g) in frozen beef from Brazil	Unknown	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	09/08/2017	2017.1188	Information for attention	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (046:H2; *stx*2+ /25g) in frozen roast beef from Uruguay	Detained by operator	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	27/07/2017	2017.BJZ	Border rejection	Border control – consignment detained	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+) in chilled beef fillets and roast beef from Brazil	Re-dispatch	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	19/07/2017	2017.BJA	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (0103; *stx*+; *eae*+ and 0146) in frozen beef from Brazil	Import not authorised	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	05/07/2017	2017.0964	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+ /25g) in chilled lamb chops from the Netherlands	Destruction	Distribution to other member countries	Serious

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Meat and meat products (other than poultry)	23/06/2017	2017.BDR	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in frozen beef from Brazil	Unknown	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	19/06/2017	2017.BCP	Border rejection	Border control – consignment detained	Spain	Shigatoxin-producing *Escherichia coli* (0:145; *stx*1+; *stx*2+; *eae*+ /25g) in frozen beef meat from Uruguay	Re-dispatch	Product not (yet) placed on the market	Undecided
Meat and meat products (other than poultry)	14/06/2017	2017.0847	Alert	Consumer complaint	Austria	Shigatoxin-producing *Escherichia coli* (O133:H21; *stx*1+; *stx*2+; *hly*+ /25g) in frozen minced beef from Hungary	No stock left	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	09/06/2017	2017.0823	Alert	Company's own check	Belgium	Shigatoxin-producing *Escherichia coli* (*stx*+, *eae*+ /25g) in chilled vacuum-packed beef from the Netherlands	Seizure	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	07/06/2017	2017.0805	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+) in frozen kangaroo striploins from Australia	Destruction	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	06/06/2017	2017.0796	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in chilled vacuum-packed beef tenderloin from Argentina, packaged in the Netherlands	Unknown	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	26/05/2017	2017.0730	Alert	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+; *eae*+ /25g) in chilled lamb rolls from the United Kingdom	Detained by operator	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	22/05/2017	2017.AXJ	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+; *eae*– /25g) in frozen beef from Brazil	Import not authorised	Product not (yet) placed on the market	Undecided
Meat and meat products (other than poultry)	22/05/2017	2017.AXK	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (*stx*+ *eae*+) in frozen beef from Brazil	Import not authorised	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	15/05/2017	2017.0649	Alert	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in chilled lamb loins from the United Kingdom	Seizure	Distribution to other member countries	Serious

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Meat and meat products (other than poultry)	27/04/2017	2017.0535	Alert	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+ /25g) in chilled lamb meat from the United Kingdom	Withdrawal from recipient(s)	Product (presumably) no longer on the market	Undecided
Meat and meat products (other than poultry)	26/04/2017	2017.0528	Alert	Company's own check	Belgium	Shigatoxin-producing *Escherichia coli* (*stx*1+, *stx*2+, *eae*+ /25g) in chilled beef lungs and oesophagus from Belgium	Informing recipients	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	21/04/2017	2017.0514	Alert	Official control on the market	Belgium	Shigatoxin-producing *Escherichia coli* (*stx*+, *eae*+) in chilled lamb from the Netherlands, slaughtered in Belgium	Informing consignor	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	28/02/2017	2017.0254	Information for attention	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*2+ O-nt:H46 /25g) in chilled beef from Uruguay	Destruction	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	21/02/2017	2017.AHY	Border rejection	Border control – consignment detained	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+ O87:H8) in frozen deer meat from New Zealand	Import not authorised	Product not (yet) placed on the market	Undecided
Meat and meat products (other than poultry)	17/02/2017	2017.0211	Alert	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*2+ O113:H21 /25g) in frozen leg of lamb without bone from Uruguay	Unknown	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	01/02/2017	2017.0140	Alert	Border control – consignment released	Italy	Shigatoxin-producing *Escherichia coli* in chilled boneless beef cuts (*Bos taurus*) from Uruguay	Withdrawal from the market	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	26/01/2017	2017.0106	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in frozen kangaroo striploins from Australia	Withdrawal from the market	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	02/01/2017	2017.0001	Alert	Official control on the market	Austria	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+) in deer sausage from Austria	Public warning – press release	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	29/12/2016	2016.1823	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in frozen kangaroo silverside from Australia, via Belgium	Informing consignor	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	29/12/2016	2016.BSY	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* in frozen beef from Brazil	Re-dispatch	Product not (yet) placed on the market	Undecided

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Meat and meat products (other than poultry)	28/12/2016	2016.BSV	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* in frozen beef from Brazil	Re-dispatch	Product not (yet) placed on the market	Undecided
Meat and meat products (other than poultry)	23/12/2016	2016.1809	Alert	Official control on the market	Austria	Shigatoxin-producing *Escherichia coli* (*stx*2+; *eae*A+) in chilled deer meat from Austria	Withdrawal from the market	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	20/12/2016	2016.1778	Information for attention	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+; *eae*+; O157 /25g) in chilled deer meat without bone from New Zealand	Unknown	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	16/12/2016	2016.1757	Alert	Official control on the market	Latvia	Shigatoxin-producing *Escherichia coli* (*eae*a+ *stx*2+ /25g) in cold smoked sausages from Lithuania	Withdrawal from the market	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	16/12/2016	2016.1759	Alert	Official control on the market	Latvia	Shigatoxin-producing *Escherichia coli* (O103, O145; *eae*A+, *vtx*2+ /25g) in smoked sausages from Lithuania	Withdrawal from the market	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	14/12/2016	2016.BQE	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (presence /25g) in frozen beef from Brazil	Re-dispatch	Product not (yet) placed on the market	Undecided
Meat and meat products (other than poultry)	14/11/2016	2016.1555	Information for attention	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in chilled beef fillets from Paraguay	Re-dispatch	Product not (yet) placed on the market	Serious
Meat and meat products (other than poultry)	12/10/2016	2016.1401	Alert	Official control on the market	France	Shigatoxin-producing *Escherichia coli* (O26:H11 *stx*1+ *eae*+) in frozen minced beef from France	Recall from consumers	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	13/08/2016	2016.1106	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in frozen ground beef steak from Germany	Recall from consumers	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	05/08/2016	2016.1056	Alert	Official control on the market	France	Shigatoxin-producing *Escherichia coli* (O26:H11; *stx*1+; *stx*2+; *eae*+) in frozen minced meat from Ireland	Recall from consumers	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	28/07/2016	2016.1013	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in chilled beef tenderloins from Uruguay	Official detention	Distribution to other member countries	Serious

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Meat and meat products (other than poultry)	26/07/2016	2016.0999	Information for attention	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (in 2 out of 5 samples /25g) in chilled beef from Argentina	Unknown	Distribution restricted to notifying country	Undecided
Meat and meat products (other than poultry)	25/07/2016	2016.0986	Alert	Official control on the market	Belgium	Shigatoxin-producing *Escherichia coli* (*stx*2+, *eae*+ /25g) in chilled beef from Belgium	Withdrawal from the market	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	22/06/2016	2016.0814	Information for attention	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in frozen striploin roast beef from Brazil	Withdrawal from the market	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	20/06/2016	2016.0801	Information for follow-up	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+ *stx*2+) in chilled boneless beef tenderloins from Uruguay	Re-dispatch	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	31/05/2016	2016.0700	Information for follow-up	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+, 088:H25 /25g) in chilled beef burgers (*Bos taurus*) from Uruguay	Informing authorities	Distribution to other member countries	Undecided
Meat and meat products (other than poultry)	10/05/2016	2016.0589	Information for follow-up	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in chilled beef from Ireland	Re-dispatch	No distribution from notifying country	Undecided
Meat and meat products (other than poultry)	06/05/2016	2016.0572	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ /25g) in chilled bison peel knuckle from the United States, via Belgium	Withdrawal from recipient(s)	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	29/04/2016	2016.0542	Information for attention	Border control – consignment released	Germany	Shigatoxin-producing *Escherichia coli* (*stx*2+ *eae*– Ont:H14, O186:H49, O86:H51) in chilled boneless beef from Argentina	Official detention	Product (presumably) no longer on the market	Undecided
Meat and meat products (other than poultry)	26/04/2016	2016.0504	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ *eae*– /25g) in frozen kangaroo steak from Australia, via Belgium	Withdrawal from the market	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	19/04/2016	2016.0470	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*1+; O8:H16 /25g) and *Listeria monocytogenes* (presence /25g) in frozen rib eye from Argentina	Informing recipients	Distribution to other member countries	Serious

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Meat and meat products (other than poultry)	13/04/2016	2016.0441	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ 022:H8 /25g) in frozen beef from Uruguay, via Belgium	Informing recipients	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	18/03/2016	2016.ALG	Border rejection	Border control – consignment detained	Italy	Shigatoxin-producing *Escherichia coli* (*stx*1; *stx*2 /25g) in chilled beef (*Bos taurus*) from Argentina	Import not authorised	Product not (yet) placed on the market	Undecided
Meat and meat products (other than poultry)	04/03/2016	2016.0254	Alert	Company's own check	France	Shigatoxin-producing *Escherichia coli* (0157:H7 *eae*+ *stx*2+) in minced sheep meat from Spain	Detained by operator	Distribution to other member countries	Serious
Meat and meat products (other than poultry)	17/02/2016	2016.AHL	Border rejection	Border control – consignment detained	France	Shigatoxin-producing *Escherichia coli* (*stx*1+, *stx*2–, *eae*– /25g) in chilled boneless beef meat (*Bos taurus*) from Brazil	Destruction	Product not (yet) placed on the market	Undecided
Milk and milk products	26/06/2017	2017.0905	Alert	Official control on the market	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+ /25g) in raw milk sheep's cheese from France	Informing authorities	Distribution restricted to notifying country	Serious
Milk and milk products	12/06/2017	2017.0832	Information for attention	Company's own check	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+; *stx*2+; *eae*+; *e-hlya*+; *nleb*+; 4.4×10^{3} CFU/g) and coagulase-positive *Staphylococcus* (3×10^{4} CFU/g) in raw cow's milk soft cheese from France	Recall from consumers	Product (presumably) no longer on the market	Serious
Milk and milk products	07/06/2017	2017.0812	Information for attention	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (026; 0145; *stx*+; *eae*+) in chilled cow's cheese from Romania	Official detention	Product (presumably) no longer on the market	Serious
Milk and milk products	02/06/2017	2017.0771	Alert	Official control on the market	Belgium	Shigatoxin-producing *Escherichia coli* (*stx*+, *eae*+ /25g) in raw cow's milk cheese from Belgium	Public warning – press release	Distribution to other member countries	Serious
Milk and milk products	12/05/2017	2017.0635	Alert	Company's own check	France	Shigatoxin-producing *Escherichia coli* (0:26; *stx*+ *eae*+) in raw goat's cheese from France	Recall from consumers	Distribution to other member countries	Serious
Milk and milk products	24/03/2017	2017.0386	Alert	Official control on the market	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+; 0136 /25g) in roquefort cheese from France, via Luxembourg	Withdrawal from the market	Distribution to other member countries	Undecided

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject[a]	Action taken	Distribution status	Risk decision[b]
Milk and milk products	24/10/2016	2016.1459	Information for attention	Official control on the market	Norway	Shigatoxin-producing *Escherichia coli* (0113 – *stx*2+) in soft cheese from France	Informing consignor	Product (presumably) no longer on the market	Serious
Milk and milk products	27/09/2016	2016.1334	Alert	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (presence; *stx*1+ /25g) in raw milk cheese (Camembert) from France	Public warning – press release	Distribution to other member countries	Serious
Milk and milk products	29/06/2016	2016.0842	Alert	Official control on the market	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+ *eae*+ 026:H11) in goat's cheese from France	Recall from consumers	Distribution to other member countries	Serious
Milk and milk products	06/05/2016	2016.0573	Alert	Food poisoning	Italy	Shigatoxin-producing *Escherichia coli* (*stx*2+, 0111 /25g) in chilled raw cow's milk from Germany	Informing authorities	Distribution to other member countries	Serious
Milk and milk products	29/04/2016	2016.0537	Alert	Official control on the market	Germany	Shigatoxin-producing *Escherichia coli* (*stx*1+; 06:[H10] /25g) in Roquefort cheese from France	Recall from consumers	Distribution to other member countries	Serious
Milk and milk products	25/03/2016	2016.0372	Alert	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (*stx*+, *eae*+ /25g) in raw cow's milk soft cheese from France	Official detention	Distribution to other member countries	Undecided
Milk and milk products	16/03/2016	2016.0312	Alert	Food poisoning	Italy	Shigatoxin-producing *Escherichia coli* (026 *stx*1+ *stx*2+ *eae*+) in fermented cheese from Romania	Public warning – press release	Distribution to other member countries	Serious
Milk and milk products	22/02/2016	2016.0201	Alert	Official control on the market	Italy	Shigatoxin-producing *Escherichia coli* (*stx*+, *eae*+, 0:103 /25g) in raw milk cheese from France	Official detention	Distribution restricted to notifying country	Undecided
Poultry meat and poultry meat products	03/02/2017	2017.0147	Information for attention	Official control on the market	Netherlands	Shigatoxin-producing *Escherichia coli* (*stx*2+ 027:H30; *stx*1+ H25) in ostrich eye fillet from South Africa	Unknown	Product (presumably) no longer on the market	Serious

[a] Whether the results in these RASFF alerts were PCR positive only or culture confirmed is not publicly available through the RASFF Portal.

[b] Risk decision – A decision is made by the reporting country as to whether the notification concerns a serious risk or not.

Data source: **European Commission** (n.d.) RASFF portal. Brussels: European Commission. Available at: https://ec.europa.eu/food/safety/rasff/portal_en

APPENDIX 3B RASFF alerts notified to the EC in 2016 and 2017 (until end of September 2017) relating to the detection of EPEC (n=9) in food. Tables exported from RASFF Portal.

Product category	Date	Reference	Notification type	Notification basis	Notified by	Subject	Action taken	Distribution status	Risk decision[a]
Meat and meat products (other than poultry)	31/07/2017	2017.1125	Information for attention	Company's own check	Germany	Enteropathogenic *Escherichia coli* (presence /25g) in chilled beef from Germany	Withdrawal from the market	Distribution restricted to notifying country	Undecided
Meat and meat products (other than poultry)	14/12/2016	2016.1742	Alert	Official control on the market	France	Enteropathogenic *Escherichia coli* (*eae*+, *stx*–, 026:H11) in frozen minced beef meat from Ireland	Withdrawal from the market	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	11/11/2016	2016.1552	Alert	Official control in non-member country	Latvia	Enteropathogenic *Escherichia coli* (*eae*A+ /25g) in smoked sausages from Lithuania	Withdrawal from the market	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	17/10/2016	2016.1426	Alert	Official control on the market	France	Enteropathogenic *Escherichia coli* (0103:H2, *stx*–, *eae*+) in frozen minced beef from Ireland	Recall from consumers	Distribution restricted to notifying country	Serious
Meat and meat products (other than poultry)	22/09/2016	2016.1314	Alert	Official control on the market	Latvia	Enteropathogenic *Escherichia coli* (*eae*A+ /25g) in smoked sausages from Lithuania	Withdrawal from the market	No distribution from notifying country	Serious
Meat and meat products (other than poultry)	08/09/2016	2016.1253	Alert	Official control on the market	France	Enteropathogenic *Escherichia coli* (026:H11 *eae*+ *stx*–) in frozen halal minced beef from Ireland	Recall from consumers	Distribution restricted to notifying country	Serious
Milk and milk products	22/08/2017	2017.1257	Alert	Company's own check	France	Enteropathogenic *Escherichia coli* (*eae*+; serotype 026:H11) in raw cow's milk cheese from France	Recall from consumers	Distribution to other member countries	Serious
Milk and milk products	31/08/2016	2016.1216	Alert	Company's own check	France	Enteropathogenic *Escherichia coli* (026:H11 *eae*+ *stx*–) in raw milk cheese from France	Recall from consumers	Distribution to other member countries	Serious
Milk and milk products	04/08/2016	2016.1049	Alert	Company's own check	France	Enteropathogenic *Escherichia coli* (026:H11; *stx*1–; *stx*2-; *eae*+) in raw milk cheese from France	Recall from consumers	Distribution to other member countries	Serious

[a] Risk decision – A decision is made by the reporting country as to whether the notification concerns a serious risk or not.

Data source: **European Commission** (n.d.) RASFF portal. Brussels: European Commission. Available at: https://ec.europa.eu/food/safety/rasff/portal_en

APPENDIX 4 Results of selected research studies on the prevalence of STEC and *E. coli* (stx–) in RTE fresh produce. Results should be interpreted with caution, as different methodologies were used.

Year	Food type	Country	Number of isolates culturally recovered/ number of samples tested (% positive)	Reference
2015	Pre-packaged nuts, seeds and dried fruit	Ireland	0/821 (0%) STEC	FSAI (2018) Survey of the microbiological safety of ready-to-eat, pre-packaged nuts, seeds and dried fruit (15NS1) https://www.fsai.ie/nuts_seeds_dried_fruit_micro_survey/
2013	Pre-cut and pre-packaged fresh herbs and salad leaves	Ireland	0/247 (0%) STEC 0/397 (0%) *E. coli* (*stx*–) O26 1/403 (0.25%) *E. coli* (*stx*–) O157 (*stx* genes not detected in the isolate)	FSAI (2015) Survey of the microbiological safety of ready-to-eat, pre-cut and pre-packaged fresh herbs and salad leaves from retail establishments in Ireland (13NS7) https://www.fsai.ie/publications_survey_salad_leaves/
2007	Fruit and/ or vegetable juices and smoothies	Ireland	0/436 (0%) *E. coli* (*stx*–) O157	FSAI (2007) 2nd National Microbiological Survey 2007 (07NS2) Bacteriological Safety of Fruit and/ or Vegetable Juices and Smoothies https://www.fsai.ie/uploadedFiles/Monitoring_and_Enforcement/Monitoring/Surveillance/Micro%20Surveillance%2007NS2.pdf
2004	Lettuce, fresh herbs, tomatoes	Canada	0/530 (0%) STEC 0/188 (0%) STEC 0/141 (0%) STEC	Arthur *et al*. (2007)
2004	Sprouts	United States of America	1/200 (0.5%) *E. coli* (*stx*–) O157	Samadpour *et al*. (2006)
2000–2001	Various ready-to-eat foods taken at retail level	Argentina	49/500 (9.8%) *E. coli* strains of which 10/49 (20.4%) characterised as STEC from soft or cottage cheese (n=7), chicken or meat with sauce (n=2) and vegetables with mayonnaise (n=1)	Balagué *et al*. (2006)

Year	Food type	Country	Number of isolates culturally recovered/ number of samples tested (% positive)	Reference
2000–2002	Fresh herbs, leafy greens	United States of America	0/184 (0%) *E. coli* (*stx*–) O157 0/124 (0%) *E. coli* (*stx*–) O157	Johnston *et al*. (2005)
2000	Organic cress, lettuce, watercress	United Kingdom	0/492 (0%) *E. coli* (*stx*–) O157	Sagoo *et al*. (2001)

APPENDIX 5 Summary of research studies in Ireland on STEC and *E. coli* (*stx*–) in dairy production

Year of sampling	Matrix	Methodology	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (n=number of isolates)	Virulence genes present in cultured isolates (number of isolates)	Reference
2013–2014	Milk filter and raw milk from 40 lactating cows from two dairy herds	Screening of samples using quantitative real-time PCR. Isolates cultured from PCR-positive samples.	Milk filter used during the milking session and bulk tank raw milk sample taken every second month between August 2013 and July 2014	0157 026	STEC not detected	Not applicable	Murphy *et al.* (2016)
2012–2013	Raw milk filters (n=211 farms)	In-house modified method based on ISO 16654:2001	Raw milk filters (n=190)	0157 026	**STEC (n=12; 6.3%)** ***E. coli* (*stx*–) (n=19; 10%)** **2 serotypes:** 0157:H7 (n=1) 026 (n=18)	**026 isolates** *stx*1, *stx*2, *eae* and *hlyA* (n=5) *stx*1, *eae* and *hlyA* (n=5) *stx*2, *eae* and *hlyA* (n=2) *eae* and *hlyA* (n=1)	FSAI (2015)
2007–2008	Raw milk filters and raw milk bulk tank samples on dairy farms (n=60 farms)	Screening by real-time PCR for *stx*1 and *stx*2 genes, followed by sero-specific real-time PCR. Isolates cultured from PCR-positive samples.	117 milk filters, 120 bulk tank raw milk samples	0157 026 0111 0103 0145	STEC not detected	Not applicable	Lynch *et al.* (2012)
2004–2005	Milk filters from bovine (n=56 herds), caprine (n=13 herds) and ovine (n=5 flocks) milk production holdings, the majority of which were supplying raw milk for farmhouse cheese production	Culture method to isolate *E. coli* 0157, 026 and 0111 followed by PCR to confirm virulence genes.	161	0157 026 0111	**STEC (n=9; 5.6%)** ***E. coli* (*stx*–) (n=44; 27.3%)** **2 serotypes:** 0157 (n=27) 026 (n=17)	**0157 isolates** *stx*2, *eae* and *hlyA* (n=5) **026 isolates** *stx*1, *eae* and *hlyA* (n=4) *eae* only (n=7)	Murphy *et al.* (2007)
2001–2003	In line milk filters (n=97 dairy farms)	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	536	0157	**STEC 0157 (n=16; 3%)**	**0157 isolates** *stx*1, *stx*2, *eae* and *hlyA* (n=4) *stx*2, *eae* and *hlyA* (n=12)	Murphy *et al.* (2005)

APPENDIX 6 Selected studies on inactivation of STEC and *E. coli* (*stx*–) in cheese food production processes

Process	Inoculation level	STEC and *E. coli* (*stx*–)	Number surviving	Reference
Manufacture of smear-ripened cheese from raw milk	33 CFU/mL into raw milk	*E. coli* (*stx*–) O157:H7	Number decreased to <1 CFU/g and <10 CFU/g in the rind and core, respectively, after 21 days. Viable cells detectable by enrichment after 90 days ripening. For a 1 $\log_{10}$ cycle reduction (*D*-value) in the rind and core, 7 and 14 days determined to be required, respectively.	Maher *et al.* (2001)
Manufacture and aging of Gouda and stirred-curd Cheddar cheeses made from raw milk	20 CFU/mL into raw milk	*E. coli* O157:H7 (3 strains, 2 of those linked to outbreaks)	Counts increased to approximately 145 CFU/g on day 1, then dropped significantly over 60 days to mean levels of 25 and 5 CFU/g in Cheddar and Gouda, respectively. Levels stayed below 5 CFU/g after an average of 94 and 108 days in Gouda and Cheddar, respectively, yet remained detectable for more than 270 days in both cheese types.	D'Amico *et al.* (2010)
Manufacture and storage of white brined cheese made from pasteurised milk. Cheeses prepared with and without a starter culture and stored in 10% or 15% NaCl brine at 10 and 21 °C for 28 days.	10^7 CFU/g	*E. coli* (*stx*–) O157:H7	Numbers were reduced by 2.6 and 3.4 $\log_{10}$ CFU/g in cheese stored in 10% and 15% NaCl brine, respectively, in the presence of starter lactic acid bacteria (LAB) and by 1.4 and 2.3 $\log_{10}$ CFU/g, respectively, in the absence of starter LAB at 10 °C. There was survival in cheese stored in both brines at 10 and 21 °C regardless of the presence of starter LAB after 28 days, although the latter significantly enhanced *E. coli* O157:H7 reduction in cheese or its brine at 10 °C.	Osaili *et al.* (2014)
Production and ripening of semi-hard raw milk cheese	10–10^3 $\log_{10}$ CFU/g	STEC O2:H27 STEC O26:H11 STEC O91:H21	Six of 16 cheeses made from raw milk at a low spiking level and 13 of 16 cheeses made at the high spiking level contained more than 10 CFU/g of STEC at the end of the 16-week ripening process.	Peng *et al.* (2013)
Manufacture and ripening of Camembert cheese made with raw milk standardised by microfiltration	10^3 CFU/mL into milk	Acid-resistant (AR) and non-acid-resistant (NAR) STEC strains: O6:H10, OntH8, O166:H28, O11:H43, O6:H1, O174:H8	STEC numbers decreased but small numbers survived the manufacture and ripening process (20 days). The biggest decrease was observed for an NAR STEC strain (O174:H8) whose counts reached 10 CFU/g at 20 days. The other AR or NAR STEC strains were all counted at levels ranging from 10^2 to 10^4 CFU/g.	Montet *et al.* (2009)
Manufacture of five types of raw milk cheese: Blue-type (sheep's milk) Lactic cheese (goat's milk) Uncooked pressed cheese with short ripening time (cow's milk) Uncooked pressed cheese with long ripening time (cow's milk) Cooked cheese (cow's milk)	10^2 CFU/mL into milk	STEC 0157:H7 STEC 026:H11 STEC 0103:H2 STEC 0145:H28	The behaviour of STEC during cheesemaking and ripening varied according to the cheesemaking schemes. Two physicochemical factors (sudden, rapid acidification and high temperature) inhibited the growth of the STEC during the first hours of cheesemaking. Serotype effect seen and the hypothesis that serotypes 026:H11, 0103:H2 and 0145:H28 may be better competitors than serotype 0157:H7. For blue-type cheese and uncooked pressed cheese with long ripening time, viable strains were still isolated (after enrichment or not) at day 240. For uncooked pressed cheese with short ripening time, the concentrations of the different STEC strains (ranging from 3.3 to 5 $\log_{10}$ CFU/g depending on the strain) remained constant during ripening and storage until the end, at day 40. For cooked cheese throughout the ripening (120 days) for both the core and the rind, strains could be isolated only after enrichment. For lactic cheese, STEC levels remained detectable after enrichment during ripening and storage (60 days) for four of the eight strains inoculated into the raw goat's milk.	Miszczycha *et al.* (2013)

APPENDIX 7 Comparison of detection methods for *stx* genes by PCR and isolation of STEC in cheese

Cheese type	Methodology	Number of samples	*stx* positive by PCR (%)	STEC culturally recovered (%)	Reference
Raw milk cheese	Enriched samples were screened by PCR for *stx*1 and *stx*2. Isolates were cultured from PCR-positive samples with hybridisation. Isolates were confirmed as *E. coli* by API® (analytical profile index) ID strip range (BioMérieux, Marcy l'Etoile, France).	1,039	13.1	3.1	Vernozy-Rozand *et al*. (2005)
Raw milk soft cheese	Enriched samples were screened by PCR for *stx*1 and *stx*2. Isolates were cultured from PCR-positive samples with hybridisation. Isolates were confirmed as *E. coli* by API®.	80	10.0	6.3	Zweifel *et al*. (2010)
Raw milk semi-hard and hard cheese	Enriched samples were screened by PCR for *stx*1 and *stx*2. Isolates were cultured from PCR-positive samples with hybridisation. Isolates were confirmed as *E. coli* by API®.	1,422	5.4	1.7	Zweifel *et al*. (2010)
Uncooked and soft raw milk cheeses	Enriched samples were screened by PCR for a range of genes, including *stx*. Isolates were obtained by IMS and colony hybridisation, and confirmed as *E. coli* by API®.	400	29.8	3.8	Madic *et al*. (2011)
Cheese – unspecified	Enriched samples were screened by PCR. Isolates were cultured from PCR-positive samples with hybridisation. Isolates were confirmed as *E. coli* by API®.	603	10.0	1.0	Pradel *et al*. (2000)
Raw milk cheese	Enriched samples were screened by PCR. Isolates were cultured from PCR-positive samples with hybridisation. Isolates were confirmed as *E. coli* by API®.	180	30.6	11.7	Fach *et al*. (2001)
Pasteurised cheese	Enriched samples were screened by PCR. Isolates were cultured from PCR-positive samples with hybridisation. Isolates were confirmed as *E. coli* by API®.	45	8.9	2.2	Fach *et al*. (2001)

APPENDIX 8 Results of selected research studies in Ireland on STEC and *E. coli* (*stx*–) on meat carcases and raw meat

Year of sampling	Matrix	Methodology	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2010	Bovine carcase at slaughter plant	Screened by PCR for *stx*1 and *stx*2. Samples PCR positive for *stx*1 and/or *stx*2 were cultured for STEC detection.	450	Strains isolated from *stx*-positive samples serotyped and examined for the presence of genes associated with virulence	**STEC (n=5; 1.1%)** **4 serotypes:** O13:H2 (n=1) O26:H11 (n=2) O113:H4 (n=1) O168:H8 (n=1)	Not investigated	**non-O157 isolates:** *stx*1, *stx*2 and *eae* (n=1) *stx*1 and *eae* (n=2) *stx*1 only (n=1) *stx*2 only (n=1) (other virulence genes were present in these isolates in varying combinations)	Monaghan *et al.* (2012)
2007–2008	Beef carcase at slaughter plant	Screened by real-time PCR for *stx*1 and *stx*2, followed by sero-specific real-time PCR. Isolates cultured from PCR-positive samples.	n=301 carcase swabs analysed for 0157 and 0111 n=402 carcase swabs analysed for 026, 0103 and 0145	0157 026 0111 0103 0145	**STEC (n=4; 1.3%)** **1 serotype:** 0157 (n=4) ***E. coli* (*stx*–) (n=46; 11.4%)** **4 serotypes:** 0157 (n=7) 026 (n=4) 0103 (n=33) 0145 (n=2)	Yes Below limit of detection	**0157 isolates:** *stx*1 or *stx*2 and *eae* (n=4)	Thomas *et al.* (2012)
2012	Beef and sheep minced meat and minced meat products	Not stated	Beef samples (n=172) Sheep samples (n=70)	0156 026 0111 0103 0145	In beef: *E. coli* (*stx*–) 0157 (0.58%) and *E. coli* (*stx*–) 026 (1.16%)	Not investigated	Not stated	Yearsley *et al.* (2011)
2001–2004	Beef carcase at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	132	0157	**STEC 0157 (n=4; 3%)**	Range: 0.7–1.41 $\log_{10}$ CFU/g	**0157 isolates:** *stx*1, *stx*2 and *eae* (n=1) *stx*1 and *eae* (n=2) *stx*2 and *eae* (n=1)	Carney *et al.* (2006)
1997–1998	Beef carcase at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	250	0157	**STEC 0157 (n=0)** ***E. coli* (*stx*–) 0157:H7 (n=4; 1.6%)**	Not investigated	**0157 isolates:** *eae* and *hlyA* (n=4)	McEvoy *et al.* (2003)
2005–2006	Lamb carcases at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	Pre-chill carcase swabs (n=400) Post-chill carcase swabs (n=400)	0157	**STEC 0157 (n=7; 0.9%)** ***E. coli* (*stx*–) 0157:H7 (n=10; 1.25%)** Pre-chill (n=6) Post-chill (n=4)	Not investigated	**0157 isolates:** *stx*1, *stx*2 and *eae* (n=1) *stx*2 and *eae* (n=6) *eae* only (n=3)	Lenahan *et al.* (2007)

Year of sampling	Matrix	Methodology	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2004	Pig carcase at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	480	0157	**STEC 0157 (n=1; 0.21%)**	Not investigated	**0157:H7 isolate** *vt*1, *eaeA* and *hlyA* (n=1)	Lenahan *et al.* (2009)
2001–2004	Beef trimmings at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	1,351	0157	**STEC 0157 (n=31; 2.3%)** ***E. coli* (*stx*–) 0157:H7 (n=32; 2.4%)**	Range: <0.7–1.61 log_{10} CFU/g	**0157 isolates** *stx*1, *stx*2, *eae* and *hlyA* (n=2) *stx*1, *eae* and *hlyA* (n=14) *stx*2, *eae* and *hlyA* (n=15)	Carney *et al.* (2006)
2001–2004	Head meat at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	100	0157	**STEC 0157 (n=3; 3%)**	Range: 0.7–1.00 log_{10} CFU/g	**0157 isolates** *stx*2, *eae* and *hlyA* (n=3)	Carney *et al.* (2006)
2001–2002	Retail minced beef and beef burgers	Culture method to isolate *E. coli* 0157 followed by PCR to confirm virulence genes	1,533	0157	**STEC 0157 (n=43; 2.8%)**	Range: <0.52–4.03 log_{10} CFU/g	**0157:H7 isolates** *stx*1, *stx*2, *eae* and *hlyA* (n=41) *stx*1, *eae* and *hlyA* (n=1) *stx*2, *eae* and *hlyA* (n=1)	Cagney *et al.* (2004)
2004	Retail minced beef	Culture method to isolate *E. coli* 026 and 0111 and PCR to confirm virulence genes	800	026 0111	***E. coli* (*stx*–) 026 (n=2; 0.25%)**	Not investigated	Not detected	Murphy *et al.* (2005)

APPENDIX 9 Results of selected research studies in Ireland on the prevalence of STEC and *E. coli* (*stx*–) on the hide/fleece of food-producing animals

Year of sampling	Matrix	Methodology	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2010	Bovine hide at slaughter plant	Screened by PCR for *stx*1 and *stx*2. Samples positive for *stx*1 and/or *stx*2 cultured for STEC.	450	Strains isolated from *stx*-positive samples serotyped and examined for genes associated with virulence	**STEC (n=25; 5.6%)** ***E. coli* (*stx*–) (n=35; 7.8%)** **10 serotypes:** O5:H- (n=3) O33:H11 (n=1) O55:H11 (n=1) O113:H4 (n=5) O128:H8 (n=14) O136:H12 (n=3) O138:H48 (n=3) O150:H2 (n=1) O168:H8 (n=2) ONT:H11 (n=2)	Not investigated	**non-O157 isolates:** *stx*1, *stx*2 and *eae* (n=1) *stx*1 and *eae* (n=3) *stx*1 only (n=18) *stx*2 only (n=12) *stx*1 and *stx*2 (n=1) (other virulence genes present in these isolates in varying combinations)	Monaghan *et al.* (2012)
2007–2008	Bovine hide at slaughter plant	Screened by real-time PCR for *stx*1 and *stx*2, followed by sero-specific real-time PCR. Isolates cultured from PCR-positive samples.	n=301 hide samples analysed for O157 and O111 n= 402 hide samples analysed for O26, O103 and O145	O157 O26 O111 O103 O145	**STEC (n=55; 13.7%)** ***E. coli* (*stx*–) (n=219; 54.5%)** **4 different serotypes:** O157 (n=63) O26 (n=27) O103 (n=119) O145 (n=10)	Range: below limit of detection to 110 CFU/cm^2	**O157 isolates:** *stx*1 or *stx*2 and *eae* (n=54) **O26 isolates:** *stx*1 or *stx*2 and *eae* (n=1)	Thomas *et al.* (2012)
2008–2009	Sheep fleece at slaughter plant	Screening by real-time PCR for *stx*1 and *stx*2, followed by sero-specific real-time PCR. Isolates cultured from PCR-positive samples.	500	O157 O26 O111 O103 O145	**STEC (n=10; 2%)** ***E. coli* (*stx*–) (n=94; 18.8%)** **4 different serotypes:** O157 (n=4) O26 (n=5) O103 (n=84) O145 (n=1)	O103 in n=6 fleece samples. Range: 120 to 1200 CFU/g fleece. Remainder below limit of detection (<100 CFU/g)	**O157 isolates:** *stx*1 or *stx*2 and *eae* (n=4) **O26 isolates:** *stx*1 or *stx*2 and *eae* (n=5)	Thomas *et al.* (2013)
2001–2004	Bovine hide	Culture method to isolate *E. coli* O157 followed by PCR to confirm serogroup and virulence genes	1,500	O157	**STEC O157 (n=98; 6.5%)** ***E. coli* (*stx*–) O157 (n=109; 7.3%)**	n= 82 samples Range: 0.13 to 4.24 $\log_{10}$ CFU/100 cm^2	**O157 isolates:** *stx*1, *stx*2, *eae* and *hlyA* (n=6) *stx*2, *eae* and *hlyA* (n=91) *stx*1 only (n=1) *eae* and *hlyA* (n=2)	O'Brien *et al.* (2005)
2005–2006	Lamb fleece samples at slaughter plant	Culture method to isolate *E. coli* O157 followed by PCR to confirm serogroup and virulence genes	400	O157	**STEC (n=22; 5.5%)** ***E. coli* (*stx*–) O157 (n=23; 5.75%)**	Not investigated	**O157 isolates:** *stx*1, *stx*2 and *eae* (n=4) *stx*2 and *eae* (n=18) *eae* only (n=1) (other virulence genes present in these isolates in varying combinations)	Lenahan *et al.* (2007)

APPENDIX 10 Results of selected research studies in Ireland on carriage and shedding of STEC and *E. coli* (*stx*–) by food-producing animals

Year of sampling	Matrix	Method	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2014	Bovine recto-anal junction of dairy herd (2 × farms). Repeat samples over 1 year.	Screening of samples using quantitative real-time PCR. Isolates cultured from PCR-positive samples.	**Farm A** (n=305) **Farm B** (n=224)	0157 026	**Farm A:** STEC (n=18; 5.9%) *E. coli* (*stx*–) (n=20; 6.6%) representing 2 different serotypes: 0157 (n=15) 026 (n=5) **Farm B:** STEC (n=16; 7.1%) *E. coli* (*stx*–) (n=17; 7.6%) 2 different serotypes: 0157 (n=8) 026 (n=9)	**Farm A:** 1 animal super-shedding 026 (≥10000 CFU/g) **Farm B:** 2 animals super-shedding 0157 (≥10000 CFU/g) and 1 animal super-shedding 026 (≥10000 CFU/g)	**Farm A 0157 isolates:** *stx*1, *stx*2 and *eae* (n=13), *eae* only (n=2) **Farm A 026 isolates:** *stx*2, *eae* and *hlyA* (n=2) *stx*2 and *eae* (n=3) **Farm B 0157 isolates:** *stx*2 and *eae* (n=5) *eae* and *hlyA* (n=1) *stx*2 only (n=2) **Farm B 026 isolates:** *stx*2 and *eae* (n=3) *stx*1, *stx*2 and *eae* (n=5) *stx*1, *stx*2, *eae* and *hlyA* (n=1)	Murphy *et al.* (2016)
2007–2008	Bovine rectal faecal swabs, milk filters and bulk tank samples on dairy farms (n=60 farms)	Real-time PCR screening of samples for *stx*1 and *stx*2 genes, followed by sero-specific real-time PCR. Isolates cultured from PCR-positive samples.	600 rectal faecal samples, 117 milk filters, 120 bulk tank milk samples	0157 026 0111 0103 0145	**STEC (n=10; 1.2%)** ***E. coli* (*stx*–) (n=57; 6.8%)** **4 different serotypes:** 0157 (n=10) 026 (n=12) 0103 (n=26) 0145 (n=19)	Not investigated	**026 isolates:** *stx*1, *stx*2, *eae* and *hlyA* (n=1) *eae* only (n=3) **0103 isolates:** *stx*1 only (n=1) *eae* and *hlyA* (n=1) **0157 isolates:** *stx*1, *eae* and *hlyA* (n=4)	Lynch *et al.* (2012)

Year of sampling	Matrix	Method	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2008–2009	Bovine faeces and slurry from beef farms (n=12 farms)	Screening of samples by multiplex PCR for *stx*1 and *stx*2, *eae* and *hlyA*. Isolates cultured from PCR-positive samples.	650	Strains isolated from *stx*-positive samples serotyped and examined for genes associated with virulence	**STEC (n=84; 12.9%) 33 different serotypes:** O-:H- (n=3) O-:H10 (n=6) O-:H11(n=2) O-:H12 (n=1) O-:H14 (n=1) O-:H16 (n=1) O-:H18 (n=7) O-:H21 (n=1) O-:H46 (n=1) O-:H48 (n=1) O2:H+ (n=1) O2:H25 (n=1) O2:H27 (n=4) O2:H32 (n=1) O3:H12 (n=1) O26:H11 (n=2) O33:H11 (n=1) O69:H- (n=1) O76:H34 (n=1) O88:H8 (n=1) O113:H4 (n=1) O113:H36 (n=3) O118:H16 (n=1) O136:H12 (n=1) O150:H8 (n=1) O153:H+ (n=1) O153:H40/44 (n=1) O157:H7 (n=26) O157:H16 (n=1) O171:H2 (n=4) OR:H18 (n=1) OX18:H38 (n=2) OX18:H+ (n=3)	Not investigated	**O157:H7 isolates:** *stx*1, *stx*2, *eaeA* and *hlyA* (n=4) *stx*2, *eaeA* and *hlyA* (n=22) **O157:H16 isolates:** *stx*1, *stx*2, *eaeA* and *hlyA* (n=1) **non-O157 isolates:** *stx*1 only (n=3) *stx*1 and *eae* (n=2) *stx*2 only (n=49) *stx*2 and *eae* (n=3) (other virulence genes were present in these isolates in varying combinations)	Ennis *et al.* (2012)

Year of sampling	Matrix	Method	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2007–2008	Bovine faeces and soil on beef farms (n=20 farms)	Screened by PCR for *stx*1 and *stx*2. Samples positive for *stx*1 and/or *stx*2 cultured for STEC.	Faeces (n=1200) Soil (n=600)	Strains isolated from *stx*-positive samples serotyped and examined for the presence of genes associated with virulence	**STEC (n=23; 1.9% faecal and n=4; 0.7% soil samples)** ***E. coli* (*stx*–) (n=107; 5.9%) 21 different serotypes:** O2:H27 (n=13) O6:H8 (n=1) O13/O150:H2 (n=2) O20:H19 (n=1) O26:H11 (n=14) O86:H21 (n=1) O109:H5 (n=1) O113:H4 (n=31) O116:H28 (n=6) O119:H5 (n=6) O136:H2 (n=1) O136:H16 (n=2) O145:H28 (n=1) O168:H8 (n=9) O168:H27 (n=1) O171:H2 (n=4) O174:H21 (n=7) ONT:H4 (n=3) ONT:H17 (n=1) ONT:H18 (n=1) ONT:H27 (n=1)	Not investigated	**non-0157 isolates:** *stx*1 only (n=15) *stx*1 and *eae* (n=10) *stx*2 only (n=44) *stx*1, *stx*2 and *eae* (n=9) *stx*1 and *stx*2 (n=29) (other virulence genes were present in these isolates in varying combinations)	Monaghan *et al.* (2011)
2007–2008	Bovine faeces at slaughter plant	Screened by real-time PCR for *stx*1 and *stx*2, followed by sero-specific real-time PCR. Isolates cultured from PCR-positive samples.	n=301 faecal samples analysed for 0157 and 0111 n=402 faecal samples analysed for 026, 0103 and 0145	0157 026 0111 0103 0145	**STEC (n=20; 5%)** ***E. coli* (*stx*–) (n=53) 4 different serotypes:** 0157 (n=8) 026 (n=8) 0103 (n=34) 0145 (n=3)	Range: Below limit of detection to 1300 CFU/cm^2)	**0157 isolates:** *stx*1 or *stx*2 and *eae* (n=65) **026 isolates:** *stx*1 or *stx*2 and *eae* (n=7) **0103 isolates:** *stx*1 or *stx*2 and *eae* (n=3) **0145 isolates:** *stx*1 or *stx*2 and *eae* (n=3)	Thomas *et al.* (2012)

Year of sampling	Matrix	Method	Number of samples	Serogroup examined	STEC and *E. coli* (*stx*–) serogroup culturally recovered (number of isolates)	Quantitative data	Virulence genes present in cultured isolates (number of isolates)	Reference
2008–2009	Pooled rectal swabs, carcase swabs and minced meat samples at bovine and ovine slaughter plants	Culture method (ISO 16654:2001) to isolate *E. coli* 0157 followed by PCR to confirm serogroup and virulence genes	Bovine rectal swabs (n=304); bovine carcase swabs (n=304) Ovine rectal swabs (n=103); ovine carcase swabs (n=103) Beef minced meat (n=77) and sheep minced meat (n=14)	0157	**STEC 0157 (n=45; 5%)** ***E. coli* (*stx*–) 0157 (n=65; 7.2%)** Bovine rectal swabs (n=31) Bovine carcase swabs (n=17) Ovine rectal swabs (n=7) Ovine pooled carcase swabs (n=7) Bovine minced meat (n=1) Ovine minced meat (n=1)	Not investigated	**0157 isolates:** *stx*2 and *eae* (n=41) *stx*1, *stx*2 and *eae* (n=4) *eae* only (n=9)	Prendergast *et al.* (2011)
2005–2006	Lamb faeces at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm serogroup and virulence genes	400	0157	*E. coli* (*stx*–) 0157 was not detected in ovine faeces (n=0)	Not investigated	Not applicable	Lenahan *et al.* (2007)
2004	Pig faeces at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm serogroup and virulence genes	480	0157	**STEC 0157 (n=3; 0.63%)**	Not investigated	**0157 isolates:** *stx*1, *stx*2, *eae, hlyA, tir, katP, espA, espB, espF, esp, etpD* (n=3)	Lenahan *et al.* (2009)
1997–1998	Bovine faeces at slaughter plant	Culture method to isolate *E. coli* 0157 followed by PCR to confirm serogroup and virulence genes	250	0157	**STEC 0157 (n=6; 2.4%)**	Not investigated	**0157 isolates:** *stx*1, *stx*2, *eae* and *hlyA* (n=2) *stx*1, *eae* and *hlyA* (n=1) *stx*2, *eae* and *hlyA* (n=3)	McEvoy *et al.* (2003)

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