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Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC

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Abstract

The provisional molecular approach, proposed by EFSA in 2013, for the pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) has been reviewed. Analysis of the confirmed reported human STEC infections in the EU/EEA (2012–2017) demonstrated that isolates positive for any of the reported Shiga toxin (Stx) subtypes (and encoding *stx* gene subtypes) may be associated with severe illness (defined as bloody diarrhoea (BD), haemolytic uraemic syndrome (HUS) and/or hospitalisation). Although strains positive for *stx2a* gene showed the highest rates, strains with all other *stx* subtypes, or combinations thereof, were also associated with at least one human case with a severe clinical outcome. Serogroup cannot be used as a predictor of clinical outcome and the presence of the intimin gene (*eae*) is not essential for severe illness. These findings are supported by the published literature, a review of which suggested there was no single or combination of virulence markers associated exclusively with severe illness. Based on available evidence, it was concluded that all STEC strains are pathogenic in humans, capable of causing at least diarrhoea and that all STEC subtypes may be associated with severe illness. Source attribution analysis, based on 'strong evidence' outbreak data in the EU/EEA (2012–2017), suggests that 'bovine meat and products thereof', 'milk and dairy products', 'tap water including well water' and 'vegetables, fruit and products thereof' are the main sources of STEC infections in the EU/EEA, but a ranking between these categories cannot be made as the data are insufficient. Other food commodities are also potentially associated with STEC infections but rank lower. Data gaps are identified, and are primarily caused by the lack of harmonisation in sampling strategies, sampling methods, detection and characterisation methods, data collation and reporting within the EU.

Keywords: STEC, pathogenicity, methods, ranking associated foods, source attribution

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Summary

The European Commission (EC) requested that European Food Safety Authority (EFSA) provides a scientific opinion that reviews the new information available since 2013 and uses this to assess if the molecular approach to defining Shiga toxin-producing *Escherichia coli* (STEC) pathogenicity, proposed in the EFSA STEC opinion published in that year, is still the most appropriate and if not, whether it could be further revised. EFSA was also asked to provide updated information on the methods that may be used to detect and characterise STEC in humans, animals, feed and food and to rank relevant food commodities in terms of their associated STEC risk of human infection. Finally, the European Commission also requested EFSA to identify relevant data gaps when addressing the previous questions and provide recommendations on how these data gaps could be filled.

A literature review was used to gather scientific publications, reports and official documents published between 2012 and 2019. The information obtained was used by the working group (WG) experts to develop the pathogenicity assessment and to describe the detection and typing methods currently available. Ranking food commodities based on the associated public health risk from STEC was performed using source attribution models and data from a range of sources. The data used in this opinion included; [1] the data reported to the European Surveillance System (TESSy) on STEC in humans between 2012 and 2017; [2] the data reported in the zoonoses database on the occurrence of STEC in food, feed and animals (2012–2017) and [3] the data reported in the zoonoses database on the occurrence of strong and weak evidence STEC food-borne and waterborne outbreaks. Furthermore, data were obtained from public health national reference laboratories in 29 EU/EEA Member States (MSs) on STEC in humans and from the national contact points of the National Reference Laboratories in 28 MSs on STEC in food, feed and animals, using questionnaires. Analysis of the information available in the peer-reviewed literature and data in the TESSy database (2012–2017) suggested that serogroup, intimin (*eae*) variant or Stx toxin subtype could not be used to predict clinical outcome. Intimin was present in the majority but not all STEC infections associated with severe illness (bloody diarrhoea (BD), haemolytic uraemic syndrome (HUS) and/or hospitalisation). There is currently insufficient data to test for an association between intimin variant and disease outcome. All Stx toxin subtypes were associated with some cases of severe illness suggesting all STEC strains are potentially associated with BD, HUS and/or hospitalisation. Thus, the molecular approach for categorisation of STEC pathogenicity can be revised with all STEC being considered to be pathogenic and capable of causing severe illness. Future analysis, using tools such as whole genome sequencing (WGS), has the potential to identify virulence genes or gene combinations that are more often associated with severe illness, but any prediction of clinical outcome will always be uncertain as other factors, e.g., gene expression in the bacteria and the immune status of the host are also important.

Current methods for detecting STEC in humans, animal, feed and food samples are described as are their limitations, especially with respect to lack of sensitivity for detecting STEC in food samples and animal faeces. Success in harmonisation of detection methods used for testing food samples has been obtained as most laboratories now use the ISO TS 13136 method. However, currently the method only allows detection of *stx1* and/or *stx2* and does not include *stx* subtyping. Harmonisation of sampling strategies, sampling methods, testing of human and animal samples and strain characterisation across the EU would greatly progress our understanding of this pathogen.

Source attribution analysis based on 'strong evidence outbreaks' suggested that 'bovine meat and products thereof', 'milk and dairy products', 'tap water including well water' and 'vegetables, fruit and products thereof' are the main sources of STEC infections in the EU. However, there are several uncertainties associated with this analysis, as using 'strong evidence outbreak' data only means the data set is limited. Thus, it is not possible to provide a ranking within these four different food commodities. Moreover, other food commodities are also potentially associated with STEC infections, but rank lower.

Despite many years of research and testing of animals, food, feed and human samples, there are still many unanswered questions about STEC. The current data gaps that prevent a more comprehensive assessment of any association between an individual gene or combinations of genes, and disease outcome include comprehensive virulence gene profiles for human STEC isolates (as a minimum, data are lacking on the presence/absence of specific Shiga toxin subtypes) and metadata on humans infected with STEC including age, immune status, therapeutic treatments, medical history, etc. More accurate ranking of foods to target specific food chains for STEC control interventions, including potential microbiological criteria/performance objectives, would require more comprehensive knowledge of the prevalence and concentrations of STEC in animals and food in the EU. Moreover,

data on the sources and transmission routes of STEC infections in humans would have to be identified as would data on person-to-person transmission, dose-response and the virulence gene profiles in STEC isolates from food, feed and animals.

Questionnaires sent to relevant laboratories in the EU on surveillance/testing of STEC in humans and in food, feed and animals provide an important insight into why these data gaps exist and how they could be addressed. STEC testing within the EU should be harmonised including sampling strategies, sampling methods and reporting. All MSs should use the same case definition and outbreak investigation systems. Moreover, it should be a mandatory requirement to report all data (animal, food, feed and human) to EFSA/ECDC. Whole genome sequencing (WGS) technologies would facilitate these improvements for typing strains.

The main conclusions include: [1] the molecular approach, described in EFSA 2013, for the pathogenicity assessment of STEC has been revised to consider Stx subtypes; [2] all STEC strains are pathogenic in humans, capable of causing at least diarrhoea and, based on the analysis of the *stx* subtypes and the presence/absence of the *eae* gene, all STEC subtypes may be associated with severe illness, i.e. HUS, BD and/or hospitalisations; [3] *stx2a* showed the highest rates of HUS, hospitalisation and BD; however, all other *stx* subtypes, or combinations thereof, were also associated with at least one of these severe illness outcomes; [4] the presence of intimin (*eae* gene) was an aggravating factor, but this virulence factor was not always essential for severe illness, suggesting that there is an alternative mechanism of attachment; [5] there is a range of methods (e.g. immunological and molecular methods) available for the detection of STEC but their effectiveness are limited by the need to demonstrate that the signals identified are derived from a live bacterial cell, which, in turn, is affected by lack of selectivity of culture media with respect to the ability to discriminate between STEC and other *E. coli*; [6] at EU level, the methodology for STEC detection in food is substantially harmonised. For example, the international standard ISO TS 13136:2012 has been used to test the 97.4% of the food samples reported to EFSA in 2017; [7] there are a range of characterisation methods including sero, phage and molecular typing. At EU level, the methodology for STEC characterisation in food isolates is not currently standardised, and [8] data on strong evidence outbreaks reported to EFSA from 2012 to 2017 were analysed by an established source attribution method and it was concluded that 'bovine meat and products thereof', 'milk and dairy products', 'tap water including well water' and 'vegetables, fruit and products thereof' are the main food commodities causing human STEC outbreaks. Other food commodities are potentially associated with STEC infections but rank lower.

Recommendations to fill STEC data gaps in the EU include; [1] harmonisation of sampling (surveillance strategies, case definition, number of samples tested, etc.) and testing (although most laboratories in the different MS use the ISO TS 13136:2012 method for testing food and feed samples); [2] MSs should develop national guidelines for the detection of STEC in human samples and the characterisation of isolated strains. Clinical detection and characterisation methods should be harmonised across the EU. Additionally, the use of WGS to type the isolated STEC is strongly recommended; [3] the objectives for STEC surveillance should be changed to ensure all MSs collect data on all STEC cases and not just HUS cases. Public health and other laboratories supporting the healthcare system in MSs should be encouraged to isolate STEC from all STEC patients and undertake WGS analysis using a methodology that is harmonised across the EU. Extensive metadata (age, immune status, therapeutic treatments, medical history, etc.) for each patient infected with STEC in individual MSs should also be collected and all this data/information forwarded to ECDC and [4] the STEC reporting system in the EU should be reviewed focusing on user-friendly and efficient sharing and reporting systems.

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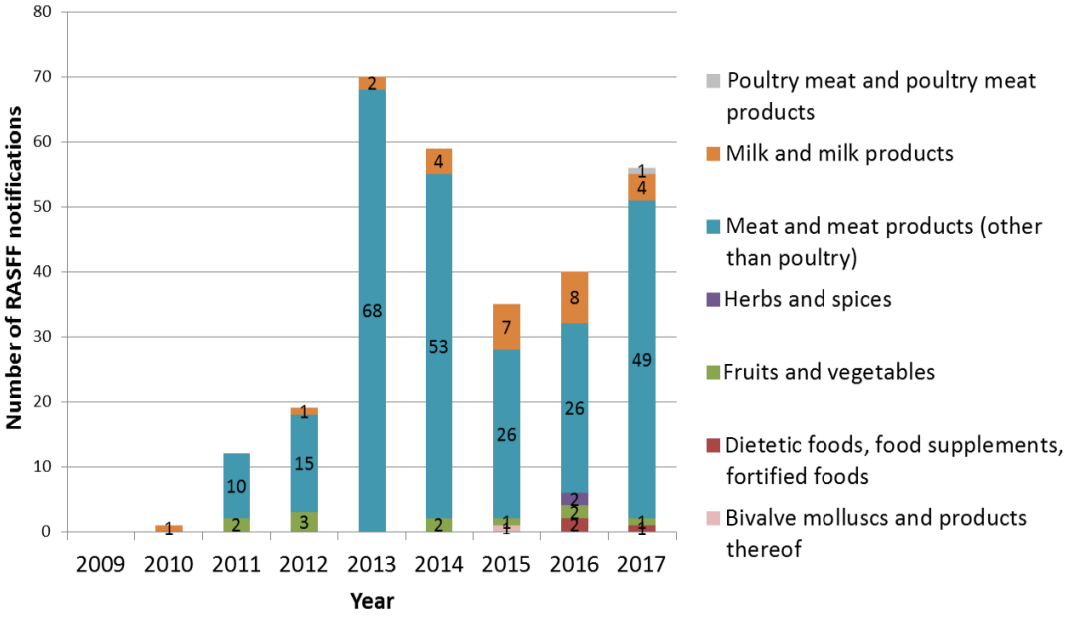
1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Shiga toxin-producing *Escherichia coli* (STEC), also known as verocytotoxin-producing *E. coli* (VTEC), is one of the most common causes of gastrointestinal illness around the world. These food-borne pathogenic bacteria are frequently associated with severe forms of infection including haemorrhagic colitis and haemolytic uraemic syndrome. As reported by the last edition of the European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks, in 2016, most of the STEC foodborne outbreaks with reported known food vehicle were associated with the consumption of food of animal origin (meat, milk and milk products) and with tap water (including well water).

The last major STEC outbreak occurred in 2011 from sprouts and, since then, most Member States (MS) have significantly increased the number of official controls aiming at detecting the presence of STEC in food placed on the market. These controls concern both imported and domestically produced foodstuffs. The following chart shows the number of RASFF notifications issued due to food contamination with STEC by MSs following official controls over the last 10 years.



The complexity of STEC relates to the difficulty of defining when a given strain is or is not pathogenic. The plasticity of its genome, resulting in the acquisition of virulence properties from other organisms, normally by means of translocation on phages, means that new and emerging strains can appear in an unpredictable way over time. The major 2011 outbreak of *E. coli* O104:H4 is an example of the genomic variability referred to above and has challenged the concept of STEC seropathogenicity, in particular the seropathotype approach proposed by Karmali and colleagues in 2003 (Journal of Clinical Microbiology, 41, 4930-4940).

In April 2013, the European Food Safety Authority (EFSA) published a scientific opinion[1] on 'VTEC-seropathotype and scientific criteria regarding pathogenicity assessment'. This opinion acknowledged that the seropathotype classification by Karmali does not define pathogenic STEC. Furthermore, it concluded the impossibility to fully define human pathogenic STEC or identify factors for STEC that absolutely predict the potential to cause human disease. A molecular approach based on genes encoding virulence characteristics additional to the presence of *stx* genes was proposed and could be the basis for a common risk assessment approach, which could assist the Competent Authorities (CA) of MS in conducting a case-by-case assessment when confronted with positive STEC results and in taking the appropriate measures to ensure that the risk for consumers is reduced as much as possible.

[1] https://www.efsa.europa.eu/en/efsajournal/pub/3138

Both the 2007 opinion[2] on 'Monitoring of VTEC and identification of human pathogenic VTEC types', as well as the later 2015 opinion[3] on 'Public health risks associated with Enteroaggregative *E. coli* (EAEC) as food-borne pathogen' gave some recommendations to improve the monitoring of animal populations and foodstuffs for these pathogens.

Except for sprouts, no other STEC food safety criteria have been laid down in Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.[4] Currently there is no harmonised EU risk management approach as regards STEC findings.

At the occasion of the European Commission Working Group (WG) meeting on Microbiological Criteria of 2nd October 2017, the European Commission together with Member States' experts of the WG identified the need: firstly, to update the scientific information on the risk posed by STEC in foods and to carry out risk ranking of different foods; and, secondly, to identify possible control options and considerations on potential microbiological criteria and/or targets at different stages of the food chain.

This mandate is focused on the first need identified above.

The outcome of this mandate should be discussed between risk assessors and risk managers in order to decide how to proceed on a second mandate, which should request (i) the identification of possible control options, and (ii) considerations on potential microbiological criteria/performance objectives and/or targets at different stages of the food chain.

EFSA is asked to provide an update of the scientific opinions on 'monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types' (EFSA Journal 2007;579:1–61) and on 'VTEC-seropathotype and scientific criteria regarding pathogenicity assessment' (EFSA Journal 2013;11(4):3138), with regard to the aspects relevant to the terms of reference below.

In particular EFSA is asked to:

1) Review the new body of knowledge available for pathogenicity assessment of STEC, and refine, if needed, the molecular approach for the categorisation of STEC strains proposed in the EFSA 2013 Opinion.
2) Review the microbiological methods for the detection and characterisation of human pathogenic STEC in animals and food.
3) Analyse available data on human foodborne STEC cases in the EU and rank different food commodities based on the public health risk.
4) To provide recommendations to fill the data gaps identified in the above assessment.

EFSA is invited to liaise with ECDC in the scope of this mandate, in particular to ensure that all relevant human monitoring data are made available to support this mandate.

1.2. Interpretation of the Terms of Reference

In 2013, EFSA published a scientific opinion that concluded the following: (1) the seropathotype approach did not define pathogenic STEC (pathogenicity being defined as 'the ability to cause disease (i.e. harm the host) or otherwise induce pathological change in a susceptible host); (2) it was not possible, given the current state of knowledge, to fully define pathogenic STEC and (3) a molecular approach based on the presence of genes encoding virulence factors could be the basis for future pathogenicity assessment. This molecular approach divided STEC into three groups based on the presence of the *eae* or *aaiC* and *aggR* genes and serogroup as follows:

Group I: *eae*-positive or (*aaiC* and *aggR*)-positive isolates belonging to serogroups O157, O26, O103, O145, O111 and O104 – high risk of diarrhoea and severe illness defined as HUS/HC.

Group II: *eae*-positive or (*aaiC* and *aggR*)-positive isolates belonging to any other serogroup– high risk of diarrhoea and unknown risk of severe illness defined as HUS/HC.

Group III: *eae*-negative and (*aaiC* and *aggR*)-negative isolates belonging to any other serogroup – unknown risk of diarrhoea and severe illness defined as HUS/HC.

At that time, the Panel highlighted that: (i) this proposed molecular approach must be regarded as provisional because the screening of STEC for the presence of *eae, aaiC,I* and *aggR* is not routinely undertaken by all laboratories reporting data to TESSy; and (ii) the potential risk for severe illness needs epidemiological studies for confirmation. The molecular approach proposed in 2013 included *aaiC* and *aggR* genes due to the major 2011 outbreak, which was caused by a highly virulent cross-pathotype (EAEC-STEC) strain carrying these genes.

[2] https://www.efsa.europa.eu/en/efsajournal/pub/579
[3] https://www.efsa.europa.eu/en/efsajournal/pub/4330
[4] OJ L 338, 22.12.2005, p. 1–26.

ToR 1 was interpreted as a request to undertake a pathogenicity assessment, including a review of the new information and analyse the data available since 2013 on STEC pathogenicity in the scientific and grey literature as well as relevant human, animal and food databases. The specific objectives of this review were: [1] to determine if the data now available supported the concept that there are STEC strains (defined by a specific combination of virulence genes) that are/are not associated with severe illness, and [2] if this was the case, to identify the specific virulence gene combinations (including attachment factors) that define pathogenicity. In line with the definition of pathogenicity as an 'ability' to harm the host and not a 'probability' to do that (see Glossary), it was agreed within the Working Group and the BIOHAZ Panel that the predictions about clinical outcome(s) should be provided on an absolute rather than a probabilistic level. For the purpose of this exercise, disease outcomes included diarrhoea (D), bloody diarrhoea (BD), haemolytic uraemic syndrome (HUS) and/or hospitalisation (Hosp), with all of these, except D, being considered as indicative of severe illness. However, due to a lack of data on hemorrhagic colitis (HC), this clinical outcome has been excluded in the analysis of Section 3.1.2.2.

ToR 2 was considered as a request to update the information provided on microbiological methods for the detection and characterisation of human pathogenic STEC in animals and food since the EFSA 2013 Opinion.

ToR 3 was interpreted as a request to rank different relevant food commodities (including drinking water) in the European Union (EU) using relevant recent (2012–2017, inclusive) data, in terms of the risk for humans of becoming infected with STEC as a result of consuming such foods.

ToR 1 (pathogenicity assessment), ToR 2 (microbiological methods) and ToR 3 (risk ranking of foods) require considerable data and information, some of which may not be currently available. Even where data are available, differences in sampling, testing and reporting prevents direct comparison and ToR 4 were interpreted as a request to identify the data and knowledge gaps that might inhibit answering ToRs 1 to 3. ToR 4 was considered as a request to identify the data gaps when addressing ToR 1 to ToR 3. The answer to this ToR was to include recommendations on how data gaps should be addressed.

1.3. Additional information

Escherichia coli are facultative anaerobic Gram-negative rods that form part of the normal gastrointestinal microbiota in humans and animals. Although most are harmless, pathogenic variants cause either enteric (diarrhoeagenic) or extra-intestinal infections in humans. The former are referred to as diarrhoeagenic *E. coli* (DEC) and the latter as extra-intestinal pathogenic *E. coli* (ExPEC). The ExPEC cause urinary tract infections, and (to a lesser extent) peritonitis, mastitis, septicaemia, meningitis and Gram-negative pneumonia.

The diarrhoeagenic *E. coli* are divided into seven pathotypes based on virulence traits and mechanism of pathogenicity and include the Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely Adherent *E. coli* (DAEC) and Adherent Invasive E. coli (AIEC). A description of STEC is provided below with similar information provided for each of the other six pathotypes in Annex A. The main virulence genes and mechanisms of pathogenicity for all *E. coli* pathotypes are further summarised in Table A.1 (also Appendix A).

A complication in this generic *E. coli* pathotype scheme is the emergence of cross-pathotype strains. These may be defined as strains harbouring pathogenicity genes associated with more than one pathovar, e.g. EAEC strains carrying *stx* genes. These arise when the *stx* genes, which are encoded on a bacteriophage, are transferred to other pathotypes of *E. coli*. It could be argued that all STEC are cross-pathotype strains with the majority arising from EPEC. Regardless of the original pathotype, once an *E. coli* strain carries the *stx* gene(s) it may be considered an STEC and thus included in the assessment undertaken in this Opinion.

ToR 1, an assessment of the association between specific virulence gene/gene combinations and severe illness (BD and/or HUS with/without hospitalisation) requires consideration of a range of factors related to the STEC strain, infected human hosts and dose-response, all of which are discussed in this section after a brief description of STEC.

1.3.1. STEC

STEC are zoonotic pathogens, transferred to humans via contaminated food and water. Individual cases and outbreaks have also been associated with direct animal contact (e.g. farm visits),

environmental contamination and human-to-human transfer via the faecal-oral route. STEC infections are characterised by the production of Shiga toxins (Stx), so called because of their similarity with the toxin produced by *Shigella dysenteriae* serotype 1. These are also referred to as verocytoxins (VT) because of their cytotoxicity for Vero cells. There are 2 major types (Stx1 and Stx2) and the *stx* toxin genes are carried by lambdoid bacteriophages integrated into the *E. coli* chromosome. The *stx1* gene has four subtypes (a, c, d and e) and *stx2* has 12 (a to l) (see Section 3.1.2.2). Strains with more than one *stx1* subtype have not been described. However, a given strain may carry an *stx1* and an *stx2* subtype gene, or more than one *stx2* subtype. This is more common when the subtypes are *stx2a*, *stx*2c or *stx2d*.

Many STEC are attaching and effacing (A/E) bacteria, which carry the *eae* gene on the locus of enterocyte effacement (LEE) and form distinctive lesions on the surfaces of intestinal epithelial cells. Moreover, the outcome of infection is dependent on several strain and host factors. Symptoms range from mild uncomplicated to severe bloody diarrhoea and complications may include HUS.

Historically, Enterohaemorrhagic *E. coli* (EHEC) were considered a subset of STEC associated with haemorrhagic colitis. EHEC were usually LEE positive although they may also have encompassed LEE-negative strains within serovars such as O91:H21, O104:H4 and O113:H21, all of which were also associated with haemorrhagic colitis (HC). However, the EHEC terminology is now obsolete. It was based on the previous opinion from the 1980s that only certain types of STEC were able to cause severe disease and that these were homogeneously identified by the presence of *eae*, and the possession of certain LPS. Based on the majority of the currently available literature, this designation should be replaced with STEC.

The most common STEC serogroup associated with human illness is O157 and its molecular mechanisms of pathogenesis have been well studied. Three genetic lineages (I, II and I/II) of *E. coli* O157:H7 have been described. It is suggested that these arose from the result of a geographical spread of an ancestral clone and subsequent regional expansion (Kim et al., 2001; Yang et al., 2004; Franz et al., 2018). More recent studies revealed differences between the three lineages including Stx-encoding bacteriophage insertion sites (Besser et al., 2007), Stx2 expression (Dowd and Williams, 2008) and stress resistance (Lee et al., 2012), as well as lineage-specific polymorphisms (Bono et al., 2007). To investigate the propensity of different STEC O157:H7 strains to cause serious illness, further subtyping schemes have been developed which subdivided the population into nine clades based on single nucleotide polymorphisms (SNPs) (Manning et al., 2008; Riordan et al., 2008). In terms of clade typing, lineage II corresponded to clade 7, lineage I/II corresponded to clade 8 and lineage I corresponded to clades 6 through 1 as suggested previously (Eppinger et al., 2011). Subsequent *in vitro* studies showed varied adherence and virulence factor expression between different clades (Abu-Ali et al., 2010) and whole genome studies elucidated further potential virulence determinants (Eppinger et al., 2011).

The most common non-O157 serogroups associated with human illness in Europe include O26, O103, O91, O146 and O145 and in the USA O26, O45, O103, O111, O121 and O145 (Brooks et al., 2005; USDA, 2012; EFSA and ECDC, 2019). In 2011, O104 caused a major outbreak in Europe. This was a strain consisting of an enteroaggregative *E. coli* (EAEC) strain, probably originating in Africa, that had acquired the Stx2 encoding bacteriophage (Bielaszewska et al., 2011; Frank et al., 2011). Moreover, between 1992 and 2012, four distinct EAEC-STEC strains of different serotypes were described that caused one small outbreak and six sporadic cases of HUS.

The whole genome sequence of the Phi-191 phage found in O111:H10 isolated in France in 1992 was identical to that of the Stx2-phage P13374 present in the EAEC-STEC O104:H4 strain isolated during the 2011 European outbreak 20 years later (Grande et al., 2014). Furthermore, it was also almost identical to those of the other Stx2-phages of EAEC O104:H4 strains described so far and to the phage present in a Stx2-producing EAEC of serotype O127:H4 identified during a small HUS outbreak in Italy in 2013 (Tozzoli et al., 2014). Conversely, the Phi-191 phage appeared to be different from the Stx2-phage carried by the EAEC O111:H21 isolated in Northern Ireland in 2012 (Morabito et al., 1998; Iyoda et al., 2000; Scavia et al., 2008; Dallman et al., 2012). Similar strains have also been identified outside the EU (Carbonari et al., 2019).

1.3.2. Horizontal gene transfer, Stx phages, pathogenicity islands and the emergence of more virulent STEC and cross-pathotype strains

Mobile genetic elements (MGEs) such as plasmids, bacteriophages, transposons, pathogenicity islands (PAIs) and insertion sequence (IS) elements play a major role in the evolution of *E. coli*.

Plasmids are highly diverse and may possess genes for antimicrobial resistance, virulence, regulation and adhesins. Through the process of conjugation, plasmids can transfer small or large fragments of DNA between bacteria and convey those traits to the recipient.

Some bacteriophages have the capacity to mobilise genes, as demonstrated by the enormous fraction of phage particles in faeces that contain bacterial DNA. Through lysogenic conversion of resident intestinal bacteria, phages may introduce new phenotypic traits, such as antimicrobial resistance and the ability to produce exotoxins (Breitbart et al., 2003). Shiga toxin-converting bacteriophages (Stx phages) carry the *stx* gene and have the capability to lysogenise non-pathogenic bacterial strains and convert them into STEC. Stx-phages, therefore, represent highly mobile genetic elements that play an important role in the expression of Stx and in horizontal gene transfer and genome diversification of STEC. One example is the Stx-producing EAEC O104:H4 strain mentioned above. It has been hypothesised that this strain may have originated from a genetically primitive lineage of *E. coli* in a confined geographical area but evolved via several independent streams of horizontal gene exchange (Bezuidt et al., 2011; Bielaszewska et al., 2011; Rasko et al., 2011).

Data from Central Europe and Italy show that O26:H11 strains have evolved from producing Stx1 only, to Stx1 and Stx2, and more recently, Stx2 only. The latter being more virulent than the progenitor Stx1 only strains (Allerberger et al., 2003; Bielaszewska et al., 2007, 2013). In the US, mostly Stx1-producing O26 strains have been found in foods and isolation of the strain producing Stx2 alone strain has thus far, rarely occurred.

Frequent loss of *stx* genes in clinical isolates of STEC have been observed upon subcultivation (Karch et al., 1992) and Stx-negative *E. coli* O157:H7/H- variants may occur at a low frequency in patients with diarrhoea or HUS (Schmidt et al., 1999). The loss and gain of Stx-encoding phages from *E. coli* in the human intestine or during cultivation can result in strains with different pathotypes. Such strains can present challenges to DNA fingerprinting (such as PFGE), resulting in variable diagnostics that has clinical, epidemiological and evolutionary implications.

Free and infectious Stx-encoding phages can be found in high densities in healthy human faecal samples, in environments polluted with human and animal faeces and also in foods (Muniesa and Jofre, 2004; Imamovic and Muniesa, 2011; Martinez-Castillo et al., 2013). Other enterobacterial species known to acquire Stx phages include *Shigella dysenteriae* type 1, *S. flexneri*, *S. sonnei*, *Citrobacter freundii*, *E. albertii*, *Acinetobacter haemolyticus*, *Aeromonas caviae* and *Enterobacter cloacae* (Beutin et al., 1999; Herold et al., 2004; Grotiuz et al., 2006; Alperi and Figueras, 2010; Ooka et al., 2012; Brandal et al., 2015a; Carter et al., 2016; Khalil et al., 2016).

More than 170 PAIs carrying important virulence properties have been annotated as genomic islands (GIs) in the sequences of the STEC O157:H7 strains EDL933 and Sakai (Hayashi et al., 2001; Perna et al., 2001). One of these PAIs carries the locus for enterocyte effacement (LEE) which has the genes necessary for the attaching and effacing lesion. Another PAI, designated O island 122 (OI-122) carries the large virulence gene cluster *efa1-lifA* (Klapproth et al., 2000; Nicholls et al., 2000; Stevens et al., 2002) and has frequently been found in STEC strains associated with severe human disease (Karmali et al., 2003; Morabito et al., 2003; Konczy et al., 2008). OI-122 has multiple other functions and appears to be involved in cell adhesion, immunosuppression, disruption of epithelial barrier function and intestinal colonisation (Klapproth and Meyer, 2009).

PAI OI-57 is also important as it harbours *adfO*, a putative virulence gene for adhesion and *ckf*, which encodes a putative killing factor for the bacterial cell. OI-57 is present in the majority of the STEC genomes and in a proportion of human enteropathogenic *E. coli*, suggesting it could be involved in the attaching-and-effacing colonisation of the intestinal mucosa (Imamovic et al., 2010).

A more complete description of many of the additional MGEs is beyond the scope of this assessment, but a few examples of MGE-derived recombinant strains, also referred to as cross-pathotype strains are described below.

EAEC-STEC: *E. coli* O104:H4 from the major 2011 outbreak in Europe (mainly Germany and France) with Stx2a subtype, pAA (the virulence plasmid encoding genes for AAF/I, AggR and SepA), ESBL antibiotic resistance plasmid, chromosomal genes for Aat (dispersin translocator), SigA (IgA protease-like homologue) and Pic (Serine protease precursor) (Boisen et al., 2014, 2015).

EPEC-STEC: *E. coli* serotypes O26:H11, O55:H9 and O80:H2 with *stx2f* from patients with HUS in Austria and Italy having the EPEC-associated *efa1* gene that resides on the pathogenicity island OI-122, the STEC plasmid genes *ehxA*, *espP* and *katP*, and intimin types ξ (xi) or β (beta) (Grande et al., 2016). A less well-characterised *stx2f*-positive O8:H19 isolate from a patient with HUS in the Netherlands was also positive for the *eae* gene but negative for *ehxA* (Friesema et al., 2015).

ExPEC-STEC: *E. coli* O80:H2 have been reported from France and Spain with *stx2a*, *stx2c* or *stx2d*, intimin gene *eae*-ξ, and at least four genes characteristic of pS88 (*sitA*, *cia*, *hlyF* and *ompT*), and other genes associated with extraintestinal virulence (*iss*, *iroN* and *cvaA* genes) (Soysal et al., 2016). Thirteen O2:H6 strains with sequence type ST141 had *stx2b*, *saa* and ExPEC-associated genes *vat*, *clb* Island, *cdiAB*- and *ybt* clusters; 12 also had *iro* and 10 had α-*hly*, *cnf1*, the *pap* cluster and *hek*, and nine also had *sfa*II cluster (Bielaszewska et al., 2014).

ETEC-STEC: *E. coli* O2:H27 with *stx2a*, *ehxA* and *estIa* (gene for heat stable toxin) was isolated from two people (one had diarrhoea while the other was asymptomatic), and O101:H- with *stx2a*, *ehxA*, *estIa* and *eae* was isolated from a case of HUS in Finland (Nyholm et al., 2015). An *E. coli* O159:HUT, ST171, with *stx2a*, *elt* for heat labile toxin and the ETEC colonisation factor CS12 was isolated from a patient with diarrhoea in Korea (Oh et al., 2017). Four O15:H16, five O175:H28, two O136:H- and one ONT:H16 human clinical isolates from Germany were positive for *stx2g* and *estIa* (the O15:H16 strains were also positive for the plasmid encoded *astA* and *espP*) (Prager et al., 2011). In 2017, an O2:H27 ETEC-STEC cross-pathotype strain, associated with a case of HUS, was identified in Northern Italy (Michelacci et al., 2018). This isolate was LEE negative and carried the *stx2* gene. More recently 4 ETEC-STEC cross-pathotype strains, carrying the *stx2a* and *stx2e* genes, were isolated from diarrhoeal patients in Sweden (Bai et al., 2019).

1.3.3. Human factors

In addition to the characteristics of the STEC strain, there are other factors that influence the occurrence and severity of clinical infection (Russo et al., 2015). The human or host factor is especially important and more than any other factor, age is associated with a higher risk of severe infection with STEC. Multivariate analyses have indicated odds ratios (ORs) for the risk of HUS of 11.4 (Ethelberg et al., 2004), 16.2 (crude) and 10.4 (adjusted) in children ≤ 7 years (Persson et al., 2007), and 9.6, 11.5 and 12.6 for age categories ≤ 5, 6–12 and ≥ 75 respectively (De Rauw et al., 2018). The OR was 16.7 in children ≤ 5 years in Norway in a model including all factors analysed in a multivariate analysis (Brandal et al., 2015b). Age-associated susceptibility has also been observed for different types of STEC. The proportion of patients < 5 years old among patients infected with STEC of the *stx2* (i.e. *stx2a*) genotype was significantly higher than that among patients infected with STEC of the *stx2c* genotype and that among patients infected with STEC harbouring *stx2d* or *stx2e* (Friedrich et al., 2002). In a study of the risk of HUS associated with *stx2d,* patients infected with this Shiga toxin subtype alone, were substantially older (as indicated by median age) than those infected with strains harbouring *stx2d* in combination with other *stx* genes (Bielaszewska et al., 2006).

The impact of human individual susceptibility is also indicated by reports of asymptomatic STEC carriers (Stephan and Untermann, 1999). A study of faecal samples from 5590 asymptomatic workers from the Swiss meat processing industry reported that 3.5% were positive for *stx* genes, 47 STEC strains were isolated of which some also had the *eae* gene, including one isolate of the O157:H7 serotype (Stephan et al., 2000). Similarly, a study from Northern Italy examined faecal samples from 350 asymptomatic farm workers from 276 dairy farms and 50 abattoir workers from 7 different facilities and found 1.1% of the farm workers to have O157:H7 strains that had *eae* and *stx1*, *stx2* or both (Silvestro et al., 2004). All these individuals were adults and although they were asymptomatic, they were considered to pose a health risk to younger individuals. Indeed, in at least one case, an asymptomatic mother with an *eae*-negative O146:H28 *stx2b* strain had transmitted the strain to her child, resulting in neonatal HUS (Stritt et al., 2013).

Other evidence on the effects of human factors include a case from Finland, where an *eae*-negative, *stx1c*-positive O78:H- strain was isolated from the faecal samples of five healthy members of the same family (Lienemann et al., 2012). However, while the parents and the older siblings had no symptoms, the 2-year-old child developed HUS.

A retrospective study in Japan indicated that adult women (older than 20 years of age) were significantly more at risk of developing HUS than adult men, and associated this with significantly more frequent immunodetection of Gb3 in female than in male distal and collecting renal tubules (Fujii et al., 2016).

Patients with underlying diseases or other predisposing factors may also be more susceptible to developing severe disease. For example, a 65-year-old woman died after infection with two rare serotypes, O9ab:H- and O101:H-, both positive for *stx2e* (not usually associated with severe human illness) as she had other underlying illnesses including diarrhoea caused by *Clostridium difficile*, bacteraemia with an O9ab:H- *E. coli* and influenza A (Thomas et al., 1994) and a 65-year old man

under immunosuppressive therapy post transplantation developed HUS with a fatal outcome after infection with an *stx2e*- and *eae*-harbouring O51:H49 strain found both in the stool and in the blood (Fasel et al., 2014). Similarly, the severity of STEC infections can also be due to a synergistic effect with other organisms. In a 2001–2010 survey of 1800 non-O157 infections, 3.6% of the cases were attributed to multiple aetiology infections (Luna-Gierke et al., 2014). In several of these, patients were co-infected with a non-O157 STEC and O157:H7, *Cryptosporidium* or *Campylobacter*.

These examples suggest that human genetics and individual susceptibility (including immunosuppression, underlying disease and comorbidity) can greatly affect disease outcome.

1.3.4. Dose-response for STEC

Stx is the main virulence factor of STEC, but Stx is seldom produced in foods, unless it has undergone severe time-and-temperature abuse sufficient to result in spoilage which will usually render the food unfit for consumption. Significant production of Stx1 in milk and ground beef, when these samples have been subjected to vigorous aeration at 37°C for 48 h, has been demonstrated (Weeratna and Doyle, 1991). However, these conditions are seldom encountered in normal food production processes. Food-borne STEC infections typically occur as a result of ingesting food and/or other vehicles contaminated with STEC, binding of the organism to intestinal epithelial cells and the expression of Shiga toxin. After exposure, the probability of infection depends on the number of STEC cells ingested (Teunis et al., 2004) and also the type of food matrix, which may protect the STEC cells during passage through the gastrointestinal tract. The risk of life-threatening illness in humans and the absence of an animal model that replicates human pathology preclude experimental determination of STEC dose-response. Estimates of dose-response have been made for STEC O157:H7 using outbreak data, specifically the amount of contaminated food consumed by people who did or did not become ill. Exposure estimates have also been reported from three outbreaks as 2–45 cells in salami (Tilden et al., 1996), less than 700 cells in beef patties (Tuttle et al., 1999) and 31–35 cells in pumpkin salad with seafood sauce (Teunis et al., 2004). These estimates are reinforced by reports of STEC O157:H7 levels, expressed either as Colony Forming Units (CFU) or Most Probable Number (MPN), in a variety of foods involved in outbreaks e.g. in raw milk cheeses, 5–10 CFU/g (Strachan et al., 2001) and 0.0037–0.0095 MPN/g (Gill and Oudit, 2015) and in beef patties 1.45 MPN/g (Hara-Kudo and Takatori, 2011) and 0.022 MPN/g (Gill and Huszczynski, 2016). In one food-borne outbreak, one in four children was estimated to be infected upon exposure to a single viable cell of STEC O157, for adults this estimate was one in six (Teunis et al., 2004). The high transmission rate of STEC in childcare centres and among family members also suggests high infectivity. In a report of 3-year-old identical twins that were infected with the same O157:H7 strain but differed in outcomes, where one case resulted in HUS, but not in the other (Inward et al., 1993), the authors speculated that differences in the number of cells ingested may have impacted on the different disease outcomes observed in the twins.

It is unknown whether the dose-response relationship of STEC that use intimin (encoded by *eae*) for attachment varies between strains belonging to different O groups. An investigation of an STEC outbreak involving serotypes O145:H28 and O26:H11 in ice cream found concentrations of 2.4 MPN/g for O145 and 0.03 MPN/g for O26 (Buvens et al., 2011). In an outbreak of STEC O111:H- associated with fermented sausage, the estimated exposure dose was 1 cell per 10 g (Paton et al., 1996). This indicates that the probability of infection upon exposure to other STEC strains may approach that of O157:H7.

While the Stx is the primary virulence factor for STEC, the presence of the Stx-encoding gene does not necessarily indicate expression. STEC pathogenesis is highly complex and involves overexpression of a range of genes including some whose gene product is not directly involved in virulence (e.g. metabolism associated genes) (Gardette et al., 2019). Moreover, the environment in which the bacteria are grown may influence virulence gene expression. Co-culturing O157:H7 strains with commensal *E. coli*, e.g. can increase Stx2 production and the virulence of O157:H7 strains in mice, suggesting that there is a synergistic effect with intestinal microbiota (Goswami et al., 2015). Clade 8 of O157:H7 has been shown to overexpress Stx2 (Ogura et al., 2015) and are more often associated with severe human infections (Neupane et al., 2011).

2. Data and methodologies

2.1. DATA

In this section, the data available for addressing ToRs 1 and 3 are summarised. As some of these data sets were incomplete and/or not considered representative, they were excluded from the assessment (as indicated).

2.1.1. Human STEC data reported to TESSy (2012–2017)

Human cases of STEC infections are reported by EU MSs and EEA countries in accordance with Decision No 1082/2013[5]. The cases are reported annually to The European Surveillance System (TESSy) held by the European Centre for Disease Prevention and Control (ECDC) database in accordance with the EU case definition for STEC/VTEC infections (Decision 2018/945/EU).[6] In 2017, all 28 EU MSs, Iceland and Norway reported STEC data. All countries, except Portugal, have reported STEC human data during 2012–2017. Portugal started reporting in 2015. In 2017, 14 of the 30 EU/EEA countries used the latest case definition (EU, 2012), 9 countries reported in accordance with the previous case definition (EU, 2008) and 7 countries reported using other definitions, or did not specify which case definition they used. Due to differences in national surveillance systems, notification rates are not directly comparable between MSs. However, the notification rate is the closest estimate to a population-based incidence rate in the EU/EEA and currently represents the best available data at the EU level.

Data on STEC from 2012 to 2017 in EU/EEA were extracted on 7 December 2018 from TESSy and used for the pathogenicity assessment (ToR 1). Only confirmed cases, as defined in the EU case definition, were used for analyses. Countries reported their data to TESSy, either from combined notification/laboratory databases or from separate databases for notification and laboratory data. For countries reporting from two separate data sets, the laboratory data set with most complete set of microbiological variables was selected for the analyses to avoid duplicate cases. This comprised 29,945 human cases representing 81% of the total (37,197) STEC cases reported in the EU/EEA from 2012 to 2017. Epidemiological and microbiological variables included in the extraction and further analysed. In this data set, the clinical outcome (D or BD) was reported for 70% of confirmed STEC cases from 28 MSs; HUS status (yes or no) was reported on for 73% of cases from 23 MSs; hospitalisation (yes or no) was reported on for 42% of cases with 21 MSs providing this information; intimin *eae* gene (presence or absence; completeness 60%; number of reporting MSs: 22), *stx1* gene (presence or absence; completeness 64%; number of reporting MSs: 24), *stx1* subtype (completeness of *stx1*-positive cases 19%; number of reporting MSs: 23), *stx2* gene (presence or absence; completeness 64%; number of reporting MSs: 24), *stx2* subtype (completeness of *stx2*-positive cases 24%; number of reporting MSs: 23). These completeness numbers were calculated in terms of proportion of confirmed STEC cases with the information reported for each of the variables considered. The completeness of the data set in terms of the proportion of confirmed STEC cases reported with this information compared to the 3,942 STEC cases with 'full' virulence profile (as understood in this opinion with *eae presence/absence*, *stx1 subtype* and *stx2 subtype)* was for clinical outcome (bloody diarrhoea or diarrhoea; completeness 73%; number of reporting MSs: 10), hospitalisation (completeness 57%; number of reporting MSs: 13) and HUS (completeness 81%; number of reporting MSs: 13).

2.1.2. Occurrence of STEC in food, feed and animals from the zoonoses database (2012–2017)

Monitoring of STEC along the food chain by MS is mandatory under Directive 2003/99/EC[7]. Reporting of data on the occurrence of STEC along the food chain annually to EFSA by MSs and EEA countries is also mandated under the same framework. In the next subsections, the data available

[5] Decision No 1082/2013/EU of the European Parliament and of the Council of 22 October 2013 on serious cross-border threats to health and repealing Decision No 2119. OJ L 293, 5.11.2013, p. 1–15.

[6] Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. OJ L 170, 6.7.2018, p. 1–74.

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

from the testing of sprouts (Regulation (EC) No 2073/2005[8]), monitoring of food and animals (Directive 2003/99/EC[7]) are discussed. Although the data sets were not considered useful for answering ToR 3, they nonetheless were used as indicative of the data gaps that currently exist (ToR 4).

2.1.2.1. STEC data as reported in compliance with Regulation (EC) No 2073/2005, STEC food safety criterion for sprouts at the retail level

The only existing regulatory limit (microbiological criterion) for STEC in a food commodity is set out for sprouted seeds (sprouts) in Regulation (EC) No 2073/2005[8]. This food safety criterion applies to sprouts and the results must be compliant with 'not detected in 25 grams' of STEC O157, O26, O111, O103, O145 and O104:H4, for sprouts placed on the market during their shelf-life. The production of these data is not fully harmonised across MS, because the sampling objectives, the place of sampling and the sampling frequency applied vary or are interpreted differently between MS. Data are also generated by the National Competent Authorities conducting inspections to verify whether the (food business) operators implement correctly the legal requirements and in particular food hygiene. It is important to note that these official monitoring data allow for descriptive summaries to be made at EU level (Boelaert et al., 2016), but they are not suitable for trends analyses, because a reference (study) population is mostly absent and because the sampling, being risk-based, is non-representative.

2.1.2.2. Other STEC monitoring data from foods and animals

The monitoring data regarding STEC in foods other than sprouts and monitoring data regarding STEC in animals originate also from the reporting obligations of MS under Directive 2003/99/EC[7], which stipulates that MSs must investigate the presence of STEC at the most appropriate stage of the food chain. The directive is not explicit about the sampling strategy and the data generated by MSs are based on investigations with non-harmonised sampling and different analytical methods. Moreover, the directive does not indicate strict details of the mandatory reporting requirements. Therefore, STEC monitoring data according to Directive 2003/99/EC[7] are not comparable between MSs and preclude subsequent data analysis like assessing temporal and spatial trends at the EU level. Sampling biases and inaccuracies due to limited numbers of tested samples prevents an estimated of prevalence. The use of laboratory analytical methods testing for STEC O157 only leads to biased STEC prevalence estimations or biased STEC serogroup frequency distributions at the EU level. Thus, the data from the monitoring of STEC in food have not been used to answer to ToR 3 in this opinion.

The latest reported STEC monitoring data, for the year 2017 were on 21,574 units of food (batches or single samples) tested by 25 MS. Compared with 2016, this was a moderate increase in the number of samples tested and in the number of reporting MSs, suggesting an increased awareness at the EU level of the necessity to monitor for this pathogen in food in accordance with EU Directive 2003/99/EC[7]. In 2017, 2,310 units from animals (animals or herds or flocks) were tested for the presence of STEC, confirming the decrease in the testing of animal samples observed in 2016. The variability in the sampling strategies is a serious weakness in the data set and is likely to introduce a selection bias in the estimates of STEC prevalence or STEC serogroup distribution, hindering spatial and temporal trends analyses.

Out of the total number of samples tested in 2017, 98 official single samples taken both at retail and at processing by the Competent Authorities of six MS as part of official controls based on Regulation 2073/2005[8] were reported, with no positive results. During previous years, 2013–2016, zero or a single sample was found to be positive.

The proportion of the top five STEC serogroups (O157, O26, O103, O111 and O145) identified in food and animal samples was estimated by considering only the reported STEC monitoring results obtained using the analytical method CEN ISO TS 13136:2012 (ISO, 2012) which can be used to detect any STEC. This subset of data can be considered homogeneous and may facilitate a more comparable estimation of the level of contamination of the different food categories with these STEC serogroups. In previous years, an increasing trend in the adoption of this standard by the MSs for food testing was observed, and the percentage of food samples tested using the CEN ISO TS 13136:2012 standard (ISO, 2012) in 2016 increased to 91.5% (EFSA and ECDC, 2017). In 2017, this figure increased further to 97.4% (EFSA and ECDC, 2018). The remaining 2.6% of the assays were carried out using methods targeting STEC O157 only.

[8] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

In 2017, 23 MSs provided data on the detection of STEC in food obtained using the CEN ISO TS 13136:2012 method (ISO, 2012) on 21,011 out of the total 21,574 samples analysed. Four hundred samples tested positive for the presence of STEC (1.9%). The STEC belonging to the top five serogroups accounted for 10.7% of the STEC isolated from food (43 out of the 400 isolates reported). The percentages of the top five serogroups reported in food in 2017 were: O157 (0.12% of 21,011 samples tested and 6.2% of all samples that were STEC positive), O103 (0.05% of 21,011 samples tested and 2.7% of the positive samples), O26 (0.02% of 21,011 samples tested), O111 and O145 (< 0.01%).

The data on occurrence of STEC in food and animals reported during the period from 2012 to 2017 were extracted from the EFSA's zoonoses database on 16 November 2018 and was used to answer ToR3.

2.1.3. Occurrence of strong and weak evidence food-borne and waterborne outbreaks where STEC has been implicated (2012–2017)

Monitoring of food-borne outbreaks by MSs and EEA countries and the annual reporting to EFSA is mandatory under Directive 2003/99/EC[7]. Based on this Directive 2003/99/EC[7], the reporting of serotyping and virulence gene information of the causative agent of the food-borne outbreaks is not mandatory and this level of detail is usually not reported to EFSA. Food-borne outbreak data reporting is based on harmonised specifications, which have been increasingly applied in the EU since 2007. The current system is known as the European Union Food-borne Reporting System (EU-FORS) and has been in operation since 2010. Outbreaks are categorised as having 'strong evidence' or 'weak evidence' based on the strength of proof implicating a suspected food vehicle as the cause of the outbreak (EFSA, 2014). For the former, it is compulsory to report a detailed data set, while for the latter this is not mandatory, but voluntary. This categorisation is therefore important to represent the level of uncertainty associated with the identification of the potential implicated vehicle, contributory factors and source. The evaluation of the strength of evidence implicating a suspected food vehicle in food-borne outbreaks as being strong or weak, is based on the assessment of all available types of evidence related to illness and exposure information (i.e. microbiological, epidemiological, descriptive, environmental and based on tracing-back of the investigated foodstuffs) and according to the EU-FORS guidance and the last published manual for reporting on food-borne outbreaks (FBO) (EFSA, 2014). It should be noted that the monitoring of FBO by EU MSs and EEA countries under Directive 2003/99/EC[7] also includes waterborne outbreaks where the implicated vehicle has been identified as tap water, including well water. Water in bottles is considered under another category, i.e. 'Drinks, including bottled water'. Due to the lack of mandatory harmonisation of the national food-borne outbreak investigation systems, differences in the number and type of reported outbreaks are to be interpreted with caution as these may not necessarily reflect the level of food contamination among MS; rather they may indicate differences in the sensitivity of the national surveillance systems in identifying and investigating food-borne outbreaks.

This lack of harmonisation prevents comparison between MSs, even when analysing data originating from mandatory data elements in the reporting tool, such as; data on the outbreak strength (strong evidence food-borne outbreak; yes or no), the causative agents, the food vehicle, the nature of evidence (information on the evidence supporting the food-borne outbreak) and the outcome variables number of outbreaks, the number of human cases (illnesses), the number of hospitalisations and the number of deaths. The reporting tool also provides optional data elements; whether the outbreak was mixed or not, the type of food-borne outbreak (i.e. general/household), the place of consumption/exposure, the place of origin of the problem leading to contamination of food, the origin of the food vehicle and factors that may have contributed (e.g. cross-contamination, inadequate heat treatment, etc.). Even more caution is needed when interpreting these optional data. MSs may not systematically collect or report food-borne outbreak data. For example, MSs may not report on the extent of a food-borne outbreak (general/household) or not report on household outbreaks at all. This reporting (or detection) bias impacts not only on the outbreak reporting rate for these MSs, being on average lower compared to MSs that do report, but also on the mean number of illnesses involved in single outbreaks, a statistic that varies greatly across MS. MS not reporting on household outbreaks were observed to have, on average, a higher mean number of illnesses involved in single outbreaks.

The data on STEC food-borne outbreaks reported during the period from 2012 to 2017 were extracted from the EFSA's zoonoses database on 11 October 2018 and used to answer ToR 3. Only the data on the food vehicles coming from strong evidence outbreaks were used to have a more robust

attribution of the food commodities concerned by STEC contamination. Weak evidence outbreaks are defined by only having weak evidence as regards the particular food vehicle and were not considered suitable for food vehicle ranking.

2.1.4. Other relevant data collected by questionnaire

2.1.4.1. Questionnaire on STEC in humans (Appendix B)

To answer ToR4, information was gathered on the current system and methods for testing and characterisation of STEC in humans using a questionnaire that was drafted by the WG in close collaboration with the ECDC FWD and Zoonoses programme and its' coordination committee (see Annex B). In the questionnaire, the responder was asked to describe the STEC diagnostics system, if there were national guidelines for the detection of STEC in humans, cases/symptoms when STEC is tested for, detection and typing methods, characterisation of the isolates, and reporting STEC typing data to TESSy. The questionnaire was divided into two parts. Part A included multiple choice option questions about primary STEC diagnostics in humans and targeted the local/regional level laboratories and part B covered STEC diagnostics at the public health national reference laboratory level. The questionnaire was circulated to the STEC contact points of the public health national reference laboratories in 30 countries (28 EU MSs, Iceland and Norway) in the FWD and Zoonoses network in January 2019 using the EU survey tool. By the 29 March, 27 of 28 MS, Iceland and Norway had responded to the questionnaire.

2.1.4.2. Questionnaire on STEC in food, feed and animals (Appendix C)

The WG also drafted a questionnaire to obtain data from EU MSs and EEA/EFTA countries on the diagnostics and characterisation of STEC strains isolated from food, feed and animals (Appendix C). The questionnaire included questions about the method used for STEC detection, accreditation of used methods and characterisation of the STEC isolates (virulence gene profiling, serotype antimicrobial resistance (AMR) profile, WGS). The questionnaire was distributed using the EU survey tool on 17 December 2018 to the national contact points of the National Reference Laboratories (NRLs) of 28 MS, Iceland, Norway and Switzerland. By the 28 April 2019, 28 Member States plus Iceland, Norway and Switzerland had replied to the questionnaire.

2.2. Methodologies

2.2.1. Databases for literature reviews

Relevant documents from 2012 to 2017 (inclusive) were identified and reviewed. These included EFSA scientific opinions and reports, guidance documents, ISO standards, scientific papers including review papers, books chapters, non-peer-review papers known by the experts themselves or retrieved through non-systematic searches as well as reports and opinions from different national food authorities on characterisation/source attribution/risk assessment/risk profile of STEC. In addition, manual searching of the reference list of these documents was performed to identify additional relevant information.

2.2.2. Approach for answering ToRs

2.2.2.1. Approach to answer ToR 1 and ToR2

A literature search was used to gather scientific publications, reports and official documents relevant to the ToRs in this opinion. This focused on publications between 2012 and 2019 (inclusive) and was reliant on the experts in the WG searching for relevant peer-reviewed papers, scientific reports, book chapters, etc. that was further developed using 'footnote chasing' until sufficient coverage of the subject area was achieved. Relevant information, publications and suggestions were also provided by members of the EFSA BIOHAZ Panel. For ToR 1 (pathogenicity assessment) information was sought on the different approaches previously developed, and the key concepts were reviewed and developed using new information on the association between specific virulence genes and the clinical outcome of specific cases. The TESSy data (2012–2017, inclusive) were also analysed to identify any association between *stx* subtypes and severe illness. For the purpose of this exercise, disease outcomes included diarrhoea (D), bloody diarrhoea (BD), haemolytic uraemic syndrome (HUS) and/or hospitalisation (Hosp), with all of these, except D, being considered as indicative of severe

illness. Due to a lack of data on haemorrhagic colitis (HC), this clinical outcome was excluded in the analysis of the TESSy data. The molecular approach proposed in 2013 included *aaiC* and *aggR* genes in response to the major 2011 outbreak, which was caused by a highly virulent cross-pathotype (EAEC-STEC) strain carrying these genes. The *aaiC* and *aggR* genes were not included in this Opinion because since 2011, hybrid strains carrying these genes have rarely been associated with causing illness, and there is very limited data on their prevalence in the TESSy database.

For ToR 2 (microbiological methods) information was collated from the scientific literature and other relevant sources on culture based, cell culture, immunological and molecular/PCR methods used for testing different sample types. Testing methodologies as well as approaches for strain characterisation and typing were reviewed using the expertise and experience in the WG.

2.2.2.2. Approach to answer ToR3

To answer ToR 3, the ranking of food commodities based on the associated public health risk from STEC in the EU/EEA during 2012–2017 was performed using human food-borne illness source attribution models. Source attribution is defined as the partitioning of the human disease burden of one or more food-borne illnesses to specific sources, in this case food commodities (Pires et al., 2009). Source attribution methods analyse data from food/animal monitoring and/or public health registries to estimate the relative contribution of different sources for disease. Source attribution approaches for STEC were suggested in a review of the applicability of source attribution methods for different pathogens (Pires, 2013). Based on this review, and after an overview of the available data and review of recent studies, an epidemiological method, namely analysis of data from outbreak investigations, was selected. The source attribution method applied was based on a previously published method (Pires et al., 2012), modified and applied to the STEC data set. The principle was to attribute human illnesses to food sources on the basis of the number of outbreaks that were caused by each of these foods.

Data on outbreak investigations reported to EFSA from 2012 until the end of 2017 were analysed. This included recent data collected after the major 2011 outbreak, when MSs became more aware of the problem and better data became available. As previously stated, only data on outbreaks reported with strong evidence were used, as those reported with weak evidence were not sufficiently informative. Due to the limited availability of data, there was no segregation by country or EU region. The results of the analysis were compared with the output of recently published studies that provide information on the sources of STEC infections, including global and regional analysis of STEC outbreak data, a systematic review of case–control studies of sporadic infections, and the source attribution based on expert elicitations performed by WHO/FERG (Hald et al., 2016; Devleesschauwer et al., 2019; Pires et al., 2019).

To categorise foods, the food categorisation scheme defined under the Zoonoses Catalogue was applied (Table D.1 in Appendix D). The level of subcategorisation within each main food category varied. For example, meat and meat products were subdivided into 'bovine meat and products thereof', 'pig meat and products thereof' and 'broiler meat and products thereof' but all fruits and all vegetables were grouped in a category 'vegetables, fruit and products thereof'. Food categories in the catalogue that do not correspond to single food ingredients, specifically 'bakery products', 'buffet meals', 'mixed food', 'other foods', 'sweets and chocolate', 'canned food products' and 'drinks, including bottled water', were not included in the model. Type of processing or degree of cooking (i.e. raw, undercooked, well done) was not included in the categorisation scheme. Seven outbreaks that reported only one human case for each of these outbreaks were excluded because they did not match the definition of outbreak. The data set included a majority (52 out of 57) of outbreaks associated with 'simple foods' (i.e. foods constituted by ingredients belonging to one food category), with a few outbreaks associated with 'complex foods' (i.e. foods constituted by ingredients belonging to multiple food categories). Including outbreaks associated with complex foods in the analysis would require partitioning these to the food categories with highest likelihood of causing the outbreaks and estimating underlying uncertainty. However, as the number of complex food-associated outbreaks was limited, only simple food commodities that were considered feasible as a source for STEC were included in the analysis. Also due to lack of data, all outbreaks were considered equally important and the relative importance of the food sources implicated in outbreaks causing severe illness (i.e. HUS) or fatalities was not estimated.

We defined attribution proportions as the probability that a given outbreak was caused by a specific source, defined as the proportion of outbreaks caused by each source across all countries and the whole study period. Hence, the number of ill people implicated in the outbreaks was not considered in the analysis to avoid potential overestimation of the importance of sources that caused large

outbreaks. Pires et al. (2010) compared the results of source attribution analyses using outbreak data reported to EFSA performed by the number of ill people implicated in reported outbreaks and by the number of outbreaks, and concluded that the first may lead to an overestimation of the importance of the sources when extrapolating to the whole population. This can happen when the data are sparse, if some of the reported outbreaks are very large, or if the sources causing outbreaks and sporadic cases in general are different.

The uncertainty in the defined probability was quantified using a Dirichlet distribution, informed by S_i, the number of single-food outbreaks caused by a given source *i.* To allow for estimating the uncertainty around attribution proportion estimates for sources that have not been implicated in any outbreak, the Dirichlet was used with a uniform prior density, which means that it is assumed that all sources that were regarded as a feasible source of an STEC outbreak were considered equally likely as a source for an STEC outbreak, before considering the outbreak data. Hence, we defined the probability distribution as Dirichlet (S_1 + 1, S_2 + 1, …, S_k + 1) for all k sources considered, k corresponding to all food categories in the Zoonoses Catalogue (Walley, 1996). We used a Monte Carlo simulation approach to estimate the uncertainty around attribution proportions. This simulation was implemented using the software R 3.5.1 (R Core Team, 2018); the source code is given in Appendix E. The resulting uncertainty distribution was summarised by its mean and a 95% uncertainty interval (UI) given by the distribution's 2.5th and 97.5th percentiles.

The model was implemented using the software R 3.5.1 (R Core Team, 2018).

2.2.2.3. Approach to answer ToR4

The answer to ToR 4 (gap analysis) was based on the limitations (information and hard data) encountered when addressing the other ToRs and the answers to two questionnaires; [1] STEC monitoring in food, feed and animals sent to the STEC EURL network of NRLs and [2] STEC monitoring in humans sent to the ECDC's food and waterborne disease (FWD) and zoonoses network and the public health NRLs. In addition to identify data gaps when addressing ToR 1 to ToR 3, recommendations were provided on how these data gaps should be filled.

2.2.2.4. Uncertainty analysis

The uncertainty in this Opinion was investigated in a qualitative manner following the procedure detailed in the EFSA guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018). The sources of the main uncertainties were identified, and for each of these, the nature or cause of the uncertainty was described by the experts (Appendix F).

For ToR 3, uncertainty intervals were derived for the source attribution proportions. As the source attribution performed is based on a limited data set in which several MSs are underrepresented, and has, for example, the underlying assumption that outbreaks are representative for all STEC cases, the ranking obtained may be biased and the factual uncertainty about the ranking of food commodities may be larger than expected. This was taken into account by combining the results obtained in the source attribution with results from other studies, where the working group made an informal expert judgement on the final ranking.

3. Assessment

3.1. Pathogenicity assessment of STEC

3.1.1. Serotype

In the EU, the top five serogroups in human STEC infections in the period under review were O157, O26, O103, O91 and O145 (TESSy data, 2012–2017). Serogroups most frequently associated with severe STEC infections (HUS, hospitalisation or BD) were O157 and O26. Serogroups O111, O80 and O145 were among the five-most-commonly reported serogroups in HUS cases, serogroups O145, O103 and O111 in the hospitalised cases, and serogroups O103, O145 and O91 in BD cases (Table 1). In total, 49, 88 and 95 different O-serogroups were reported in HUS, hospitalised and BD cases, respectively.

Table 1: STEC serogroup distribution for human cases associated with HUS, BD or hospitalisation (TESSY data, 2012–2017)

Serogroup	Number of HUS cases	%	Serogroup	Number of hospitalised cases	%	Serogroup	Number of BD cases	%
157	634	38.4	**157**	2,753	60.4	**157**	4,245	71.6
26	403	24.4	**26**	705	15.5	**26**	582	9.8
111	85	5.1	**145**	137	3.0	**103**	162	2.7
80	74	4.5	**103**	107	2.4	**145**	159	2.7
145	68	4.1	**111**	97	2.1	**91**	64	1.1
55	48	2.9	**146**	51	1.1	**146**	51	0.9
121	44	2.7	**91**	33	0.7	**111**	49	0.8
103	42	2.5	**55**	32	0.7	**128**	32	0.5
91	17	1.0	**5**	26	0.6	**5**	28	0.5
104	6	0.4	**174**	21	0.5	**55**	27	0.5
Other	232	14.0	**Other**	598	13.1	**Other**	532	9.0
Total	1,653	100.0	**Total**	4,560	100.0	**Total**	5,931	100.0

In the USA, the main STEC serogroups associated with human illness are O157, O26, O45, O103, O111, O121 and O145 (CDC (2018).The detection of any of these STEC serogroups in food has been considered to provide an early indication of the potential for serious illness, if that food is consumed without further treatment that inactivates *E. coli*. However, the presence of serogroups other than those previously mentioned cannot be taken as indicative of the presence of non-pathogenic STEC. An early STEC seropathotype classification, developed by Karmali and colleagues, was based on serotype association with human epidemiology and haemolytic uraemic syndrome (HUS) (Karmali et al., 2003). STEC O157:H7 and O157:NM, which are associated with large outbreaks and cause HUS were assigned to seropathotype A. O26:H11, O103:H2, O111:NM, O121:H19 and O145:NM which also cause large outbreaks (but less often than O157) and HUS were assigned to seropathotype B while O91:H21, O104:H21, O113:H21, O5:NM, O121:NM and O165:H25 found in sporadic cases (including HUS) were considered to be seropathotype C strains. Seropathotype D included serotypes associated with diarrhoea but not outbreaks or HUS while E strains had never been associated with human illness. In 2011, the BIOHAZ Panel concluded 'that the Karmali seropathotype classification does not define pathogenic STEC nor does it provide an exhaustive list of pathogenic serotypes' (EFSA BIOHAZ Panel, 2013b). This conclusion was based on the fact that the higher prevalence of certain serogroups in severe disease cases may be due to a higher prevalence in the environment, animals and/or food. The data may also be skewed by bias in detection with testing methods more suitable for detecting certain serogroups such as O157. Moreover, STEC virulence genes are often present on mobile genetic elements, which can be lost or transferred, and the same serotype often carries different virulence genes and hence can cause different kinds of disease. One example is O26:H11, which has shifted *stx* profile from a rarely HUS-associated *stx1* profile to a more common HUS-associated *stx2a* profile over the past two decades). O157:H7 is another example, where the hypervirulent (and HUS associated) clade 8 has a particular Stx2a encoding bacteriophage inserted into insertion site *argW* on the chromosome whereas the clade 7, which is rarely associated with HUS, has an Stx2c encoding phage inserted into *sbcB* (Ogura et al., 2015) (as described below). Just O grouping – without the full serotype – can be even more misleading. For example, the O group O145 has been shown to comprise at least three different serotypes: STEC O145:H28 and O145:H25 that possess distinct *eae* variants γ and θ, respectively (Sonntag et al., 2004), and an atypical EPEC O145:H34 with the full LEE pathogenicity Island (Carter and Pham, 2018).

Thus, while serotype is important in epidemiological tracking, including incidence, emergence of new clones, and in the detection and investigation of outbreaks, it is not possible to exclude pathogenicity or the possibility of severe illness based on serogroup. All serogroups should therefore be considered to be pathogenic and potentially associated with severe illness.

3.1.2. Virulence genes

STEC infection is a complex process involving the expression of many genes. It starts with ingestion of sufficient bacterial cells to cause illness. Once ingested, these cells must pass through the stomach and small intestine, where survival is dependent on the *ure*, *ecf, katP* and the *stcE* gene products. Upon reaching the large intestine attachment occurs, initiated by contact with enterocytes through fimbrial adhesins encoded by *hcp*, *ecp* and *efa* which triggers expression of the LEE genes (*eae*, *tir*, *espA*, *espB*, *espC* and *espD*) which encode for the formation of attaching and effacing (A/E) lesions. In non-LEE STEC other genes, such as those encoded on the pO113 megaplasmid (*saa*, *lpf* and *sab* genes) may provide an alternative mechanism for attachment. Once attached the STEC cells must multiply, necessitating the expression of a range of genes involved in metabolism and related functions (*araB, nirB*, *gabT*, *glpB*, *trpA*, *ybaT*, *yjeH*, *agaW, btuR*, *pdxA*, *azoR*, *fadA*, *yjbB*, *caa*, *mhpR*, *araC*, *ascG*, *yjiR*, *nor*, *ytfE*, *yhiI*, *mdtE*, *mdtM* (*yjiO*), *yhbU*, *yghU*, *degQ*, etc. (Gardette et al., 2019)). At this stage, the STEC cells may produce Shiga toxin encoded by the *stx* genes.

The minimum combination of genes required to cause severe illness is unknown and even if it was established there are many contributory factors such as gene expression levels, alternative genes performing similar functions, host factors (as previously discussed), etc. However, investigative studies in combination with epidemiological data suggest general trends, especially in the relationship between adherence, Shiga toxin genes and other virulence genes or gene combinations and disease outcome. These will be described in the following subsections.

3.1.2.1. Adherence factors

The vast majority of STEC known to cause HC or HUS have virulence factors that enable attachment to intestinal epithelial cells, and these adherence factors are generally considered essential for severe illness and perhaps even for non-bloody diarrhoea. The principal adherence factor in STEC is the intimin protein coded by the *eae* gene that resides on the locus of enterocyte effacement (LEE) pathogenicity island. Intimin is also a virulence factor of Enteropathogenic *E. coli* (EPEC) and it is crucial in the A/E lesion that has been demonstrated for EPEC and LEE-positive STEC strains (Kaper et al., 2004). The *eae* gene is highly polymorphic, with over 34 different genetic variants (alleles) (Horcajo et al., 2012; Lacher et al., 2016) designated by Greek letters. For example, STEC O157:H7 carries the γ (gamma)-*eae* allele, O26:H11 often have ß- (beta)-*eae* and O121:H19 have ε (epsilon)-*eae*. However, the prevalence of the different intimin variants is highly variable and there is very limited data on the association of different intimin types and the severity of disease. Thus, there is currently insufficient data to assess whether or not specific variants are associated with specific disease outcomes.

The initial stages of infection include: 1) connection of the cytoplasm of the STEC cell to the cytosol of the host enterocyte using a type III secretion system (TTSS), which is essentially a molecular syringe through which secreted proteins pass from the bacterial cells to the host cell cytoplasm; 2) passage of the Tir (translocated intimin receptor) protein (*tir/espE* gene product) through the TTSS and insertion into the host cell membrane; 3) formation of an intimin bridge between the bacterial and host cell; 4) injection of a range of proteins encoded by *rtx*, *hlyA, fepC* and *efa1/lifA* and a range of non-LEE effector molecules into the host cell which serve a variety of functions including haemolysis, repression of the host lymphocyte response, inhibition of phagocytosis, invasion, cytotoxicity and iron transportation; 5) activation of the signalling pathways in the host cell which results in the destruction of microvilli and the remodelling of the host cell cytoskeleton into pedestals. These are known as A/E lesions which mediate intimate attachment between the STEC and host cell, often with accumulation of polymerised actin directly beneath the adherent bacteria.

LEE-negative (i.e. *eae*-negative) STEC have also been implicated as causes of dysentery and HUS (Newton et al., 2009). Indeed, an STEC O113:H21 strain was first isolated from a child with HUS in 1983 (Karmali et al., 1983) and this serotype later caused a cluster of HUS cases in Australia (Paton et al., 2001). STEC O91:H21 strains that are also LEE-negative have been implicated in HUS in Germany (Mellmann et al., 2009). LEE-negative STEC strains probably have other means or mechanisms for adherence (Dytoc et al., 1994). The O113:H21 strains have the STEC agglutinating adhesin (Saa) (Paton et al., 2001). Saa was also found in 13 cross-pathotype ExPEC-STEC strains of serotype O2:H6 from cases of UTI and diarrhoea (Bielaszewska et al., 2014). However, there is no significant correlation between the presence of Saa and HUS in humans (Jenkins et al., 2003).

A small subset of STEC carries *aggR* encoding a bacterial transcriptional regulator, and the defining factor for typical EAEC strains. *aggR* is located on the pAA plasmid and controls the expression of the

aggregative adherence fimbriae (AAF), dispersin, the dispersin translocator Aat and the Aai type VI secretion system. AAFs are regarded as the principle adhesin in *E. coli* harbouring *aggR*.

The *sab* gene that codes for an outer membrane autotransporter protein that enhances biofilm formation (Herold et al., 2009) is also thought to be an adherence factor. Molecular characterisation of other STEC strains have identified *paa*, *efa1*, *ompA*, *lpfA* and other genes that code for adhesins (Kaper et al., 2004).

The plasmid-borne *toxB* gene also codes for an adhesin and is found in O157:H7 and many LEE-positive STEC, including strains of O26, O121 and O145 O groups as well as in EPEC (Tozzoli et al., 2005). The *toxB* gene-encoded adhesin is thought to contribute to the adherence properties of O157:H7 serotype. However, like the other adhesin genes mentioned, the precise role of these factors in the virulence mechanism of LEE-negative STEC strains has not been fully determined, so are often regarded as putative virulence factors and their prevalence varies among STEC strains (Feng et al., 2017).

More recently, a report has described an 86-kb mosaic PAI composed of four modules that encode 80 genes, including novel and known virulence factors associated with adherence and autoaggregation (Montero et al., 2017). The PAI has been named Locus of Adhesion and Autoaggregation (LAA), and phylogenomic analysis using whole genome sequencing (WGS) shows that LAA PAI appears to be exclusively present in a subset of emerging LEE-negative STEC strains, including strains isolated from HC and HUS cases. The authors suggest that the acquisition of LAA PAI is a recent evolutionary event, which may have contributed to the emergence of these STEC strains (Montero et al., 2017).

Based on the above information, it may be concluded that while the majority of STEC associated with severe illness (BD, HC, HUS, hospitalisation and/or death) carry the *eae* gene, there are alternative mechanisms of attachment and many cases involving *eae*-negative strains have also been associated with severe illness. Moreover, *eae*-positive strains have caused mild illness (D). Thus, *eae* is not a definitive marker for severe illness but may, in combination with other virulence genes, be considered a general indicator that a given strain has a high potential to cause severe illness.

3.1.2.2. Shiga toxin subtypes

3.1.2.2.1. Information from the peer-reviewed publications

STEC are characterised by the production of Shiga toxins (Stx), located on lysogenic lambdoid bacteriophages (Stx-phages), or remnants thereof. The genes encoding these toxins are expressed primarily when the phage is replicating during the lytic cycle (Wagner et al., 2001), which may be triggered after exposure to DNA-damaging agents or certain antibiotics. Stx1 phages have an additional regulator (p*stx1*), which is modulated by the iron concentration in the surrounding environment (Wagner et al., 2002). Shiga toxins are AB5 protein toxins consisting of a single enzymatic A subunit in non-covalent association with five identical B subunits, which are responsible for binding to the Gb3 cellular receptor found in several organs including the kidneys, liver, brain and pancreas. Once bound to the receptor, the A subunit (which functions as an RNA N-glycosidase) is internalised and inhibits protein synthesis by removing an adenine base from the 28S ribonucleic acid of the 60S ribosomal subunit (Gyles, 2007), the underlying cause of HUS or kidney damage. It is generally accepted that there are two main types, designated Stx1 and Stx2, with three Stx1 (Stx1a, Stx1c and Stx1d) and seven Stx2 (Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g) subtypes reported (Scheutz et al., 2012). However, as outlined below, the total number of subtypes is – at the time of this publication – up to four Stx1 subtypes (Stx1a, Stx1c, Stx1d and Stx1e) and 12 Stx2 subtypes (Stx2a–Stx2l). The nomenclature of the Stx toxins and subtypes is based on comparison of the amino acid sequences. However, the typing uses DNA methodology, either as PCR or WGS and is therefore based on the nucleotide sequences and designated as *stx* subtypes and variants. Based on similarity analyses of both nucleotide and amino acid sequences, other subtypes have been proposed. A novel subtype of Stx1, Stx1e (accession number KF926684), with limited reactivity with anti-Stx1 antibodies has been found in *Enterobacter cloacae* but not in *E. coli* (Probert et al., 2014). Also, provisional designations have been proposed (Lacher et al., 2016) for two new Stx2 subtypes, *stx2h* (GenBank AM904726) and *stx2i* (GenBank FN252457), but the proposed sequence of *stx2h* (AM904726) was found to be identical to the already published subtype *stx2e*-O8-FHI-1106-1092 (Scheutz et al., 2012).

More recent analyses of new types submitted either in the public domain or to the International Centre for Reference and Research at SSI in Copenhagen and maximum parsimony tree for both the

nucleotide (nn) and the amino acid (AA) sequences indicate that there are four new *stx2* subtypes and that the *stx2e*-O8-FHI-1106-1092 would require to be re-designated:

- Stx2h-O102-STEC293 was published by Bai et al. (2018)
- Stx2i-ONT-CB10366, Acc. No. FN252457 mentioned in Lacher et al. (2016)
- Stx2j-ONT-5447 submitted by an American group (Xiong Wang Ph.D, Sequencing and Bioinformatics Unit, Minnesota Department of Health, USA, confirmed this by e-mail 25.02.2019, personal communication).[9] The highest similarity is to nn *stx2k*-O159-12GZSW01 at 90.2 and to many of the *stx2d* subtypes. The AA similarity is 94.0 to Stx2k-O159-12GZSW01 and again similarities up to 94.4 to some of the *stx2d* subtypes.
- Stx2k-O159-12GZSW01 (Acc. No. KC339670) submitted by Meng et al. (2013).

A specific subtype of *stx2e* designated *stx2e*-O8-FHI-1106-1092 has identical primer sequences with *stx2a* and differs only by the last nucleotide in the reverse primer sequence for *stx2d* designed by Scheutz et al. (2012). The subtype *stx2e*-O8-FHI-1106-1092 has hitherto not been considered to be clinically relevant. Furthermore, it has been observed that the *stx2e* gene in the reference strain for *stx2e*-O8-FHI-1106-1092 was often lost during subculturing. It was therefore assumed that this subtype was quite rare. However, the subtype has since been reported from cases of diarrhoea in Norway (Lin C. T. Brandal, senior scientist at the Norwegian Institute of Public Health, Oslo, Norway, confirmed this by e-mail 20.02.2019, personal communication),[10] where it was originally identified. The Norwegian strains are of serotypes O8:H9, O8:H19 and O8:H30. This *stx* subtype was also found in isolates from five cases of diarrhoea in Denmark (unpublished) all in the same serotype O8:H9. The reference strain for *stx2e* O8-FHI-1106-1092 was O8:H2, has not been isolated from patients with diarrhoea. As mentioned above, a provisional designation was proposed by Lacher et al. (2016) for a new Stx2 subtype, *stx2h* (GenBank AM904726), identical to the subtype *stx2e*-O8-FHI-1106-1092 (Scheutz et al., 2012). As Stx2h had already been published (Bai et al., 2018), this particular subtype will have to be given a new designation, Stx2l, and primers will have to be designed for its detection. Preliminary testing – as well as the original testing of the reference strain (Scheutz et al., 2012) – has shown that the Danish isolates with the *stx2e*-O8-FHI-1106-1092 subtype reacts with both the *stx2a* and *stx2d* primers. As these two subtypes are significantly associated with HUS, this needs to be resolved, and would require additional validation and sequence analyses beyond the scope of this report but is underway. Such a validation should also include the other five subtypes. At present, there is no validated protocol for the detection and subtyping of these new *stx1e* and *stx2h-stx2l* subtypes. Hence, their clinical and epidemiologic relevance as well as the prevalence need to be further studied. This requires a revised protocol for their detection. Furthermore, if a protocol for their detection can be developed, new variables should be created in TESSy, which at the moment only accepts submission of *stx1a*, *stx1c* and *stx1d*, and *stx2a-stx2g*. In summary, one new *stx1e* subtype and five new *stx2* subtypes are recognised resulting in a total of four *stx1* and 12 *stx2* subtypes. Novel subtypes are likely to occur and need to be included in future detection methodology as well as to the reporting in the EU.

STEC strains can produce any of the Stx or combination of Stx subtypes but not all subtypes have been implicated in severe illness (Martin and Beutin, 2011; Hofer et al., 2012). For example, among the Stx1 group, little is known about the clinical significance the *stx1d* subtype, which is commonly found in animals but rarely in humans (Kumar et al., 2012). The Stx1a subtype is often produced by LEE-positive strains that have caused severe infections, including O157:H7, O26:H11, O111:H8 and others. Brooks et al. (2005) showed that 83% of O26, 50% of O111 and 100% of O103 strains that caused HC in the U.S. had *stx1* and *eae*; of these only one O111 strain was implicated in HUS. Consistent with these observations, O103:H2 is the second most common STEC causing infection in Norway, but is not associated with HUS (Naseer et al., 2017). These three O groups have been declared as adulterants in raw non-intact beef and intact beef products in the U.S.A. Some STEC serotypes with *stx1a* and *eae* are found in foods (Feng and Reddy, 2013) but have not been implicated in human infections. Stx1c is the most common subtype in strains isolated from sheep, wild deer and wildlife meats (Brett et al., 2003; Hofer et al., 2012; Mora et al., 2012). At least some of these specific strains do not produce intimin and are associated with asymptomatic infection or mild diarrhoea (Friedrich et al., 2003). STEC with *stx1c,* either alone or together with *stx2b*, is often isolated from wild ruminants. However, some studies have reported that 10–15% of human clinical samples from

[9] RE: A rare stx2 subtype from Non-O157 STEC. Message to Felmming Scheutz. 25.02.2019. Email.
[10] Re: SV: Detaljer om stx2l. Message to Flemming Scheutz. 20.2.2018. Email.

diarrhoeal illnesses are positive for these *stx1* subtypes (Buvens et al., 2012; de Boer et al., 2015; Brandal et al., 2015b; Fierz et al., 2017).

Studies have shown Stx2 to be more important than Stx1 in the development of HUS (Donohue-Rolfe et al., 2000). There is conflicting evidence about the *stx2* subtypes and their association with severe disease. This is further complicated by the fact that some *stx2* subtypes share high gene sequence similarities and may have been misidentified. Thus, the nomenclature for *stx* subtypes is continually being refined. Increasing use of WGS should help to clarify the associations of *stx* subtypes with severe diseases. WGS has also indicated that different *stx* subtypes are associated with different virulence profiles.

Analysis of STEC in Europe showed that *stx2b*, alone or together with *stx1c* is common in STEC from deer droppings and wildlife populations (Hofer et al., 2012; Mora et al., 2012), but did not appear to cause severe human illness (Buvens et al., 2012; de Boer et al., 2015; Brandal et al., 2015b; Fierz et al., 2017). Consistent with this finding, *stx2b*, was found in cross-pathotype ExPEC-STEC strains of serotype O2:H6 that have been associated with both urinary tract infection (UTI) and diarrhoea (Bielaszewska et al., 2014).

The *stx2e* subtype is mostly found in isolates from pigs and pork meats (Beutin et al., 2007) and is commonly associated with porcine bowel oedema disease (Beutin et al., 2008). STEC with *stx2e* have been isolated from fresh produce (Feng and Reddy, 2013) and rarely from humans. One study showed the frequency of isolation of STEC with *stx2e* to be similar among people with and without diarrhoea (Friedrich et al., 2002). Another study showed that isolation of Stx2e-producing STEC was not correlated with diarrhoeal illness (Beutin et al., 2008), suggesting that Stx2e-producing strains do not consistently cause disease in humans. However, Fasel et al. (2014) reported the isolation of STEC with *stx2e* from a HUS patient. In other studies, *stx2e* was found in serotypes O9abH- and O101:H- strains (Thomas et al., 1994) and in another study one *stx2e*- and *eae*-positive isolate was isolated from a 65-year-old person with HUS in Switzerland; the immunosusceptibility of this patient was not reported (Fasel et al., 2014).

The *stx2f* subtype has a very distinct genetic sequence from the other *stx2* subtypes and the designation *stx2f* was first applied to STEC strains isolated from pigeons (Schmidt et al., 2000), though this subtype was first reported as Shiga Like Toxin (SLT) IIva from a STEC isolated from an infant with diarrhoea (Gannon et al., 1990). Analyses of STEC isolates from wild animals, from bovine farm environments and from humans have seldom found *stx2f* (Friedrich et al., 2002; Monaghan et al., 2011; Hofer et al., 2012). Some studies suggest that STEC that produce Stx2f can cause mild diarrhoea or are asymptomatic (Prager et al., 2009; Friesema et al., 2014), but it appears to be rare (Persson et al., 2007; Hofer et al., 2012). However, a recent study reported isolation of STEC O8:H19 that carried *stx2f* and *eae* from a HUS patient in the Netherlands (Friesema et al., 2015). Three strains from HUS patients that produced Stx2f from Austria (serotype O80:H2) and Italy (serotypes O26:H11 and O55:H9) were positive for *eae*-ξ (xi) or *eae*-β (beta) *adfO*, *efa1, ehxA, espP* (Grande et al., 2016) and one O63:H6, *eae* positive was reported from Belgium (De Rauw et al., 2018). Additional information is needed to understand the association between Stx2f and severe illness.

STEC with the *stx2g* subtype was first isolated from bacteriophages in faecally contaminated water (Garcia-Aljaro et al., 2006). It was found in 8.4% of the STEC strain isolated from farm environments in one study (Monaghan et al., 2011), and also detected in some STEC strains isolated from foods (Beutin et al., 2007). STEC with *stx*2g have rarely been isolated from human samples (Beutin et al., 2007), although it was isolated from German patients with diarrhoea, fever and abdominal pain, but has not been implicated in severe diseases (Prager et al., 2011).

Several studies have indicated that toxin subtypes *stx2a* or *stx2d* are significantly associated with the risk of HC, HUS or both (Ethelberg et al., 2004; Persson et al., 2007; Mellmann et al., 2008; Buvens et al., 2012; Marejková et al., 2013; Brandal et al., 2015b; De Rauw et al., 2018). These subtypes were at least 25 times more potent than Stx2b and Stx2c in analyses on primary human renal proximal tubule epithelial cells and Vero cells (Fuller et al., 2011). In mice, the potencies of Stx2b and Stx2c were similar to Stx1, whereas Stx2a and Stx2d were 40–400 times more potent than Stx1 (Fuller et al., 2011). Moreover, Franz et al. (2015) demonstrated a positive association between *stx2a* and a range of additional virulence factors including *eae, efa1, ehxa, ent/espP2, nleB, nleB2, nleC, nleE, nleF, nleG2-3, nleG5-2, nleG9, nleH1-1, nleH1-2, saa* and *subA.* Non-LEE encoded effector (*nle*) proteins are present on pathogenicity islands encoded on prophages that may fulfil a variety of functions including inhibition of phagocytosis, invasion, cytotoxicity and bacterial attachment through effects on signal transduction pathways (Konczy et al., 2008; Naseer et al., 2017).

The Stx2d subtype, which is an indicator for clinical outcomes such as HC or HUS (Bielaszewska et al., 2006) used to be known as *stx2d*-activatable because it was activated by elastase in the intestinal mucus layer to become 10- to 1000-fold more cytotoxic (Melton-Celsa et al., 1996). In a French outbreak caused by a cross-pathotype STEC/extraintestinal pathogenic *E. coli* (ExPEC) strain of serotype O80:H2, *stx2d* in combination with other *stx* subtypes was found in 69% of the 52 strains isolated from HUS patients. Among the isolates, 62% had *stx2c/stx2d*, 7% had *stx2a/stx2d* and 31% harboured only *stx2a* (22%) or only *stx2d* (9%). All 52 strains had the intimin variant *eae*-ξ (xi), and 87% carried the *ehxA* gene (Mariani-Kurkdjian et al., 2014). Furthermore, all 52 O80:H2 strains examined shared at least four genes (*sitA*, *cia*, *hlyF* and *ompTp*) that are characteristic of the ExPEC pS88 plasmid as well as other ExPEC traits, with 98% carrying the *iss* and *iroN* genes; 96% had the *cvaA* gene; and 61% had the *iucC* and *etsC* genes (Soysal et al., 2016). A study from Spain examined 236 STEC strains isolated from patients with HUS, diarrhoea or both. Of these, 193 were *eae* positive and 43 were *eae* negative and seven (3%) were found to have *stx2d* (Sánchez et al., 2017). Further analysis showed that six of the *stx2d*-bearing strains were *eae*-negative STEC that belonged to serotypes O73:H18, O91:H21, O148:H8, O181:H49 and ONT:H21, and one was an O157:H7 strain that was also positive for *stx2c* and *eae*. A study of 32 O26:H11 sequence type (ST)-29 isolates from cases of HUS between 2010 and 2013 in France found seven isolates to be positive for *stx2d*, *eae*-β (beta) and SP_26_E (using a CRISPR-based assay), but devoid of any of the usual plasmid genes associated with O26 strains (Delannoy et al., 2015). Although these studies are suggestive that *stx2d* STEC causes severe disease, not all STEC strains with *stx2d* may causes severe infections. For example, nine patients in Norway infected with *stx2d*-positive STEC did not develop HUS (Brandal et al., 2015b). In an outbreak of gastroenteritis in Japan, both *E. albertii* and STEC O183:H18 that were *stx2d* positive were isolated, but none of the 44 patients examined developed BD or HUS (Ooka et al., 2013). Moreover, a large study of 626 STEC infections in Germany, none of the 268 HUS patients were infected with STEC that were positive for *stx2d* (Friedrich et al., 2002). At least 18 different genetic variants of the *stx2d* subtype have been identified and eight of the strains tested showed wide variations in activatability by elastase (Scheutz et al., 2012), which may account for the variability in clinical outcomes associated with *stx2d*.

Due to gene sequence similarities, *stx2a*, *stx2c* and *stx2d* can be quite difficult to discern and identify (Scheutz et al., 2012). Strains carrying *stx2c* were thought to cause severe disease and HUS (Ethelberg et al., 2004; Persson et al., 2007; EFSA BIOHAZ Panel, 2015); however, this is not certain. It has been suggested that O111 strains isolated from HUS patients were *stx2c* positive (Zhang et al., 2007). However, the alignment of the two sequenced strains showed 100% homology with the *stx2* sequences found in O157:H7 strain EDL933, which is known to have *stx2a* but not *stx2c* (Scheutz et al., 2012). Similarly, Persson et al. (2007) examined 20 STEC strains isolated from HUS patients and reported one strain that had *stx2c* alone. That strain has since been sequenced and shown to belong to clade 8 of O157:H7, which is known to have *stx2a* but not *stx2c* (Ogura et al., 2015). Lastly, Friedrich et al. (2002) did not see a statistically significant difference in the prevalence of *stx2c* genotype among STEC isolated from patients with HUS vs. diarrhoea ($p = 0.49$), nor in HUS vs. asymptomatic patients ($p = 0.74$) (Friedrich et al., 2002).

In terms of the pathogenicity assessment required in ToR1, the peer-reviewed publications suggest the following; [1] *stx1a* strains are associated with hospitalisation and BD; [2] *stx1c* and *stx1*d are usually found in *eae*-negative STEC isolates and less often associated with hospitalisations and BD; [3] 27–29% of cases with *stx2a* and 9–10% of cases with *stx2d* strains are reported as HUS. The percentages are higher for hospitalisation and bloody diarrhoea for *stx2a,* with or without *eae*; [4] *stx2b* strains are usually associated with mild illness; [4] there is uncertainty regarding *stx2c* as it has been reported to be associated with HUS, but there is evidence to suggest the subtype was actually *stx2a*; [5] *stx2e* strains are rarely found in STEC associated with human illness and, while initially thought to only cause mild illness, *stx2f* strains have recently been isolated from patients with HUS and [6] the *stx2g* subtype is also rarely associated with human illness and not usually associated with severe illness.

3.1.2.2.2. Information from the TESSY data (2012–2017)

The TESSy data (2012–2017, inclusive) included the presence of specific gene/gene combinations and/or *stx* subtypes and severe illness expressed as HUS, hospitalizations or bloody diarrhoea (BD). In that period, out of the 29,945 human STEC cases reported in the EU/EEA from 2012 to 2017, the 'full' virulence profile, as understood in the context of this opinion (*eae presence/absence*, *stx1 subgroup* and *stx2 subgroup)* was reported for 3,942 cases. Of these, the HUS status was known of 3,138

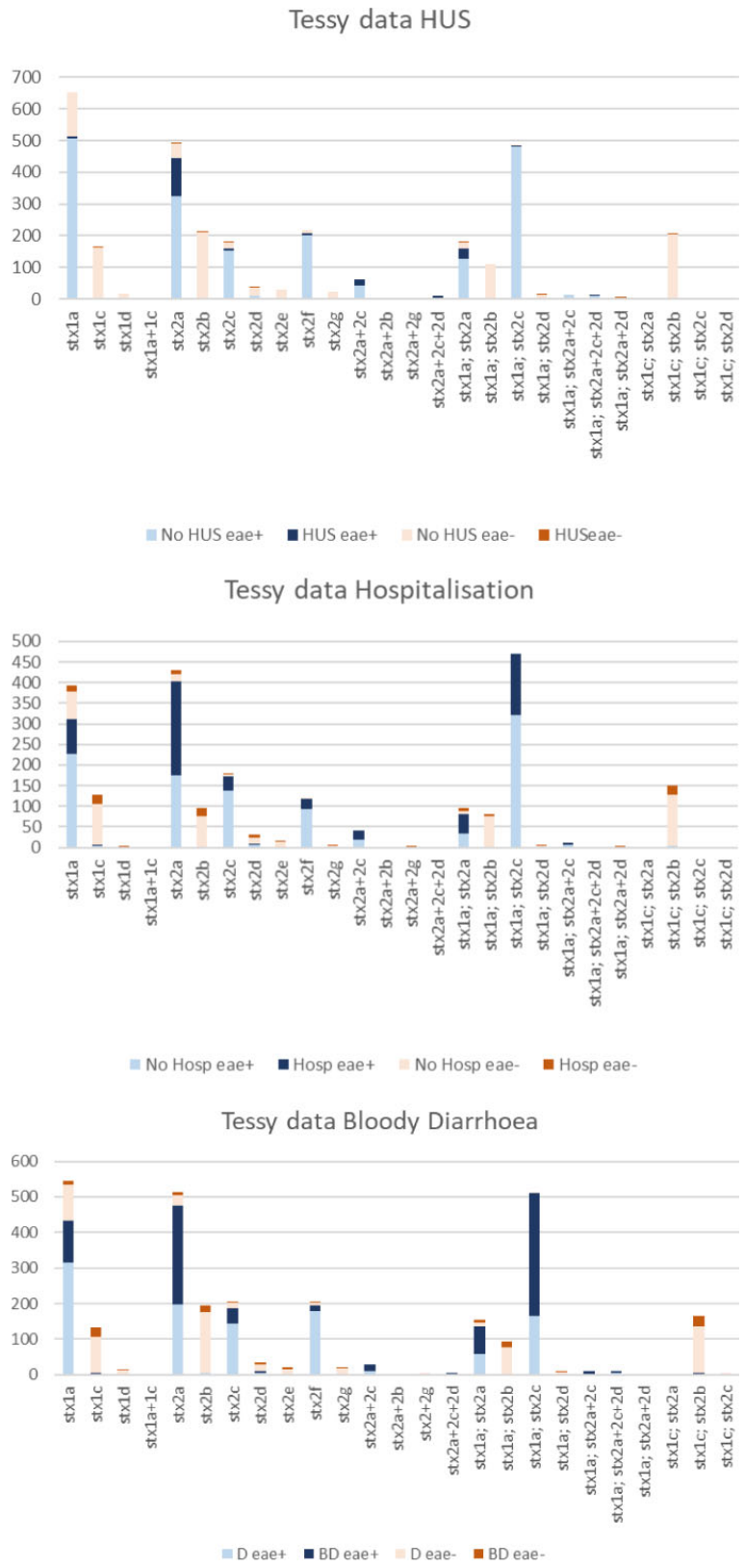
cases, the hospitalisation status was known of 2,263 cases and the clinical outcome (bloody diarrhoea) was known of 2,885 cases (data from TESSy, ECDC). Appendix G provides an overview of the associations between the presence of *eae* and/or *stx* subtypes and severe illness, expressed as HUS (Table G.1), hospitalisation (Table G.2) and BD (Table G.3) also showing the STEC cases with unknown status. The rest of this section is based on those cases with reported (known) status regarding the clinical outcome and/or hospitalisation.

To answer ToR1, the data presented in Appendix G are illustrated in Figures 1–3. Figure 1 shows the reported numbers of confirmed human STEC cases for different *stx*-genotypes, where the severe disease endpoints HUS, hospitalisation and BD have been reported.

Figure 2 shows the percentage of cases with; (a) HUS; (b) hospitalisation and (c) BD, among all reported STEC cases per *stx* subtype. The results are shown for *eae*-positive and *eae*-negative strains separately. The error bars represent the 95% credibility interval, obtained using a beta distribution with a uniform prior. Combinations with less than 10 observations were omitted, as the uncertainties of the estimates were considered too large. The results can be interpreted as an estimate of the probability that a human STEC case with the given *stx* subtype-*eae* genotype combination shows a given clinical outcome. Note that for all the *stx* subtype-*eae* genotype combinations included in the Figure, cases with hospitalisation or BD were observed.

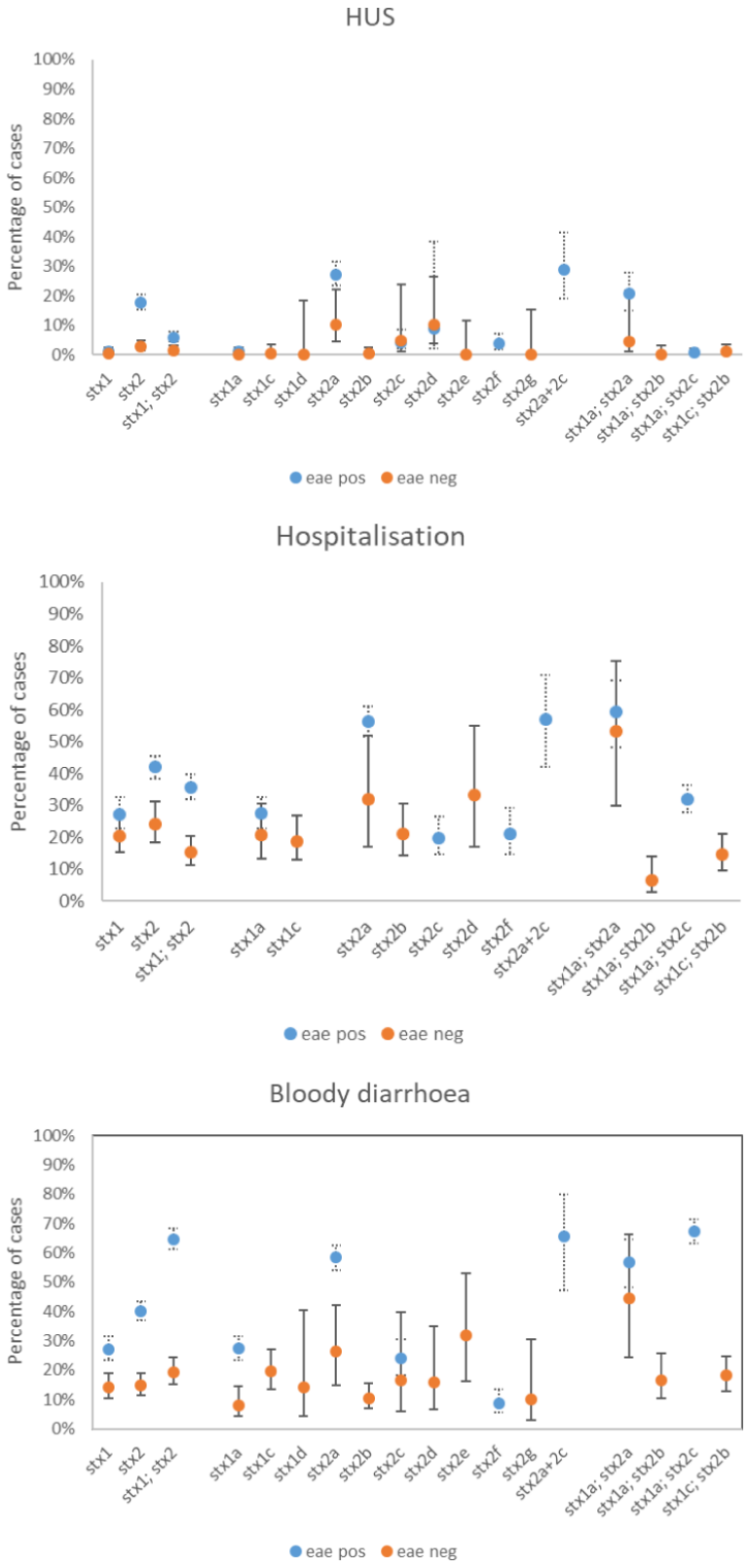
Figure 3 summarises the results of the pathogenicity assessment of STEC. The relative frequencies of observed cases of HUS, hospitalisation and BD are given for all *stx* subtype–*eae* genotype combinations with more than 20 reported cases. The colour coding shows a more intense colouring if the relative frequency is higher. It should be noted that people who are exposed to but not infected, and infected persons that do not end up as confirmed cases, are not included in the TESSy database. Moreover, cases that show severe illness may be more likely to end up as confirmed cases. Therefore, the results shown in these figures may not reflect the probability of severe illness after exposure to a strain with the indicated genotype but are the best current assessment that may be provided given these data limitations.

Overall, *stx2a*, alone and in combination with other *stx* subtypes, had the highest rates of HUS, hospitalisation and BD. In the absence of *eae*, *stx2d* was also associated with a relatively high (10.3%) HUS rate. Most Stx subtypes were associated with HUS, and although the rates may have been low, this should not be considered unimportant given the seriousness of this clinical outcome. The majority of the *stx* subtypes were also associated with hospitalisation, the exceptions being *stx2e* and *stx2g* which had too few reported hospitalisations to assign a designation. Interestingly, all of the *stx* subtypes were associated with BD. For all severe illness outcomes, the presence of intimin was an aggravating factor with the majority of STEC isolates associated with HUS, hospitalisation and/or BD carrying the *eae* gene.



The colours in the bars differentiate between *eae* subtypes and the presence and absence of the three disease endpoints (TESSy data, 2012–2017). All reported subtypes are shown.

Figure 1: Reported numbers of confirmed human STEC cases for different *stx*-genotypes, where the severe disease endpoints 'HUS', 'hospitalisation' and 'bloody diarrhoea' have been reported



Results are shown for *eae*-positive (blue) and *eae*-negative (orange) strains separately (TESSy data, 2012–2017). Error bars indicate 95% credibility intervals. Only subtypes from which more than 10 cases were observed are shown.

Figure 2: Percentage of cases with (a) HUS, (b) hospitalisation and (c) bloody diarrhoea among all reported STEC cases per *stx* subtype, as given in Appendix G

	HUS				Hospitalisation				BD			
	with *eae*	n	without *eae*	n	with *eae*	n	without *eae*	n	with *eae*	n	without *eae*	n
stx1 (all)	**1.2**	**517**	**0.3**	**316**	**27.4**	**318**	**20.3**	**207**	**27.3**	**436**	**14.1**	**255**
stx1a	1.2	512	0.0	139	27.6	312	20.7	82	27.3	432	8.0	112
stx1c	-	5	0.6	159	-	6	18.9	122	-	4	19.5	128
stx2 (all)	**17.7**	**904**	**2.7**	**365**	**42.0**	**748**	**24.3**	**173**	**40.2**	**910**	**14.8**	**325**
stx2a	27.4	446	10.4	48	56.4	404	32.0	25	58.4	476	26.3	38
stx2b	-	5	0.5	206	-	1	21.3	94	-	5	10.5	190
stx2c	4.3	161	5.0	20	19.8	172	-	8	23.9	188	-	18
stx2d	-	11	10.3	29	-	9	33.3	21	-	9	16.0	25
stx2e	-	0	0.0	29	-	0	-	16	-	0	31.8	22
stx2f	3.8	208	-	10	21.0	119	-	1	8.7	196	-	10
stx2g	-	1	0.0	21	-	0	-	6	-	1	10.0	20
stx2a+stx2c	29.0	62	-	0	57.1	42	-	0	65.5	29	-	0
stx1; stx2 (all)	**5.9**	**679**	**1.4**	**357**	**35.7**	**569**	**15.3**	**248**	**64.8**	**676**	**19.4**	**283**
stx1a; stx2a	20.8	159	4.5	22	59.3	81	-	15	56.6	136	-	18
stx1a; stx2b	-	2	0.0	108	-	2	6.4	78	-	2	16.7	90
stx1a; stx2c	0.8	485	-	2	31.9	470	-	0	67.5	510	-	2
stx1c; stx2b	-	4	1.0	202	-	5	14.6	144	-	4	18.1	160

Relative frequencies have been calculated for each *stx* subtype–*eae* genotype combination considering the n cases with reported (known) HUS/hospitalisation or BD status reported (yes or no). '–' indicates that less than n = 20 cases of the indicated *stx* subtype–*eae* combination were reported. *Stx*-subtypes for which less than n = 20 cases were reported in all columns are not shown; the complete data set is given in Appendix G. The more colour in the cell the higher the relative frequency.

Figure 3: Summary of the pathogenicity assessment of the various *stx* subtypes/*eae* types in terms of the observed relative frequency (%) in which they are found to cause severe illness expressed as either HUS, hospitalisation and/or bloody diarrhoea (BD) (TESSy data, 2012–2017)

3.1.2.2.3. Combining the peer reviewed and the TESSY data to answer ToR1

Based on both the peer-reviewed publications and TESSy data, it was concluded that serogroup, intimin (*eae*) variant or Stx toxin subtype could not be used to predict clinical outcome. Intimin was present in the majority but not all STEC associated with severe illness. There is currently insufficient data to test for an association between intimin variant and disease outcome. Although *stx2a* showed the highest rates of HUS, hospitalisation and BD, all other *stx* subtypes or combinations thereof, for which there were sufficient data, were also associated with at least one of these severe illness outcomes. Thus, it was concluded that all STEC, regardless of serogroup, *stx* subtype and/or the presence of the *eae* gene, may be associated with severe illness.

3.1.2.3. Additional virulence factors

As adherence factors and Shiga toxin subtypes were not a definitive marker for pathogenicity, the published scientific literature was further reviewed to identify other possible candidates as there are a

range of other factors associated with virulence in STEC. These include the EHEC enterohaemolysin (*hlyA/ehxA*) which releases haemoglobin from red blood cells to provide a source of iron for the bacterial cells (Beutin et al., 1989), a type II secretory pathway (*etpC-O*) (Burland et al., 1998), an extracellular serine protease, EspP (Burland et al., 1998) a catalase peroxidase (KatP), that cleaves pepsin A and human coagulation factor V contributing to the mucosal haemorrhage associated with haemorrhagic colitis (HC) in humans (Brunder et al., 1996), and a C1 esterase inhibitor (encoded by *stcE*) (Lathem et al., 2002). The *ecf* operon, on pO157, encodes enzymes involved in the synthesis of lipopolysaccharides in the cell membrane facilitating survival and persistence in adverse environments such as those encountered along the human gastrointestinal tract (Yoon et al., 2005). The *ure* gene cluster (three structural genes *ureA*, *ureB* and *ureC* and four accessory genes *ureD*, *ureE*, *ureF* and *ureG*) is involved in urease transport and processing (Mobley et al., 1995; Friedrich et al., 2005) and in colonisation and pathogenesis (Yin et al., 2013). The *hcp* genes encode a type IV pilus involved in invasion, biofilm formation and twitching motility (Xicohtencatl-Cortes et al., 2007) while Cah is an autotransporter protein found in EHEC (Torres et al., 2002). The *chuA* gene encodes an outer membrane receptor protein involved in haem utilisation (Torres and Payne, 1997; Okeke et al., 2004). The *fepB, C, D & G* genes encode ferric enterobactin transport ATP-binding proteins (Fep B, C, D & G) (Okeke et al., 2004) while the *set* gene (also known as *ent* or *sen*) encodes an enterotoxin (Afset et al., 2006). A zinc metalloprotease (*stcE/tagA*) and a subtilase cytotoxin (*subAB*), amongst others, are also reported to be involved in pathogenesis (Etcheverria and Padola, 2013). In recent years, WGS approaches have identified a number of candidate pathogenicity islands (PAIs) including OI-122 and OI-71, which encode a variable range of non-LEE encoded effector (*nle*) proteins (Nle A, B, C, D, E and F) (Naseer et al., 2017). These are encoded on prophages, common in STEC strains and are thought to fulfil a variety of functions including inhibition of phagocytosis, invasion, cytotoxicity and bacterial attachment through effects on signal-transduction pathways (Konczy et al., 2008).

Gardette et al. (2019) used a recombinase-based *in vivo* expression technology to identify genes specifically induced during the infectious process using a mouse infection model. They found 31 induced genes, termed *in vivo*-induced (ivi) genes including 13 involved in metabolism; *araB* (L-ribulokinase), *nirB* (large subunit of nitrite reductase), *gabT* (4-aminobutyrate aminotransferase, *glpB* (sn-glycerol-3-phosphate dehydrogenase), *trpA* (alpha subunit of tryptophan synthase), *ybaT* (putative nitrogen-containing metabolite transporter), *yjeH* (member of the APC superfamily of amino acid transporters), *agaW* (enzyme of the N-acetylgalactosamine phosphotransferase system), *btuR* (cobalamin adenolsyltransferase), *pdxA* (4-hydroxythreonine-4-phosphate dehydrogenase), *azoR* (NADH-aZoreductase), *fadA* (3-ketoacyl-CoA thiolase) and *yjbB* (putative transporter). Seven genes were involved in information storage and processing; *caa* (multifunctional CAA protein), *mhpR* (transcriptional activator of 3-hydroxyphenylpropionic acid catabolism), *araC* (transcriptional regulator of L-arabinose transport and catabolism), *ascG* (transcriptional repressor of arbutin and salicin transport), *yjiR* (putative transcriptional regulator), *nor* (transcriptional activator of nitric oxide reductase NorV) and a gene labelled z4799 believed to code for a putative DNA processing protein. A similar number was associated with cellular processes and signalling including *ytfE* (iron-sulfur cluster repair protein), *yhil* (putative HlyD family secretion protein), *mdtE* (multidrug efflux system protein), *mdtM* (*yjiO*) (multidrug efflux system protein), *yhbU* (putative collagenase), *yghU* (putative glutathione S-transferase) and *degQ* (serine endoprotease) while the remainder are not well characterised.

Naseer et al. (2017) tested 340 human clinical STEC isolates to identify virulence factors associated with HUS. Of these, 218 were *stx1* positive (*stx1a* n= 192, *stx1c* n = 23 & *stx1d* n = 3) while 212 carried the *stx2* gene (*stx2a* n = 90, *stx2b* n = 32, *stx2c* n = 101, *stx2d* n = 9, *stx2g* n = 2) 91 isolates were positive for both *stx1* and *stx2*. The average isolate carried 15 virulence genes (range 1–24), 2 toxin genes (range 0–4), 5 adhesins (range 0–8) and 8 MRA genes (range 0–13) and 94% of isolates carried toxin genes other than or in addition to *stx1* and/or *stx2*, including *hlyA* (85%) and *subA* (12%). Almost all isolates (99%) carried adhesion genes, including *eae* (74%), *lpf* (90%) and *iha* (71%). Moreover, the majority of isolates carried non-LEE effector protein (*nle*) genes. Further evidence for multiple virulence factor involvement in infection is provided by Franz et al. (2015), who reported a strong correlation between the presence of a range of other virulence factors and HUS including (in decreasing order) *nleG5–2, efa1, ent/espL2, ehxA, toxB, adfO, nleG2–3, nleE, cfk, ureC, nleA, nleG, nleB, eae and terB*. Interestingly, the association between *stx2a* and HUS lay between *eae* and *terB*, suggesting all these virulence markers could be considered as strong indicators of the likelihood of HUS. In a more recent study, a microbial risk assessment of 106 O157 isolates in the UK using next-generation sequencing data and machine learning identified several genetic predictors of riskier STEC clinical outcomes. These included proteins involved in initial attachment to the host cell,

persistence of plasmids or genomic islands, conjugative plasmid transfer and formation of sex pili, regulation of locus of enterocyte effacement expression, post-translational acetylation of proteins, facilitation of the rearrangement or deletion of sections within the pathogenic islands and transport macromolecules across the cell envelope (Njage et al., 2019).

While these studies provide important information on the virulence genes associated with pathogenicity, a definitive marker(s) that defines pathogenicity in terms of the severity of clinical outcome has not been identified.

3.1.3. Concluding remarks

The EFSA Opinion on STEC pathogenicity published in 2013 advocated a molecular approach in which the presence of *eae* or *aaic* and *aggR* were associated with a 'high' (for serogroups O157, O26, O103, O145, O111 and O104) or 'unknown' risk of severe illness. The inclusion of *aaic* and *aggR* was in response to the 2011 STEC O104 outbreak caused by an enteroaggregative E. *coli* (EAEC) that had acquired the *stx2a* gene. As there have been no further outbreaks caused by EAEC-STEC cross-pathotype strains, *aaic* and *aggR* are no longer considered to be virulence markers for which routine screening is recommended. Improvements in STEC detection and isolation methods have resulted in the detection of an ever-increasing range of serogroups associated with illness, including HUS, BD and hospitalisation, and thus, serogroup is no longer considered a good indicator of clinical outcome.

As EAEC-STEC cross-pathotype strains are very rare, and there is very limited data on their prevalence in the TESSy database, analyses on the association of the *aaiC* and *aggR* genes and disease outcome were not included in this opinion. Based on the analysis of the *stx* subtypes and the presence/absence of the *eae* gene (the only virulence markers for which there is, albeit limited, data available), all STEC subtypes may be associated with severe illness, i.e. HUS, BD and/or hospitalisations. Although *stx2a* showed the highest rates of HUS, hospitalisation and BD, all other Stx subtypes or combinations thereof, for which there were sufficient data, were also associated with severe illness outcomes. Intimin (*eae* gene) was an aggravating factor, but not essential for severe illness.

Based on the available evidence, it is concluded that all STEC strains are pathogenic in humans, capable of causing at least diarrhoea and, based on the analysis of the *stx* subtypes and the presence/absence of the *eae* gene, all STEC subtypes may be associated with severe illness. Currently categorisation of STEC pathogenicity based on serotype, *stx* subtype or presence of intimin is not valid.

3.2. Methods to detect and characterise STEC

3.2.1. Methods to detect STEC

3.2.1.1. Culture-based methods with selective and differential media

It is very difficult to distinguish STEC from other *E. coli* by means of phenotypic features, as the only feature that specifically identifies STEC is the production of the Shiga toxins, which may not be directly usable as a phenotypic marker to identify STEC in a mixed culture. Enrichment procedures using a range of supplements are therefore commonly applied (Bennett et al., 1995; Stephens and Joynson, 1998). However, the effectiveness of the supplements depends on the STEC strains present. Several attempts to develop selective and/or differential media to facilitate this step have been made but, although successful to a certain extent, none of the media proposed provide a one-size-fits-all solution. Due to the large variability of the *E. coli* species, none of the selective and colorimetric media currently available are suitable for all STEC types. Indeed, a large variability has been observed even within the same STEC O group (Gill et al., 2014; Brusa et al., 2016).

3.2.1.2. Cell cultures

There are two main types of cell culture-based assays: The observation of the cytotoxic activity of the Shiga toxins onto monolayers of Vero cell (VCA) and the identification of the adhesion pattern of isolated strains. The latter assay is not actually specific for STEC and has been largely replaced by the development of the more modern PCR-based assays. The VCA is instead still used in clinical diagnosis, particularly as in some cases the identification of the free faecal Shiga toxin is the only way to confirm an infection with STEC. Moreover, it is sometimes difficult to isolate STEC from a stool sample due to a late sampling or the administration of antimicrobials. However, the associated costs and the skill needed to recognise the Shiga toxin cytotoxic effect have restricted the use of the VCA to reference laboratories only.

3.2.1.3. Immunological-based methods

Immunological methods provide indirect evidence of the presence of STEC. These are based on the detection of the Shiga toxins in stool samples or culture from stool specimens. Although easy to use, these approaches have not yet been specifically developed for testing food and are thus mostly confined to use in the clinical environment. Additional immunological applications include several ELISA tests, such as the ELISA sandwich assay for the detection of Stx2f (Skinner et al., 2013) or the ELISA test to distinguish between Stx1 and Stx2 (Downes et al., 1989), both of which may be used for testing faecal cultures of stool samples. Other ELISA kits for the detection of Stx have been commercially produced and distributed (Premier EHEC, Meridian Bioscience Inc., Cincinnati, OH; ProSpecT Shiga toxin *E. coli* (STEC) Microplate Assay, Remel Inc., Lenexa, KS and the Ridascreen Verotoxin Enzyme Immunoassay r-Biopharm AG, Darmstadt, Germany) and are used in the clinical diagnosis of STEC infections.

3.2.1.4. Molecular-based methods

By far the most suitable approach for detecting STEC in clinical, animal and food samples is the identification of the Stx-coding genes. It is important to consider that the major difference between commensal *E. coli* and STEC is the presence of the Shiga toxin-coding genes acquired through horizontal gene transfer. This approach applies to both isolated strains and DNA extracted from complex samples (either stools or food samples). A metagenomics approach has recently been described for direct detection and characterisation of STEC in stool samples (Singh et al., 2019).

3.2.2. Methods to detect specific serotypes and immuno-magnetic separation

3.2.2.1. PCR-based methods

Methods based on PCR are the most appropriate approaches to detect STEC in complex matrices. The detection of the *stx* gene(s) is the only true discriminant between STEC and other *E. coli*. Furthermore, the ability to identify accessory virulence features, such as the adhesion determinants (e.g. the *eae* gene), has been used in detection methods to derive an indication of the presence of STEC strains considered to be more likely to cause severe disease in humans. A number of protocols are available in the scientific literature describing the primers' sequences and the thermal profiles to be used in endpoint PCR assays for the detection of STEC in complex matrices, such as food (Wang et al., 2002; Tzschoppe et al., 2012; Fratamico et al., 2014). An alternative to the end-point PCR is the real-time PCR, which uses fluorophores to identify the amplification product. Nowadays, real-time PCR equipment with up to five different channels is available that can identify many targets labelled with different fluorophores. In principle, any target used in end point PCR could also be adapted for the amplification in real-time PCR. Two methods based on this technology for the detection of STEC in food have been adopted as standard methodologies in food among the many that are published in the scientific literature (see Section 3.2.3). Of these, one has been translated into an international standard by the International Organization for Standardization (ISO)/European Committee for Standardization and is the CEN ISO TS 13136:2012 (ISO, 2012). The other has been developed by the USDA as an official laboratory guideline currently used in the United States for meat products (USDA, 2019) (See Section 3.2.3).

Currently, if one or more *stx* genes are detected in foods during routine testing, it does not provide sufficient evidence that viable STEC capable of causing human disease is present in the matrix. Since Stx phages can be present in foods, these may result in false-positive findings. There are alternative methods which can eliminate or significantly reduce the detection of Stx phages from non-STEC sources (see review by Quirós et al. (2015) and Martínez-Castillo and Muniesa (2014)). Moreover, the application of 0.22 or 0.45 μm low protein binding membranes that do not retain phages may facilitate the more specific detection of STEC in foods. A recent study of the performance of a panel of PCR-based commercial kits found good performance for meat samples, but not for vegetables (Costa et al., 2019).

3.2.3. Standard methods available for detection of STEC in food and feed

3.2.3.1. ISO 13136:2012

The CEN ISO/TS 13136:2012 method (ISO, 2012) requires the isolation of STEC from all samples where *stx*-genes are detected; additionally, it includes protocols for the detection of the *eae* gene and,

in positive samples, the identification of the genes associated with the five STEC serogroups O157, O145, O111, O103, O26. This additional characterisation is meant to give the operator the possibility to use, for the positive samples, an immune magnetic-based procedure to facilitate the isolation of the microorganism (see also Section 3.2.3.2). This international standard is currently under revision by the TAG18 STEC *ad hoc* group established by the CEN TC 275 WG6. The revision process is currently ongoing and only part of the new version of the standard has been agreed. In particular, the revised standard will be divided into two parts, one dedicated to the detection and isolation of STEC from food and feed while the part two will contain the specifications for the characterisation of isolated STEC strains. The CEN TC275 WG6 has voted and agreed to include in the part 2 of the standard the *stx* genes subtyping, among other features.

3.2.3.2. ISO 16654:2001

The EN ISO 16654:2001 (ISO, 2001) was the first standard on the detection of STEC in food. It was not originally designed to detect all STEC, but was used to specifically detect *E. coli* O157, which was considered as being the archetype of STEC at that time. It is based on the principle of the immune concentration of *E. coli* O157 from an enrichment culture operated by magnetic beads coupled with an antibody directed against the O157 LipoPolySaccaride (LPS) and exploits the ability of *E. coli* strains belonging to this serogroup to grow on cefixime and potassium tellurite supplemented MacConkey agar. The coupling of these features makes this method the most sensitive and robust procedure for isolating *E. coli* O157. The procedure is very specific for *E. coli* strains belonging to O157 serogroup, but does not include any step to characterise the isolates as being STEC (e.g. by detecting the Stx-coding genes or the effect of the toxin) and generally refers this activity to the reference laboratory to which the isolates should be sent for the assessment of their ability to produce the toxins or for the verification of the presence of the toxin-coding genes. In 2018, an amendment to this standard was published containing the validation data of the method (ISO, 2017) determined upon mandate of the European Commission (Mandate to CEN M/381).

3.2.4. Methods for detection of STEC in animal faeces and environmental samples

There are no methods specifically deployed to test animal faeces for the presence of STEC, and thus, the methods currently used are derived from the protocols developed for testing food, which are likely to lack sensitivity for detection of low levels of STEC found in carrier animals amongst the complex microbial population present in faecal samples. The world assembly of delegates of OIE adopted in 2008 a scheme for testing animal faeces or rectal swabs for the presence of *E. coli* O157. The approach is that specified in the ISO 16654:2001 standard (ISO, 2001) with the following modifications: The sample is mixed with the dilution of 1/10 into buffered peptone water (BPW) and incubated at 37°C for 6 h. After this pre-enrichment, the immune magnetic separation is carried out using the procedures described in ISO 16654:2001. Such a schema has been included in the OIE terrestrial manual 2016 (chapter 3.9.10 ref). Other methods are included to detect and isolate non-O157 STEC from animal faecal samples. These include combining several different selective agars (Fan et al., 2019) or the direct plating of the samples onto a solid medium such as rhamnose MacConkey Agar for STEC O26 or blood agar supplemented with calcium (indicated for all STEC). The latter method targets the haemolytic activity displayed by some STEC types. After plating the samples, the colonies are confirmed by testing a statistically relevant number of individual colonies, immunoblotting or DNA probing for the presence of *stx* genes (OIE terrestrial manual, 2016). EFSA issued in 2009 a technical specification for the monitoring and reporting of STEC in animal and food samples (EFSA, 2009). In this document, the OIE approach is proposed for testing of hides and fleece samples for the presence of *E. coli* O157 but with the increase of the pre-enrichment temperature from 37°C, as proposed by OIE, to 41.5°C. For non-O157 STEC, the ISO TS 13136 international standard (ISO, 2012) is recommended (EFSA, 2009). A new immunobead-based procedure for detection of STEC O55 strains has been developed and evaluated in the context of recent UK outbreak investigations (Kirchner et al., 2019).

This section aims to provide information on the main approaches used to test animal samples and does not intend to provide an exhaustive list of all the possible methodologies. Particularly as new analytical strategies are continuously developed by the scientific community boosted by the wealth of information provided by the modern sequencing technologies.

Animal faeces are mostly assayed in the context of surveys with the purpose to define the prevalence of STEC in specific animal species. Such animal populations are not extensively sampled for monitoring and the trend of sampling animals in the EU appears to be decreasing (EFSA and ECDC, 2017, 2018).

3.2.5. Limitations of the current STEC diagnostic methods

Testing for STEC may include culture and molecular methods. Selective enrichment, to promote the growth of STEC while inhibiting other organisms, can be achieved using combinations of antimicrobials such as novobiocin, cefixime and tellurite and selective incubation temperatures (e.g. 42°C). However, most of the current enrichment methods were developed for STEC O157 and may inhibit other STEC.

Many control strategies are informed by the presence of specific virulence gene combinations in a given isolate necessitating the isolation of the STEC. Current isolation methods have several limitations including: 1) detection limits (isolation may be difficult when the numbers of STEC present in the sample are below the detectable limits of the test); 2) selectivity as some antimicrobials commonly used, such as cefixime and tellurite, may inhibit certain STEC and 3) there is no universal phenotypic marker for all STEC and detecting STEC serogroups other than O157 may require additional screening of large numbers of individual colonies in the absence of selective or indicator plating agars. Colony blot methods have been used to increase detection of target organisms amongst large numbers of colonies on culture plates, but are now rarely used (Szakál et al., 2003). This is an area that could be revisited in light of recent developments in robotic laboratory methods.

The probability of isolating a particular STEC serogroup may be increased by using immuno-concentration using antibody-coated immuno-magnetic beads before plating on selective agar. This technology is commercially available for the five serogroups of major public health importance. However, as this method targets the O-serogroup, it does not discriminate between STEC and other *E. coli* with the same O-group. Non-selective binding has also been commonly observed with closely related serogroups or when bacterial cells are present at high concentrations, e.g. in faecal samples from carrier animals or environmental samples.

The range of molecular methods available for screening, detecting, confirming and/or characterising STEC includes the polymerase chain reaction (PCR, usually targeting *stx* genes but may also be used to characterise specific strains in terms of the virulence gene profile), real-time PCR, other PCR-based genetic methods (e.g. Loop mediated Isothermal Amplification (LAMP)) and metagenomic sequencing. All of these have several limitations including: [1] molecular methods are generally more expensive than culture-based methods and metagenomic sequencing requires a considerable investment in sequencing and ICT technologies; [2] detection of false-negative (e.g. gene variants not detected or PCR inhibitors present) and false-positive results (e.g. detection of phage or STEC DNA in the absence of viable cells); [3] different targets may be present in different cells; 4) gene detection provides no indication as to whether or not that gene may be expressed; [4] PCR plus mass spectrometry may require additional costs and the reliability of the results is dependent on the composition of the database; [5] metagenomic sequencing analysis has several limitations including being labour intensive. Thus, all PCR-positive cultures should be confirmed by culture-based isolation of the STEC, but this may be difficult if the STEC cells are present in low concentrations.

3.2.6. Characterisation and typing of STEC strains

3.2.6.1. Serotyping and molecular serotyping

Identifying the serotype of the STEC strain causing infections has been important in epidemiological tracking, including measuring incidence, tracking global emergence and detecting and investigating outbreaks. However, serological typing of *E. coli* is complex due to the large number of O and H type antigens that exist. Furthermore, not all *E. coli* isolated from foods can be serotyped. Studies characterising STEC and enterotoxigenic *E. coli* (ETEC) strains isolated from fresh produce found that over 50% of the isolates could not be typed or only yielded partial serotypes (Feng and Reddy, 2013, 2014). Moreover, most, if not all, STEC virulence factors are on mobile genetic elements that can be lost or transferred and it is not unusual to find STEC strains of the same serotype that carry different virulence genes and pose different health risks. As a result, although serotype data can be useful in identifying STEC, additional data are required to predict the human health risk.

The traditional phenotypic serotyping scheme for *E. coli*, first developed in the 1940s, provides some degree of discrimination for outbreak detection and investigation. However, establishing, maintaining and developing the scheme (comprising more than 188 O and 53 H antisera) are expensive, laborious and require specialist resources and expertise. Consequently, phenotypic serotyping is provided by a limited number of reference laboratories worldwide.

In recent years, there has been a move towards using molecular methods. Several real-time PCR methods have been developed for the rapid detection of the most common STEC O-groups in different

matrices. The European Union Reference Laboratory for *E. coli* has published a method for identification of the STEC serogroups mainly associated with human infections by real-time PCR amplification of the genes associated with the O26, O45, O55, O91, O103, O104, O111, O113, O121, O128, O145, O146 and O157 serogroups (http://old.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_11_Rev_0.pdf).

The Center for Genomic Epidemiology has developed a web-tool, *SerotypeFinder*, for WGS serotype prediction of *E. coli* based on the O and H antigen processing genes. It is a freely available web tool that requires little or no expertise in bioinformatics to operate. The database was constructed using complete O and H antigen genes from the NCBI nucleotide collection, a comprehensive set of O antigen genes reported by Iguchi et al. (2015) and WGS of *E. coli* reference strains from the International center for reference and research at SSI in Copenhagen. All database genes are compared against the genome of each test strain and the output is the predicted O and H serotype, based on the best matching genes. The same database of O and H antigen genes is also used in other web services to determine the *E. coli* serogroup from WGS either as a standalone tool or as part of a more complex bioinformatic pipeline for extensive STEC strain characterisation (https://w3.iss.it/site/aries/).

There are many advantages to adopting the *in silico* WGS serotyping approach for typing *E. coli*. Once implemented, WGS can be faster and more cost effective than traditional methods. Studies show that increasing numbers of strains of *E. coli* are reported as 'O group unidentifiable' due to antisera failing quality control procedures, unresolvable cross reactions or novel O groups. *In silico* WGS serotyping avoids the need for the resource-intensive antisera production process and the inherent quality control issues. Using WGS, problematic phenotypic cross reactions appear, for the most part, to be resolved and identifying and establishing novel O groups is much less demanding and operationally complex than producing and verifying new rabbit antisera for the phenotypic scheme. Strains of *E. coli* that are phenotypically untypable due to lack of expression of O antigens (designated 'rough') or H antigens (designated 'non-motile') are fully typable by WGS. WGS data offer valuable insights into the degree of variation in the O and H antigen encoding genes within each O and H type and the significance of cross reactions between O groups.

3.2.6.2. Phage typing

A phage typing scheme developed in Canada in the 1980s exists for STEC O157:H7, but there are no phage typing schemes for the non-O157 STEC serotypes. The scheme is not widely used in countries where non-O157 STEC serotypes are more common. Certain phage types are associated with severe disease, e.g. PT2 and PT21/28, because these types harbour the *stx2a* subtype known to be associated with progression HUS in some cases (Launders et al., 2016). The association between STEC O157:H7 phage type and severe disease is likely to vary between regions. Phage typing is rapid and inexpensive but has limited utility, being only useful for typing STEC O157:H7.

3.2.7. Subtyping and fingerprinting for epidemiology and population studies

Molecular typing methods, specifically pulse field gel electrophoresis (PFGE) (Gerner-Smidt and Scheutz, 2006) and multilocus variable number tandem repeat (VNTR) analysis (MLVA) (Byrne et al., 2014), are used by many member states for outbreak detection and investigation. More recently, certain MS have implemented whole genome sequencing for surveillance of food-borne disease caused by STEC (Joensen et al., 2014; Dallman et al., 2015; Holmes et al., 2015; Chattaway et al., 2016; Parsons et al., 2016).

WGS is a robust, reproducible approach and provides an unprecedented level of discriminatory power, lends itself to inter-laboratory comparisons of strains, including for outbreak investigations, and can be rapid and user-friendly (Abdalhamid et al., 2019).

There are a range of methods available to assess relatedness between isolates including analysis of the difference in single nucleotide polymorphisms (SNPs), gene presence or absence throughout the whole genome and gene allele differences and overall genetic similarity, e.g. comparing *k*-mers (Parsons et al., 2016).

Food-borne outbreaks may be national or international and it is vitally important that WGS typing data can be exchanged across borders (Franz et al., 2014). For *E. coli*, SNP typing and core genome and/or whole genome MLST (cgMLST/wgMLST) are currently the methods most commonly applied to phylogenetic analysis, and shared databases are in the process of being built. A whole genome MLST (wgMLST) database is in the process of being developed in PulseNet. MLST methods compare most, or

all, of the genes in bacterial genomes by creating a barcode by assigning numbers for allelic variants. With respect to the SNP typing approach, each new isolate is compared to a curated regional, national and/or international database of isolates to place it into phylogenetic clusters, so is a less standardised and transferable method than MLST-based methods (Parsons et al., 2016).

3.2.8. Concluding remarks

The current international standard, ISO TS 13136 (ISO, 2012), represents a reliable approach for the detection of STEC in food, although, as with any other methods based on molecular biology, there are limitations linked with the lower sensitivity of the isolation procedure in respect to the PCR-based screening of the enrichment samples. The method, however, is currently under revision and the application of specific steps to improve this aspect is currently being discussed.

A major overhaul of the current STEC testing and reporting for animal, food, feed and human isolates is required in the EU. A microbiological criterion has been defined for sprouts only, while the reporting of STEC presence in the remaining food commodities as well as in animal samples are only generically described in the Directive 2003/99/EC[7], a situation which should change to facilitate a better understanding of sources, pathogenicity and the emergence of novel strains. Moreover, there is an urgent requirement for harmonised sampling strategies, characterisation methods and reporting for all testing (human, animal, food and feed).

3.3. Ranking different foods in terms of STEC public health risk [ToR 3]

3.3.1. Source attribution studies of food-borne STEC-related human cases

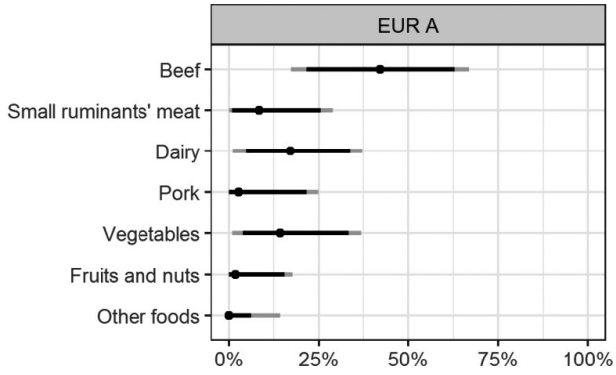
Ruminants, such as cattle, sheep, goats and deer, are the most important reservoirs of STEC (Gyles, 2007) while others including rabbits and seagulls may facilitate the cross-contamination of crops. The consumption of foods from ruminants such as cattle, including raw milk (Adams et al., 2019), is recognised as a potential source of STEC infection in humans but environmental contamination of water and vegetables, direct contact with animals and person-to-person transmission have also been identified as important routes of transmission (Karch et al., 1999; Sonntag et al., 2005).

Until recently, few source attribution studies focusing on human STEC infections had been conducted worldwide. However, a study in the UK investigated the relative contribution of pork, lamb and several beef products to *E. coli* O157 infections in Great Britain using a comparative risk assessment approach (Kosmider et al., 2010). Based on the model assumptions and available data at the time, this study estimated that beef was the sole contributor to human infection. A more recent study in the Netherlands attributed STEC infections to sources using a combined approached that allowed for identification of the most important reservoirs (livestock sources) of the pathogen and their associated risk factors (Mughini-Gras et al., 2018). The study applied a microbial subtyping approach that linked data on the STEC subtypes causing human illness and the subtypes occurring in animal reservoirs, with data from a case–control study of sporadic infections. It showed that risk factors for STEC infection may vary according to the attributable source. To address data limitations, the study used animal prevalence data from neighbouring countries. The study found supporting evidence that a growing number of unusual vehicles, including handling raw meat pet food, are associated with human infections. It is, however, not possible to apply this method at a regional or global level.

The World Health Organization's Foodborne Disease Burden Epidemiology Reference Group (WHO-FERG) estimated the relative contribution of different sources to food-borne infections globally, including STEC infections. The proportion of the burden of disease attributable to food-borne, environmental, animal contact and person-to-person transmission, as well as to specific foods in 14 World subregions, was estimated. In the absence of data-based evidence at regional or global level, FERG relied on expert elicitation to estimate these attribution proportions (Havelaar et al., 2015; Hald et al., 2016; Hoffmann et al., 2017).

In the European region-A (including countries within the European region classified as very low child and adult mortality as defined by WHO (http://www.who.int/choice/demography/mortality_strata/en/), the FERG's expert elicitation attributed 60% (95% Confidence Interval 26–83%) to food-borne transmission, 11% (95% CI 1–37%) to animal contact, 8% (95% CI 0-33%) to human-to-human contact, 7% (95% CI 0–33%) to water and 3% (95% CI 0–19%) to soil. The STEC food-borne disease burden was attributed to six food categories plus the category 'other foods' and a proportion of disease attributable to unknown categories was not estimated (Hoffmann et al., 2017). In the Eur-A

subregion, beef was estimated to be the major food source, followed by dairy products and vegetables (Figure 4).



The dot represents the median estimate; the dark black line, the 90% uncertainty interval; and the grey line the 95% uncertainty interval

Figure 4: Attribution of food-borne STEC disease burden to specific food categories in the Eur-A, based on expert elicitation (Hoffmann et al., 2017)

Recent epidemiological analyses of available data at global level have been successful in attributing STEC to sources. Under the umbrella of the Joint FAO/WHO Core Expert Meeting on VTEC/STEC, two source attribution studies were conducted: a systematic review of case–control studies, and an analysis of data from outbreak investigations (FAO/WHO, 2018). For the latter, data on all STEC outbreaks that have occurred globally were gathered via WHO contact points. Data were received from 27 countries covering the period between 1998 and 2017 and three WHO regions: the Americas (AMR), the European region (EUR) and the Western Pacific Region (WPR). Results showed that the top foods varied across regions. In the EUR, the most important sources of STEC were beef and produce (fruit and vegetables), each being estimated to be associated with 30% of illnesses (Pires et al., 2019). The systematic review of case–control studies identified 22 case–control studies of sporadic STEC infection in humans, from 10 countries within four WHO subregions, from 1985 to 2012. This study also showed regional differences, with beef as the most significant food item associated with sporadic STEC infection in Europe (Devleesschauwer et al., 2019).

3.3.2. Source attribution of human STEC cases using EFSA outbreak data

In the period 2012–2017 (inclusive), a total of 330 STEC outbreaks were reported in 18 countries of the EU/EEA, involving 2,841 cases, 463 hospitalisations and five fatalities. Of all these outbreaks reported, the food vehicle was identified for 164 outbreaks (49.7%). Most outbreaks were reported in Northern Europe. Overall, 19.4% of all outbreaks were reported as 'strong evidence', the remainder being of 'weak evidence' (Table 2). Of the 64 outbreaks reported with strong evidence, seven outbreaks reported by one single Member State from 2012 to 2014, with 'tap water, including well water' as the food vehicle and with a number of human cases reported as '1' for each of these outbreaks, were excluded from the analysis, as these data did not match the definition of an outbreak. Additionally, five outbreaks were excluded because the implicated food was 'mixed food' (one outbreak), 'other foods' (two outbreaks) or a complex food (two outbreaks: 'mixed minced beef and pork' and 'homemade spring rolls with coriander'.

Table 2: Number of reported STEC outbreaks by European Region by strength of evidence of identification of food vehicle, EU/EEA (2012–2017)

EU Region (MS reporting outbreaks)	All outbreaks (strong and weak evidence)	Outbreaks reported with strong evidence
EE (Poland, Slovakia)	5	0
NE (Denmark, Finland, Ireland, Norway, Sweden, the United Kingdom)	173	45
SE (Croatia, Italy, Malta, Portugal, Spain)	14	4

EU Region (MS reporting outbreaks)	All outbreaks (strong and weak evidence)	Outbreaks reported with strong evidence
WE (Austria, Belgium, France, Germany, the Netherlands)	138	15
Total	330	64

EE: Eastern Europe; NE: Northern Europe. SE: Southern Europe; WE: Western Europe.

The analyses of outbreak data reported with 'strong evidence' showed that 24% (95% UI 14–35%) of STEC outbreaks in the region could be attributed to 'bovine meat and products thereof', and 22% (95% UI 13–33%) to 'milk and other dairy products' (Table 3). The third contributor to STEC cases was 'tap water, including well water' (13%, 95% UI 7–22%). Note that 5 of the 8 outbreaks (63%) attributed to this food category occurred in one MS. Twelve percent (95% UI 5–20) of STEC outbreaks were attributable to 'vegetables, fruit and products thereof', and the remaining sources had an attribution proportion below 5%. The categories 'turkey meat and products thereof', 'broiler meat and products thereof', 'other, mixed or unspecified poultry meat and products thereof', 'eggs and egg products' and 'cereal products, including rice and seeds/pulses (nuts, almonds)', were not implicated in any of the strong evidence outbreaks reported in the study period, but were regarded as potential sources for STEC infections based on other evidence. The category 'broiler meat and products thereof' was implicated in one strong evidence outbreak associated with a complex food, reported as one of the ingredients in a causative source reported as 'other foods'.

Table 3: Proportion of STEC strong evidence outbreaks attributed to foods and water based on source attribution (%, mean and 95% uncertainty interval [UI])

Implicated food category (number of reported strong evidence outbreaks; number of reported countries)	Mean (%)	95% UI (%)	
Bovine meat and products thereof (15; 7)	**24**	**14**	**35**
Milk and dairy products[(a)] (14; 8)	**22**	**13**	**33**
Tap water, including well water (8; 4)	**13**	**7**	**22**
Vegetables, fruit and products thereof[(b)] (7; 3)	**12**	**5**	**20**
Other or mixed red meat and products thereof (2; 2)	6	2	13
Pig meat and products thereof (2; 1)	5	1	11
Unspecified meat[(c)] (1; 1)	3	0	8
Fish and seafood[(d)] (1; 1)	3	0	8
Herbs and spices (1; 1)	3	0	8
Sheep meat and products thereof (1; 1)	2	0	5
Cereal products including rice and seeds/pulses (nuts, almonds) (0)	2	0	5
Eggs and egg products (0)	2	0	5
Turkey meat and products thereof (0)	2	0	5
Other, mixed or unspecified poultry meat and products thereof (0)	2	0	5
Broiler meat and products thereof (0)	2	0	5

(a): Includes all foods categorised under 'milk', 'dairy products (other than cheeses)' and 'cheese' from the Zoonoses Catalogue. In at least six outbreaks, the actual source was raw milk.
(b): Includes all foods categorised under 'fruit, berries and juices and other products thereof' and 'vegetables and juices and other products thereof' under the Zoonoses Catalogue.
(c): All foods categorised under 'meat and meat products' from the Zoonoses Catalogue.
(d): Includes all foods categorised under 'fish and fish products' and 'crustaceans, shellfish, molluscs and products thereof'.

Appendix H provides an overview of the available information for the 52 strong evidence STEC outbreaks reported in the EU/EEA from 2012 to 2017. It shows that 12 Member States reported STEC food-borne outbreaks with strong evidence from 2012 to 2017. There is a large variation between reporting practices and rates between countries. Some countries did not report any outbreaks with strong evidence, whereas others provided data on a number of outbreaks with strong evidence. Apparently, the differences in reporting rates reflect differences in ability to identify and investigate food-borne outbreaks as well as reporting practices and levels of food safety.

The strength of the evidence related to an outbreak to be reported to EU level is based on an assessment of all available categories of evidence (i.e. descriptive, epidemiological or microbiological evidence) (EFSA, 2011, 2014).

Specific information regarding the genetic characterisation of the causative agent was mostly missing, serogroup is indicated when reported. For some food-borne outbreaks, no detailed information was available to be able to identify the specific implicated food vehicle and only the categories belonging from the food- and waterborne outbreaks data model of EFSA's zoonoses database were reported. No outbreaks were reported for the following food categories: 'eggs and egg products', 'turkey meat and products thereof', 'other, mixed or unspecified poultry meat and products thereof', 'broiler meat and products thereof', 'cereal products including rice and seeds/pulses (nuts, almonds)'.

The numbers of cases, hospitalisations and deaths for all of the food-borne outbreaks reported are summarised in Table 4 and this information was also considered when answering ToR 3. During 2012–2017, 52 strong evidence food-borne outbreaks caused by STEC were reported with 987 human cases, 214 hospitalisations and 4 deaths.

Table 4: Number of human cases, hospitalisations and deaths per implicated food vehicle category reported in strong evidence STEC food-borne outbreaks from 2012 to 2017

Implicated food vehicle category (number of reported strong evidence outbreaks; number of reporting countries)	**Human cases**	**Hospitalisations**	**Deaths**
Bovine meat and products thereof (15; 7)	**143**	**76**	**0**
Milk and dairy products[(a)] (14; 8)	**94**	**43**	**2**
Tap water, including well water (8; 4)	**75**	**7**	**0**
Vegetables, fruit and products thereof[(b)] (7; 3)	**575**	**73**	**2**
Pig meat and products thereof (2; 1)	6	2	0
Other or mixed red meat and products thereof (2; 2)	10	0	0
Sheep meat and products thereof (1; 1)	27	9	0
Unspecified meat[(c)] (1; 1)	2	1	0
Fish and seafood[(d)] (1; 1)	5	0	0
Herbs and spices (1; 1)	50	3	0
Total	**987**	**214**	**4**

(a): Includes all foods categorised under 'milk', 'dairy products (other than cheeses)' and 'cheese' from the Zoonoses Catalogue. In at least six outbreaks, the actual source was raw milk.
(b): Includes all foods categorised under 'fruit, berries and juices and other products thereof' and 'vegetables and juices and other products thereof' under the Zoonoses Catalogue.
(c): All foods categorised under 'meat and meat products' from the Zoonoses Catalogue.
(d): Includes all foods categorised under 'fish and fish products' and 'crustaceans, shellfish, molluscs and products thereof'.

The 'vegetables, fruit and products thereof' category was responsible for seven of the reported strong evidence outbreaks causing 575 cases, 73 hospitalisations and 2 deaths whereas the 'bovine meat and products thereof' food category was responsible for 15 of these outbreaks, causing 143 cases and 76 hospitalisations. 'Milk and dairy products' were responsible for 14 of these outbreaks causing 94 cases, 43 hospitalisations and 2 deaths and 'tap water, including well water' were responsible for 8 of these outbreaks causing 75 cases and 7 hospitalisations. There is a general tendency for the outbreaks associated with food of non-animal origin to involve more cases than those associated with food of animal origin, which is consistent with previous findings (EFSA BIOHAZ Panel, 2013a). It should also be noted that large outbreaks, and outbreaks that have a longer duration or cause serious disease, are more likely to be investigated and reported.

The following categories 'pig meat and product thereof' and 'other or mixed red meat and products thereof', 'sheep meat and products thereof', 'unspecified meat', 'fish and seafood' and 'herbs and spices' were also responsible for one or two outbreaks each.

3.3.3. Concluding remarks

Food commodities were ranked based on associated risk of STEC outbreaks in humans in the EU/EEA. Environmental exposure or direct contact with animals was therefore not considered. Source attribution analysis suggested that 'bovine meat and products thereof', 'milk and dairy products', 'tap water including well water' and 'vegetables, fruit and products thereof' were the main food vehicles of STEC infection in the EU.

As outlined in Appendix F, there are several uncertainties associated with the interpretation of the results of the source attribution of 'strong evidence' outbreaks, to represent the true risk ranking of different food commodities based on the public health risk. The impact of the small size of the data set is represented in the uncertainty interval given in Table 3, but other sources of uncertainty, such as the inconsistent reporting of food commodities, are not considered.

The majority of 'tap water including well water' outbreaks occurred in one MS, i.e. 5 out of 8 outbreaks (63%), and was most likely associated with well water, which is not frequently used as a source of drinking water in many MSs. Therefore, the finding that 'tap water including well water' is an important source of STEC may not be applicable to the EU as a whole.

Moreover, in at least 6 of the 14 outbreaks linked to milk and dairy products, the actual source was raw milk.

'Vegetables, fruit and products thereof' rank fourth in the source attribution based on strong evidence outbreaks, but is associated with the largest number of cases therein. The number of outbreaks in the data set is too small to assess whether this large number of cases is significantly associated to the source, instead of being related to other factors or being a matter of chance.

Other studies in Europe (Section 3.3.1) and the USA (NACMCF, 2019) confirm the importance of particularly 'bovine meat and products thereof' and 'vegetables, fruit and products thereof' as sources of STEC infections.

Based on the results of the source attribution (Section 3.3.2), these additional arguments and the indicated uncertainties, it is concluded that 'bovine meat and products thereof', 'milk and dairy products', 'tap water including well water' and 'vegetables, fruit and products thereof', are the main sources of STEC infections, but a ranking between these categories cannot be made. Other food commodities are also potentially associated with STEC infections but rank lower.

3.4. Data gaps [ToR 4]

Despite many years of research on STEC and the testing of animals, food, feed and human samples, there are still many unanswered questions about these pathogenic bacteria. The current data gaps prevent a more comprehensive assessment of any association between virulence gene/gene combinations and the severe disease, as required to answer ToR 1. At present, there are only limited data available on *stx* gene subtypes and the presence/absence of *eae* in the TESSy database and analysis suggests all subtypes are pathogenic and associated with severe illness.

The specific data gaps include comprehensive virulence gene profiles for human STEC isolates and metadata on humans infected with STEC including age, immune status, therapeutic treatments, medical history, etc.

To more accurately answer ToR 3, better source attribution data would be required. Such data, in combination with the true prevalence of STEC in animals, food and feed in the EU would facilitate the identification of target food chains when developing control strategies, including potential microbiological criteria/performance objectives. The only existing regulatory limit (microbiological criterion) for STEC in a food commodity is for sprouts, as defined in the Commission Regulation (EC) No 209/2013[11] amending Commission Regulation (EC) No 2073/2005[8], and STEC O157, O26, O111, O103, O145 and O104:H4 must be 'absent in 25 grams', for sprouts placed on the market during their shelf-life. However, despite the legal framework, the production of these data is not fully harmonised as different MS use different sampling plans. The monitoring data regarding STEC in foods other than

[11] Commission Regulation (EU) No 209/2013 of 11 March 2013 amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and the sampling rules for poultry carcases and fresh poultry meat Text with EEA relevance. OJ L 68, 12.3.2013, p. 19–23.

sprouts and monitoring data regarding STEC in animals, originate from the reporting obligations of MS under Directive 2003/99/EC[7], which stipulates that MSs must investigate the presence of STEC at the most appropriate stage of the food chain. The directive is not explicit about the sampling strategy and the data generated by MSs are based on investigations with non-harmonised sampling. Moreover, the directive does not indicate strict details of the mandatory reporting requirements. Therefore, STEC monitoring data according to Directive 2003/99/EC[7] are not comparable between MSs and preclude subsequent data analysis, such as assessing temporal and spatial trends at the EU level. Sampling biases and inaccuracies due to limited numbers of examined samples also preclude the evaluation of the actual prevalence or accurate prevalence estimations. Moreover, the testing of animal samples using laboratory analytical methods that detect STEC O157 only leads to biased STEC prevalence estimations or biased STEC serogroup frequency distributions in this data set.

Data on the sources of STEC infections in humans are further limited. Moreover, there is little or no data on person-to-person transmission, dose-response for different STEC serogroups and/or isolates with defined virulence gene profiles, or on the virulence gene profiles in STEC isolates from food, feed and animals. All of these data would have contributed to ToR3 and/or to develop risk-based control for STEC.

One of the main reasons STEC data are lacking is the current surveillance/monitoring strategy in the EU and the results for the questionnaires on STEC in humans (Annex E) and STEC in food, feed and animals (Annex F) provide an important insight into why these data gaps exist (see Section 3.4.1) and how they could be addressed (see Section 3.4.2).

3.4.1. Surveillance/monitoring of STEC in humans, food, feed and animals

3.4.1.1. EU surveillance data on STEC in humans

MSs have to report available technical data annually to TESSy as described in the Regulation No 851/2004[12] following the EU case definitions. The surveillance systems for STEC infections have national coverage in all countries except three (France, Italy and Spain). In some countries, surveillance is based on the clinical outcome of cases, such as BD or HUS, while others are based on laboratory results only. Heterogeneity also exists in the number of samples tested and isolates typed. There is also a lack of harmonisation across EU and EEA countries in terms of sampling strategy, strain testing (characterisation) and reporting methods.

The main findings of the questionnaire on STEC in humans are as follows (more details are provided in Appendix I):

- Most human samples are initially PCR screened for *stx1*, *stx2*, *eae*, with positive samples being further tested by PCR screening of colonies resulting from plating the enriched samples onto solid media and and/or other methods (e.g. ELISA, toxin detection).
- Less than half of the MSs have a national guideline for the detection of STEC in human samples and there is currently no harmonised approach in the methods used to detect STEC in human samples.
- All NRLs have capacity for virulence gene profiling of STEC isolates, including *stx* subtyping. Many laboratories also type for additional virulence genes and when WGS-based typing is in place, the whole array of virulence genes can be detected.
- WGS is routinely used in majority of the NRLs together with other methods. Four countries used sequencing as the only method for characterising human isolates. The molecular-enhanced surveillance system being implemented as a part of TESSy relies on MS having the capacity to produce these data.
- The criteria used when deciding if a patient should be tested for STEC infection varies between the different MSs. In most cases, patients showing relevant symptoms and (during an outbreak) close contacts are tested. Very few countries/laboratories test all patients with diarrhoea for the presence of STEC.
- More than half of the MS report a complete set of typing data to the ECDC annual data collection in TESSy.

[12] Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for disease prevention and control. OJ L 142, 30.4.2004, p. 1–11.

3.4.1.2. EU monitoring data on STEC in food, feed and animals

The main findings of the questionnaire on STEC in food, feed and animals are as follows (more details are provided in Appendix J);

- The widespread use of the CEN ISO TS 13136:2012 method (ISO, 2012) has resulted in harmonisation of testing of food, feed and animal samples.
- Most of the NRLs are capable of performing virulence genes profiling, including Shiga-toxin gene subtyping, mostly using methods published by the EURL for *E. coli*. The information on the characterisation of the STEC isolates is valuable for the proactive pathogenicity assessment of STEC strains and this capability should be replicated at the official laboratory network level.
- WGS methods are used in approximately half of the NRLs in the survey. This is an important development as it will permit the automatic uploading of the typing data to the EFSA database thus facilitating a more effective pathogenicity assessment.
- More than half of the MSs have implemented a national sampling strategy for routine STEC testing in food, feed or animal samples, mainly concerning meat, milk and sprouts.
- Most MSs do not report the full available data set to EFSA.

3.4.2. Addressing the data gaps

The data gaps that prevent a more comprehensive assessment of the pathogenicity of STEC and the establishment of microbiological criteria in relevant (e.g. beef) food chains are primarily due to inadequacies in methodology and the way in which testing is organised. This finding is based on the expertise and experience within the WG and reinforced by the answers to the questionnaires.

Current STEC detection and characterisation methods have several limitations including a lack of sensitivity and selectivity, reliability, the absence of a universal phenotypic marker for STEC, etc., all of which could be overcome using WGS technologies. WGS applications have been developed, including strain level metagenomics, that enable the identification of STEC virulence genes in a complex sample without having to isolate the bacterial cell. WGS may also be used to examine the evolution and virulence of STEC, determine strain relatedness during outbreak investigations, baseline surveys, etc. This technology, common in academic research institutions, is currently being rolled out in public health laboratories throughout Europe. Many of these laboratories are networked to the European reference laboratories and/or other food safety data collation networks, which will help facilitate a coordinated approach in the use of WGS in STEC detection and characterisation that ensures the following;

1) Methods are standardised and validated.
2) Databases are also standardised with a concerted effort to collate and build the data. Thus, laboratories involved in routine sequencing of STEC isolates should be encouraged to deposit these data. Furthermore, there should be 'open access' to the sequence data and a common approach in terms of the metadata collected which should include information about the source, strain history, epidemiological information, etc. Ideally the metadata should also be readily available in a data set that does not compromise personal or commercial confidentiality.
3) The software for analysing and interpreting the sequence data should be benchmarked and readily available.
4) Issues concerning the lack of qualified bioinformaticians should be addressed through the provision of suitable training, with programmes tailored for those working in the different areas including food, clinical, industrial and academic laboratories.

All of these developments should be complemented by better isolation methods from human, food, feed, animal and environmental samples.

Furthermore, the STEC testing framework (animals, food, feed and humans) within the EU should be harmonised including sampling strategies, sampling methods and reporting. This will require all MSs using the same case definition and outbreak investigation systems. Moreover, it should be a mandatory requirement to report all data (animal, food, feed and human) to EFSA/ECDC and this should be enforced by all MS. To achieve this issues such as; [1] a lack of expertise within a given MS; [2] communication with MSs (the person tasked with completing the reporting form is often not aware that typing data exist and/or do not have access to that data); [3] capacity and resources; [4] isolate characterisation data being used for peer-reviewed publication instead of public health gain; [5]

complexity and time-consuming nature of uploading data, will have to be addressed through the best use of ICT data management systems (including automated, real-time uploading of data from MSs) and training. It is recommended that EFSA and ECDC engage more proactively with MS with respect to the importance of their databases. Systematic review of the literature provides some indication that more STEC data are available from EU countries than is being captured by the EFSA/ECDC returns. This may be because the typing is being performed retrospectively and/or by institutions other that the national reference laboratory. It is recommended that EFSA/ECDC data are updated to include retrospective typing data.

4. Conclusions

Answer to ToR 1. Review the new body of knowledge available for pathogenicity assessment of STEC, and refine, if needed, the molecular approach for the categorisation of STEC strains proposed in the EFSA 2013 Opinion.

- The molecular approach described in EFSA 2013 for the pathogenicity assessment of STEC has been revised to consider *stx* subtypes as described below.
- Based on the available evidence, it is concluded that all STEC strains are pathogenic in humans, capable of causing at least diarrhoea. Moreover, based on the analysis of the *stx* subtypes, all STEC subtypes may be associated with severe illness, i.e. HUS, BD and/or hospitalisation. Although *stx2a* showed the highest rates of HUS, hospitalisation and BD, all other *stx* subtypes or combinations thereof, for which there was sufficient data, were also associated with at least one of these severe illness outcomes.
- The presence of intimin (*eae* gene) was an aggravating factor, but this virulence factor was not always essential for severe illness.
- The minimum combination of genes required to cause severe illness is unknown and even if it was established there are many contributory factors such as gene expression levels, alternative genes performing similar functions and host factors.

Answer to ToR 2. Review the microbiological methods for the detection and characterisation of human pathogenic STEC in animals and food.

- There are a range of methods (e.g. immunological and molecular methods) available for the detection of STEC, but their effectiveness is limited by the need to demonstrate that the signals identified are derived from a live bacterial cell, which, in turn, is affected by lack of sensitivity in the isolation step, which is necessary to demonstrate that the isolated *E. coli* possess the *stx* genes and thus to discriminate between STEC and other *E. coli.*
- At EU level, the methodology for STEC detection in food is substantially harmonised. For example, the international standard ISO TS 13136:2012 has been used to test the 97.4% of the food samples reported to EFSA in 2017.
- There are also a range of characterisation methods including sero-, phage- and molecular typing. At EU level, the methodology for STEC characterisation in food isolates is not currently standardised. However, the revision of the standard ISO TS 13136, which is ongoing, will include part 2 of the document dedicated to STEC strain characterisation, including also the advice to use more holistic characterisation approaches such as WGS.

Answer to ToR 3. Analyse available data on human food-borne STEC cases in the EU and rank different food commodities based on the public health risk.

- Data on 'strong evidence' outbreaks reported to EFSA from 2012 to 2017 were analysed by an established source attribution method and the results were critically evaluated and considered in the light of other evidence.
- It was concluded that 'bovine meat and products thereof', 'milk and dairy products', 'tap water including well water' and 'vegetables, fruit and products thereof' are the main food commodities causing human STEC infections. Other food commodities are also potentially associated with STEC infections, but rank lower.

Answer to ToR 4. To provide recommendations to fill the data gaps identified in the above assessment.

The following are recommended to fill the data gaps identified:

- There should be harmonisation of sampling (surveillance strategies, case definition, number of samples tested, etc.) and testing (although most laboratories in the different MS use the ISO TS 13136:2012 method for testing food and feed samples).
- MSs should develop national guidelines for the detection of STEC in human samples and the characterisation of isolated strains. Clinical detection and characterisation methods should be harmonised across the EU. Additionally, the use of WGS to type the isolated STEC is strongly recommended.
- The objectives for STEC surveillance should be changed to ensure that all MSs collect and collate data on all STEC cases and not just HUS cases. Public health and other laboratories supporting the healthcare system in MSs should be encouraged to isolate STEC from all patients with a positive STEC PCR or other detection test and undertake WGS analysis using a methodology that is harmonised across the EU. Extensive metadata (age, immune status, therapeutic treatments, medical history, etc.) for each patient infected with STEC in individual MSs should also be collected and all these data/information forwarded to ECDC.
- The STEC reporting in the EU should be reviewed, focusing on user-friendly and efficient sharing and reporting systems.

5. Recommendations

- Methods should be developed and validated for analysing WGS data from animal, food, feed and human isolates to identify trends and emerging STEC strains in a timely manner.
- STEC WGS operational systems need to be developed and implemented for sharing interoperable WGS data. The data should be well documented in a comprehensible and standardised way and include strain associated anonymised metadata (information about the source, strain history, epidemiological information, etc.) thus overcoming ethical, legal and/or commercial issues.
- Research and development should be undertaken on (1) the improvement of isolation methods for STEC; (2) the epidemiology of STEC in foods including food, animals and environmental sources; (3) the impact of host factors on the outcomes of STEC infection and (4) toxin expression (including the relationship between the bacteriophage encoding the Shiga toxin and the levels of production) and its transport to the final location in the human body.

References

Abdalhamid B, McCutchen EL, Bouska AC, Weiwei Z, Loeck B, Hinrichs SH and Iwen PC, 2019. Whole genome sequencing to characterize shiga toxin-producing Escherichia coli O26 in a public health setting. Journal of Infection and Public Health, 12, 884–889. https://doi.org/10.1016/j.jiph.2019.06.008

Abu-Ali GS, Ouellette LM, Henderson ST, Lacher DW, Riordan JT, Whittam TS and Manning SD, 2010. Increased adherence and expression of virulence genes in a lineage of *Escherichia coli* O157:H7 commonly associated with human infections. PLoS ONE, 5, e10167. https://doi.org/10.1371/journal.pone.0010167

Adams N, Byrne L, Edge J, Hoban A, Jenkins C and Larkin L, 2019. Gastrointestinal infections caused by consumption of raw drinking milk in England & Wales, 1992–2017. Epidemiology and Infection, 147, e281. https://doi.org/10.1017/S095026881900164X

Afset JE, Bruant G, Brousseau R, Harel J, Anderssen E, Bevanger L and Bergh K, 2006. Identification of virulence genes linked with diarrhea due to atypical enteropathogenic *Escherichia coli* by DNA microarray analysis and PCR. Journal of Clinical Microbiology, 44, 3703–3711. https://doi.org/10.1128/jcm.00429-06

Allerberger F, Friedrich AW, Grif K, Dierich MP, Dornbusch HJ, Mache CJ, Nachbaur E, Freilinger M, Rieck P, Wagner M, Caprioli A, Karch H and Zimmerhackl LB, 2003. Hemolytic-uremic syndrome associated with enterohemorrhagic Escherichia coli O26: H infection and consumption of unpasteurized cow's milk. International Journal of Infectious Diseases, 7, 42–45.

Alperi A and Figueras MJ, 2010. Human isolates of *Aeromonas* possess Shiga toxin genes (*stx1* and *stx2*) highly similar to the most virulent gene variants of *Escherichia coli*. Clinical Microbiology and Infection, 16, 1563–1567. https://doi.org/10.1111/j.1469-0691.2010.03203.x

Bai X, Fu S, Zhang J, Fan R, Xu Y, Sun H, He X, Xu J and Xiong Y, 2018. Identification and pathogenomic analysis of an *Escherichia coli* strain producing a novel Shiga toxin 2 subtype. Scientific Reports, 8, 6756. https://doi.org/10.1038/s41598-018-25233-x

Bai X, Zhang J, Ambikan A, Jernberg C, Ehricht R, Scheutz F, Xiong Y and Matussek A, 2019. Molecular characterization and comparative genomics of clinical hybrid shiga toxin-producing and enterotoxigenic Escherichia coli (STEC/ETEC) strains in Sweden. Scientific Reports, 9, 5619. https://doi.org/10.1038/s41598-019-42122-z

Bennett AR, MacPhee S and Betts RP, 1995. Evaluation of methods for the isolation and detection of *Escherichia coli* O157 in minced beef. Letters in Applied Microbiology, 20, 375–379.
Benz I and Schmidt MA, 1989. Cloning and expression of an adhesin (AIDA-I) involved in diffuse adherence of enteropathogenic *Escherichia coli*. Infection and Immunity, 57, 1506–1511.
Benz I and Schmidt MA, 1993. Diffuse adherence of enteropathogenic *Escherichia coli* strains–processing of AIDA-I. Zentralblatt fuer Bakteriologie, 278, 197–208.
Besser TE, Shaikh N, Holt NJ, Tarr PI, Konkel ME, Malik-Kale P, Walsh CW, Whittam TS and Bono JL, 2007. Greater diversity of Shiga toxin-encoding bacteriophage insertion sites among *Escherichia coli* O157:H7 isolates from cattle than in those from humans. Applied and Environment Microbiology, 73, 671–679. https://doi.org/10.1128/aem.01035-06
Beutin L, Montenegro MA, Orskov I, Orskov F, Prada J, Zimmermann S and Stephan R, 1989. Close association of verotoxin (Shiga-like toxin) production with enterohemolysin production in strains of *Escherichia coli*. Journal of Clinical Microbiology, 27, 2559–2564.
Beutin L, Strauch E and Fischer I, 1999. Isolation of *Shigella sonnei* lysogenic for a bacteriophage encoding gene for production of Shiga toxin. Lancet, 353, 1498. https://doi.org/10.1016/s0140-6736(99)00961-7
Beutin L, Miko A, Krause G, Pries K, Haby S, Steege K and Albrecht N, 2007. Identification of human-pathogenic strains of Shiga toxin-producing *Escherichia coli* from food by a combination of serotyping and molecular typing of Shiga toxin genes. Applied and Environment Microbiology, 73, 4769–4775. https://doi.org/10.1128/aem.00873-07
Beutin L, Kruger U, Krause G, Miko A, Martin A and Strauch E, 2008. Evaluation of major types of Shiga toxin 2E-producing *Escherichia coli* bacteria present in food, pigs, and the environment as potential pathogens for humans. Applied and Environment Microbiology, 74, 4806–4816. https://doi.org/10.1128/aem.00623-08
Bezuidt O, Pierneef R, Mncube K, Lima-Mendez G and Reva ON, 2011. Mainstreams of horizontal gene exchange in enterobacteria: consideration of the outbreak of enterohemorrhagic *E. coli* O104:H4 in Germany in 2011. PLoS ONE, 6, e25702. https://doi.org/10.1371/journal.pone.0025702
Bielaszewska M, Friedrich AW, Aldick T, Schurk-Bulgrin R and Karch H, 2006. Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: predictor for a severe clinical outcome. Clinical Infectious Diseases, 43, 1160–1167. https://doi.org/10.1086/508195
Bielaszewska M, Zhang W, Mellmann A and Karch H, 2007. Enterohaemorrhagic Escherichia coli O26:H11/H-: a human pathogen in emergence. Berliner und Munchener Tierarztliche Wochenschrift, 120, 279–287.
Bielaszewska M, Mellmann A, Zhang W, Kock R, Fruth A, Bauwens A, Peters G and Karch H, 2011. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infectious Diseases, 11, 671–676. https://doi.org/10.1016/s1473-3099(11)70165-7
Bielaszewska M, Mellmann A, Bletz S, Zhang W, Kock R, Kossow A, Prager R, Fruth A, Orth-Holler D, Marejkova M, Morabito S, Caprioli A, Pierard D, Smith G, Jenkins C, Curova K and Karch H, 2013. Enterohemorrhagic *Escherichia coli* O26:H11/H-: a new virulent clone emerges in Europe. Clinical Infectious Diseases, 56, 1373–1381. https://doi.org/10.1093/cid/cit055
Bielaszewska M, Schiller R, Lammers L, Bauwens A, Fruth A, Middendorf B, Schmidt MA, Tarr PI, Dobrindt U, Karch H and Mellmann A, 2014. Heteropathogenic virulence and phylogeny reveal phased pathogenic metamorphosis in *Escherichia coli* O2:H6. EMBO Molecular Medicine, 6, 347–357. https://doi.org/10.1002/emmm.201303133
Boelaert F, Amore G, Van der Stede Y and Hugas M, 2016. EU-wide monitoring of biological hazards along the food chain: achievements, challenges and EFSA vision for the future. Current Opinion in Food Science, 12, 52–62. https://doi.org/10.1016/j.cofs.2016.08.004
de Boer RF, Ferdous M, Ott A, Scheper HR, Wisselink GJ, Heck ME, Rossen JW and Kooistra-Smid AM, 2015. Assessing the public health risk of Shiga toxin-producing *Escherichia coli* by use of a rapid diagnostic screening algorithm. Journal of Clinical Microbiology, 53, 1588–1598. https://doi.org/10.1128/jcm.03590-14
Boisen N, Hansen AM, Melton-Celsa AR, Zangari T, Mortensen NP, Kaper JB, O'Brien AD and Nataro JP, 2014. The presence of the pAA plasmid in the German O104:H4 Shiga toxin type 2a (Stx2a)-producing enteroaggregative *Escherichia coli* strain promotes the translocation of Stx2a across an epithelial cell monolayer. Journal of Infectious Diseases, 210, 1909–1919. https://doi.org/10.1093/infdis/jiu399
Boisen N, Melton-Celsa AR, Scheutz F, O'Brien AD and Nataro JP, 2015. Shiga toxin 2a and Enteroaggregative *Escherichia coli*–a deadly combination. Gut Microbes, 6, 272–278. https://doi.org/10.1080/19490976.2015.1054591
Bono JL, Keen JE, Clawson ML, Durso LM, Heaton MP and Laegreid WW, 2007. Association of *Escherichia coli* O157:H7 tir polymorphisms with human infection. BMC Infectious Diseases, 7, 98. https://doi.org/10.1186/1471-2334-7-98
Brandal LT, Tunsjo HS, Ranheim TE, Lobersli I, Lange H and Wester AL, 2015a. Shiga toxin 2a in *Escherichia albertii*. Journal of Clinical Microbiology, 53, 1454–1455. https://doi.org/10.1128/jcm.03378-14
Brandal LT, Wester AL, Lange H, Lobersli I, Lindstedt BA, Vold L and Kapperud G, 2015b. Shiga toxin-producing *escherichia coli* infections in Norway, 1992–2012: characterization of isolates and identification of risk factors for haemolytic uremic syndrome. BMC Infectious Diseases, 15, 324. https://doi.org/10.1186/s12879-015-1017-6
Breitbart M, Hewson I, Felts B, Mahaffy JM, Nulton J, Salamon P and Rohwer F, 2003. Metagenomic analyses of an uncultured viral community from human feces. Journal of Bacteriology, 185, 6220–6223.

Brett KN, Ramachandran V, Hornitzky MA, Bettelheim KA, Walker MJ and Djordjevic SP, 2003. stx1c Is the most common Shiga toxin 1 subtype among Shiga toxin-producing *Escherichia coli* isolates from sheep but not among isolates from cattle. Journal of Clinical Microbiology, 41, 926–936.

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM and Strockbine NA, 2005. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. Journal of Infectious Diseases, 192, 1422–1429. https://doi.org/10.1086/466536

Brunder W, Schmidt H and Karch H, 1996. KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. Microbiology, 142(Pt 11), 3305–3315. https://doi.org/10.1099/13500872-142-11-3305

Brusa V, Pineyro PE, Galli L, Linares LH, Ortega EE, Padola NL and Leotta GA, 2016. Isolation of Shiga Toxin-Producing *Escherichia coli* from ground beef using multiple combinations of enrichment broths and selective agars. Foodborne Pathogenic Diseases, 13, 163–170. https://doi.org/10.1089/fpd.2015.2034.

Burland V, Shao Y, Perna NT, Plunkett G, Sofia HJ and Blattner FR, 1998. The complete DNA sequence and analysis of the large virulence plasmid of *Escherichia coli* O157:H7. Nucleic Acids Research, 26, 4196–4204.

Buvens G, Posse B, De Schrijver K, De Zutter L, Lauwers S and Pierard D, 2011. Virulence profiling and quantification of verocytotoxin-producing *Escherichia coli* O145:H28 and O26:H11 isolated during an ice cream-related hemolytic uremic syndrome outbreak. Foodborne Pathogenic Disease, 8, 421–426. https://doi.org/10.1089/fpd.2010.0693

Buvens G, De Gheldre Y, Dediste A, de Moreau AI, Mascart G, Simon A, Allemeersch D, Scheutz F, Lauwers S and Pierard D, 2012. Incidence and virulence determinants of verocytotoxin-producing *Escherichia coli* infections in the Brussels-Capital Region, Belgium, in 2008–2010. Journal of Clinical Microbiology, 50, 1336–1345. https://doi.org/10.1128/jcm.05317-11

Byrne L, Elson R, Dallman TJ, Perry N, Ashton P, Wain J, Adak GK, Grant KA and Jenkins C, 2014. Evaluating the use of multilocus variable number tandem repeat analysis of Shiga toxin-producing *Escherichia coli* O157 as a routine public health tool in England. PLoS ONE, 9, e85901. https://doi.org/10.1371/journal.pone.0085901

Carbonari CC, Ricciardi M, Calvo AR, Montes A, Deza NL, Conde Valentino MA, Zolezzi G, Baschkier A, Vago M, Acosta D, Manfredi EA, Miliwebsky ES, Chinen I and Rivas M, 2019. An Stx-EAEC O59:NM[H19] strain isolated from a hemolytic uremic syndrome case in Argentina. Revista Argentina de Microbiologia, https://doi.org/10.1016/j.ram.2019.03.006.

Carter MQ and Pham A, 2018. Complete genome sequences of two atypical enteropathogenic Escherichia coli O145 environmental strains. Genome Announcments, 6, https://doi.org/10.1128/genomeA.00418-18.

Carter CC, Fierer J, Chiu WW, Looney DJ, Strain M and Mehta SR, 2016. A novel Shiga toxin 1a-converting bacteriophage of *Shigella sonnei* with close relationship to Shiga toxin 2-converting phages of *Escherichia coli*. Open Forum Infectious Diseases, 3, ofw079. https://doi.org/10.1093/ofid/ofw079

CDC (Centers for Disease Control and Prevention), 2018. National Shiga toxin-producing Escherichia coli (STEC) surveillance annual report, 2016. US Department of Health and Human Services, Atlanta, Georgia. Available online: https://www.cdc.gov/ecoli/surv2016/index.html

Chattaway MA, Dallman TJ, Gentle A, Wright MJ, Long SE, Ashton PM, Perry NT and Jenkins C, 2016. Whole genome sequencing for public health surveillance of Shiga toxin-producing *Escherichia coli* other than serogroup O157. Frontiers in Microbiology, 7, 258. https://doi.org/10.3389/fmicb.2016.00258

Costa M, Sucari A, Epszteyn S, Oteiza J, Gentiluomo J, Melamed C, Figueroa Y, Mingorance S, Grisaro A, Spioussas S, Almeida MB, Caruso M, Pontoni A, Signorini M and Leotta G, 2019. Comparison of six commercial systems for the detection of non-O157 STEC in meat and vegetables. Food Microbiology, 84, 103273. https://doi.org/10.1016/j.fm.2019.103273

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M and Finlay BB, 2013. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews, 26, 822–880. https://doi.org/10.1128/cmr.00022-13

Dallman T, Smith GP, O'Brien B, Chattaway MA, Finlay D, Grant KA and Jenkins C, 2012. Characterization of a verocytotoxin-producing enteroaggregative *Escherichia coli* serogroup O111:H21 strain associated with a household outbreak in Northern Ireland. Journal of Clinical Microbiology, 50, 4116–4119. https://doi.org/10.1128/jcm.02047-12

Dallman TJ, Byrne L, Ashton PM, Cowley LA, Perry NT, Adak G, Petrovska L, Ellis RJ, Elson R, Underwood A, Green J, Hanage WP, Jenkins C, Grant K and Wain J, 2015. Whole-genome sequencing for national surveillance of Shiga toxin-producing *Escherichia coli* O157. Clinical Infectious Diseases, 61, 305–312. https://doi.org/10.1093/cid/civ318

De Rauw K, Buyl R, Jacquinet S and Pierard D, 2018. Risk determinants for the development of typical haemolytic uremic syndrome in Belgium and proposition of a new virulence typing algorithm for Shiga toxin-producing *Escherichia coli*. Epidemiology and Infection, 1–5. https://doi.org/10.1017/s0950268818002546

Delannoy S, Mariani-Kurkdjian P, Bonacorsi S, Liguori S and Fach P, 2015. Characteristics of emerging human-pathogenic *Escherichia coli* O26:H11 strains isolated in France between 2010 and 2013 and carrying the stx2d gene only. Journal of Clinical Microbiology, 53, 486–492. https://doi.org/10.1128/jcm.02290-14

Devleesschauwer B, Pires SM, Young I, Gill A and Majowicz SE, 2019. Associating sporadic, foodborne illness caused by Shiga toxin-producing *Escherichia coli* with specific foods: a systematic review and meta-analysis of case-control studies. Epidemiology and Infection, 147, e235. https://doi.org/10.1017/S0950268819001183

Donohue-Rolfe A, Kondova I, Oswald S, Hutto D and Tzipori S, 2000. Escherichia coli O157: H7 strains that express Shiga toxin (Stx) 2 alone are more neurotropic for gnotobiotic piglets than are isotypes producing only Stx1 or both Stx1 and Stx2. Journal of Infectious Diseases, 181, 1825–1829. https://doi.org/10.1086/315421

Dowd SE and Williams JB, 2008. Comparison of Shiga-like toxin II expression between two genetically diverse lineages of *Escherichia coli* O157:H7. Journal of Food Protection, 71, 1673–1678.

Downes FP, Green JH, Greene K, Strockbine N, Wells JG and Wachsmuth IK, 1989. Development and evaluation of enzyme-linked immunosorbent assays for detection of shiga-like toxin I and shiga-like toxin II. Journal of Clinical Microbiology, 27, 1292–1297.

Dytoc MT, Ismaili A, Philpott DJ, Soni R, Brunton JL and Sherman PM, 1994. Distinct binding properties of eaeA-negative verocytotoxin-producing *Escherichia coli* of serotype O113:H21. Infection and Immunity, 62, 3494–3505.

EFSA (European Food Safety and Authority), 2009. Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food) on request of EFSA. EFSA Journal 2009;7(11):1366, 43 pp. https://doi.org/10.2903/j.efsa.2007.579

EFSA (European Food Safety and Authority), 2011. Updated technical specifications for harmonised reporting of foodborne outbreaks through the European Union reporting system in accordance with Directive 2003/99/EC. EFSA Journal 2011;9(4):2101, 24 pp. https://doi.org/10.2903/j.efsa.2011.2101

EFSA (European Food Safety and Authority), 2014. Update of the technical specifications for harmonised reporting of food-borne outbreaks through the European Union reporting system in accordance with Directive 2003/99/EC. EFSA Journal 2014;12(3):3598, 25 pp. https://doi.org/10.2903/j.efsa.2014.3598

EFSA (European Food Safety and Authority), 2018. Outcome of EC/EFSA questionnaire (2016) on use of Whole Genome Sequencing (WGS) for food-and waterborne pathogens isolated from animals, food, feed and related environmental samples in EU/EFTA countries. EFSA supporting publication 2018;EN-1432, 49 pp. https://doi.org/10.2903/sp.efsa.2018.en-1432

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013a. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). EFSA Journal 2013;11(1):3025, 138 pp. https://doi.org/10.2903/j.efsa.2013.3025

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013b. Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA Journal 2013;11(4):3138, 106 pp. https://doi.org/10.2903/j.efsa.2013.3138

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2015. Public health risks associated with Enteroaggregative *Escherichia coli* (EAEC) as a food-borne pathogen. EFSA Journal 2015;13(12):4330, 87 pp. https://doi.org/10.2903/j.efsa.2015.4330

EFSA and ECDC (European Food Safety and Authority and European Centre for Disease Prevention and Control), 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal 2017;15(12):5077, 228 pp. https://doi.org/10.2903/j.efsa.2017.5077

EFSA and ECDC (European Food Safety and Authority and European Centre for Disease Prevention and Control), 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal2018;16(12):5500, https://doi.org/10.2903/j.efsa.2018.5500

EFSA and ECDC(European Food Safety and Authority),, 2019. The European Union One Health 2018 Zoonoses Report. EFSA Journal 2019;17(12):5926, 276 pp. https://doi.org/10.2903/j.efsa.2019.5926

EFSA Scientific Committee, 2018. Guidance on uncertainty analysis in scientific assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123

Eppinger M, Mammel MK, Leclerc JE, Ravel J and Cebula TA, 2011. Genomic anatomy of *Escherichia coli* O157:H7 outbreaks. Proceedings of the National Academy of Sciences of the United States of America, 108, 20142–20147. https://doi.org/10.1073/pnas.1107176108

Etcheverria AI and Padola NL, 2013. Shiga toxin-producing *Escherichia coli*: factors involved in virulence and cattle colonization. Virulence, 4, 366–372. https://doi.org/10.4161/viru.24642

Ethelberg S, Olsen KE, Scheutz F, Jensen C, Schiellerup P, Enberg J, Petersen AM, Olesen B, Gerner-Smidt P and Molbak K, 2004. Virulence factors for hemolytic uremic syndrome, Denmark. Emerging Infectious Diseases, 10, 842–847. https://doi.org/10.3201/eid1005.030576

European Union, 2008. Commission Decision of 28/IV/2008 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. Available online: https://ec.europa.eu/health/ph_threats/com/docs/1589_2008_en.pdf

European Union, 2012. Commission Implementing Decision 2012/506/EU of 8 August 2012 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. Aailable online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:262:0001:0057:EN:PDF

Fan R, Shao K, Yang X, Bai X, Fu S, Sun H, Xu Y, Wang H, Li Q, Hu B, Zhang J and Xiong Y, 2019. High prevalence of non-O157 Shiga toxin-producing Escherichia coli in beef cattle detected by combining four selective agars. BMC Microbiology, 19, 213. https://doi.org/10.1186/s12866-019-1582-8

FAO/WHO, 2018. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring. Microbiological Risk Assessment Series. Rome. Available online: http://www.fao.org/3/ca0032en/CA0032EN.pdf.Place

Fasel D, Mellmann A, Cernela N, Hachler H, Fruth A, Khanna N, Egli A, Beckmann C, Hirsch HH, Goldenberger D and Stephan R, 2014. Hemolytic uremic syndrome in a 65-Year-old male linked to a very unusual type of stx2e- and eae-harboring O51:H49 shiga toxin-producing *Escherichia coli*. Journal of Clinical Microbiology, 52, 1301–1303. https://doi.org/10.1128/jcm.03459-13

Feng PC and Reddy S, 2013. Prevalences of Shiga toxin subtypes and selected other virulence factors among Shiga-toxigenic *Escherichia coli* strains isolated from fresh produce. Applied and Environment Microbiology, 79, 6917–6923. https://doi.org/10.1128/aem.02455-13

Feng PC and Reddy SP, 2014. Prevalence and diversity of enterotoxigenic Escherichia coli strains in fresh produce. Journal of Food Protection, 77, 820–823. https://doi.org/10.4315/0362-028x.jfp-13-412

Feng PCH, Delannoy S, Lacher DW, Bosilevac JM, Fach P and Beutin L, 2017. Shiga toxin-producing serogroup O91 *Escherichia coli* strains isolated from food and environmental samples. Applied and Environment Microbiology, 83, https://doi.org/10.1128/aem.01231-17

Fierz L, Cernela N, Hauser E, Nuesch-Inderbinen M and Stephan R, 2017. Characteristics of shigatoxin-producing *Escherichia coli* strains isolated during 2010–2014 from human infections in Switzerland. Frontiers in Microbiology, 8, 1471. https://doi.org/10.3389/fmicb.2017.01471

Frank C, Werber D, Cramer JP, Askar M, Faber M, an der Heiden M, Bernard H, Fruth A, Prager R, Spode A, Wadl M, Zoufaly A, Jordan S, Kemper MJ, Follin P, Muller L, King LA, Rosner B, Buchholz U, Stark K and Krause G, 2011. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. New England Journal of Medicine, 365:1771–1780. https://doi.org/10.1056/nejmoa1106483

Franz E, Delaquis P, Morabito S, Beutin L, Gobius K, Rasko DA, Bono J, French N, Osek J, Lindstedt BA, Muniesa M, Manning S, LeJeune J, Callaway T, Beatson S, Eppinger M, Dallman T, Forbes KJ, Aarts H, Pearl DL, Gannon VP, Laing CR and Strachan NJ, 2014. Exploiting the explosion of information associated with whole genome sequencing to tackle Shiga toxin-producing Escherichia coli (STEC) in global food production systems. International Journal of Food Microbiology, 187, 57–72. https://doi.org/10.1016/j.ijfoodmicro.2014.07.002

Franz E, van Hoek AH, Wuite M, van der Wal FJ, de Boer AG, Bouw EI and Aarts HJ, 2015. Molecular hazard identification of non-O157 Shiga toxin-producing *Escherichia coli* (STEC). PLoS ONE, 10, e0120353. https://doi.org/10.1371/journal.pone.0120353

Franz E, Rotariu O, Lopes BS, MacRae M, Bono JL, Laing C, Gannon V, Soderlund R, van Hoek A, Friesema I, French NP, George T, Biggs PJ, Jaros P, Rivas M, Chinen I, Campos J, Jernberg C, Gobius K, Mellor GE, Chandry PS, Perez-Reche F, Forbes KJ and Strachan NJC, 2018. Phylogeographic analysis reveals multiple international transmission events have driven the global emergence of Escherichia coli O157:H7. Clinical Infectious Diseases, 69, 428–437. https://doi.org/10.1093/cid/ciy919

Fratamico PM, Wasilenko JL, Garman B, Demarco DR, Varkey S, Jensen M, Rhoden K and Tice G, 2014. Evaluation of a multiplex real-time PCR method for detecting shiga toxin-producing Escherichia coli in beef and comparison to the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology laboratory guidebook method. Journal of Food Protection, 77, 180–188. https://doi.org/10.4315/0362-028x.Jfp-13-248

Friedrich AW, Bielaszewska M, Zhang WL, Pulz M, Kuczius T, Ammon A and Karch H, 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. Journal of Infectious Diseases, 185, 74–84. https://doi.org/10.1086/338115

Friedrich AW, Borell J, Bielaszewska M, Fruth A, Tschape H and Karch H, 2003. Shiga toxin 1c-producing *Escherichia coli* strains: phenotypic and genetic characterization and association with human disease. Journal of Clinical Microbiology, 41, 2448–2453.

Friedrich AW, Kock R, Bielaszewska M, Zhang W, Karch H and Mathys W, 2005. Distribution of the urease gene cluster among and urease activities of enterohemorrhagic *Escherichia coli* O157 isolates from humans. Journal of Clinical Microbiology, 43, 546–550. https://doi.org/10.1128/jcm.43.2.546-550.2005

Friesema I, van der Zwaluw K, Schuurman T, Kooistra-Smid M, Franz E, van Duynhoven Y and van Pelt W, 2014. Emergence of Escherichia coli encoding Shiga toxin 2f in human Shiga toxin-producing E. coli (STEC) infections in the Netherlands, January 2008 to December 2011. Eurosurveillance Weekly, 19, 26–32.

Friesema IH, Keijzer-Veen MG, Koppejan M, Schipper HS, van Griethuysen AJ, Heck ME and van Pelt W, 2015. Hemolytic uremic syndrome associated with *Escherichia coli* O8:H19 and Shiga toxin 2f gene. Emerging Infectious Diseases, 21, 168–169. https://doi.org/10.3201/eid2101.140515

Fujii J, Mizoue T, Kita T, Kishimoto H, Joh K, Nakada Y, Ugajin S, Naya Y, Nakamura T, Tada Y, Okabe N, Maruyama Y, Saitoh K and Kurozawa Y, 2016. Risk of haemolytic uraemic syndrome caused by shiga-toxin-producing *Escherichia coli* infection in adult women in Japan. Epidemiology and Infection, 144, 952–961. https://doi.org/10.1017/s0950268815002289

Fuller CA, Pellino CA, Flagler MJ, Strasser JE and Weiss AA, 2011. Shiga toxin subtypes display dramatic differences in potency. Infection and Immunity, 79, 1329–1337. https://doi.org/10.1128/iai.01182-10

Gannon VP, Teerling C, Masri SA and Gyles CL, 1990. Molecular cloning and nucleotide sequence of another variant of the *Escherichia coli* Shiga-like toxin II family. Journal of General Microbiology, 136, 1125–1135. https://doi.org/10.1099/00221287-136-6-1125

Garcia-Aljaro C, Muniesa M, Jofre J and Blanch AR, 2006. Newly identified bacteriophages carrying the *stx2g* Shiga toxin gene isolated from *Escherichia coli* strains in polluted waters. FEMS Microbiology Letters, 258, 127–135. https://doi.org/10.1111/j.1574-6968.2006.00213.x

Gardette M, Le Hello S, Mariani-Kurkdjian P, Fabre L, Gravey F, Garrivier A, Loukiadis E and Jubelin G, 2019. Identification and prevalence of in vivo-induced genes in enterohaemorrhagic Escherichia coli. Virulence, 10, 180–193. https://doi.org/10.1080/21505594.2019.1582976

Gerner-Smidt P and Scheutz F, 2006. Standardized pulsed-field gel electrophoresis of Shiga toxin-producing *Escherichia coli*: the PulseNet Europe Feasibility Study. Foodborne Pathogenic Diseases, 3, 74–80. https://doi.org/10.1089/fpd.2006.3.74

Gill A and Huszczynski G, 2016. Enumeration of *Escherichia coli* O157:H7 in outbreak-associated beef patties. Journal of Food Protection, 79, 1266–1268. https://doi.org/10.4315/0362-028x.Jfp-15-521

Gill A and Oudit D, 2015. Enumeration of *Escherichia coli* O157 in outbreak-associated gouda cheese made with raw milk. Journal of Food Protection, 78, 1733–1737. https://doi.org/10.4315/0362-028x.jfp-15-036

Gill A, Huszczynski G, Gauthier M and Blais B, 2014. Evaluation of eight agar media for the isolation of shiga toxin-Producing *Escherichia coli*. Journal of Microbiological Methods, 96, 6–11. https://doi.org/10.1016/j.mimet.2013.10.022

Goswami K, Chen C, Xiaoli L, Eaton KA and Dudley EG, 2015. Coculture of *Escherichia coli* O157:H7 with a Nonpathogenic *E. coli* strain increases toxin production and virulence in a Germfree Mouse model. Infection and Immunity, 83, 4185–4193. https://doi.org/10.1128/iai.00663-15

Grande L, Michelacci V, Tozzoli R, Ranieri P, Maugliani A, Caprioli A and Morabito S, 2014. Whole genome sequence comparison of *vtx2*-converting phages from Enteroaggregative Haemorrhagic *Escherichia coli* strains. BMC Genomics, 15, 574. https://doi.org/10.1186/1471-2164-15-574

Grande L, Michelacci V, Bondi R, Gigliucci F, Franz E, Badouei MA, Schlager S, Minelli F, Tozzoli R, Caprioli A and Morabito S, 2016. Whole-genome characterization and strain comparison of VT2f-producing *Escherichia coli* causing hemolytic uremic syndrome. Emerging Infectious Diseases, 22, 2078–2086. https://doi.org/10.3201/eid2212.160017

Grotiuz G, Sirok A, Gadea P, Varela G and Schelotto F, 2006. Shiga toxin 2-producing *Acinetobacter haemolyticus* associated with a case of bloody diarrhea. Journal of Clinical Microbiology, 44, 3838–3841. https://doi.org/10.1128/jcm.00407-06

Gyles CL, 2007. Shiga toxin-producing *Escherichia coli*: an overview. Journal of Animal Science, 85, E45–E62. https://doi.org/10.2527/jas.2006-508

Hald T, Aspinall W, Devleesschauwer B, Cooke R, Corrigan T, Havelaar AH, Gibb HJ, Torgerson PR, Kirk MD, Angulo FJ, Lake RJ, Speybroeck N and Hoffmann S, 2016. World Health Organization estimates of the relative contributions of food to the burden of disease due to selected foodborne hazards: a structured expert elicitation. PLoS ONE, 11, 35. https://doi.org/10.1371/journal.pone.0145839

Hara-Kudo Y and Takatori K, 2011. Contamination level and ingestion dose of foodborne pathogens associated with infections. Epidemiology and Infection, 139, 1505–1510. https://doi.org/10.1017/s095026881000292x

Harrington SM, Dudley EG and Nataro JP, 2006. Pathogenesis of enteroaggregative *Escherichia coli* infection. FEMS Microbiology Letters, 254, 12–18. https://doi.org/10.1111/j.1574-6968.2005.00005.x

Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, Praet N, Bellinger DC, De Silva NR, Gargouri N, Speybroeck N, Cawthorne A, Mathers C, Stein C, Angulo FJ and Devleesschauwer B and World Health Organization Foodborne Disease Burden, 2015. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. Plos Medicine, 12, 23. https://doi.org/10.1371/journal.pmed.1001923

Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han CG, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tobe T, Iida T, Takami H, Honda T, Sasakawa C, Ogasawara N, Yasunaga T, Kuhara S, Shiba T, Hattori M and Shinagawa H, 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Research, 8, 11–22.

Hernandes RT, Elias WP, Vieira MA and Gomes TA, 2009. An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiology Letters, 297, 137–149. https://doi.org/10.1111/j.1574-6968.2009.01664.x

Herold S, Karch H and Schmidt H, 2004. Shiga toxin-encoding bacteriophages–genomes in motion. International Journal of Medical Microbiology, 294, 115–121. https://doi.org/10.1016/j.ijmm.2004.06.023

Herold S, Paton JC and Paton AW, 2009. Sab, a novel autotransporter of locus of enterocyte effacement-negative shiga-toxigenic *Escherichia coli* O113:H21, contributes to adherence and biofilm formation. Infection and Immunity, 77, 3234–3243. https://doi.org/10.1128/iai.00031-09

Hofer E, Cernela N and Stephan R, 2012. Shiga toxin subtypes associated with Shiga toxin-producing *Escherichia coli* strains isolated from red deer, roe deer, chamois, and ibex. Foodborne Pathogenic Diseases, 9, 792–795. https://doi.org/10.1089/fpd.2012.1156

Hoffmann S, Devleesschauwer B, Aspinall W, Cooke R, Corrigan T, Havelaar A, Angulo F, Gibb H, Kirk M, Lake R, Speybroeck N, Torgerson P and Hald T, 2017. Attribution of global foodborne disease to specific foods: findings from a World Health Organization structured expert elicitation. PLoS ONE, 12, 26. https://doi.org/10.1371/journal.pone.0183641

Holmes A, Allison L, Ward M, Dallman TJ, Clark R, Fawkes A, Murphy L and Hanson M, 2015. Utility of whole-genome sequencing of *Escherichia coli* O157 for outbreak detection and epidemiological surveillance. Journal of Clinical Microbiology, 53, 3565–3573. https://doi.org/10.1128/jcm.01066-15

Horcajo P, Dominguez-Bernal G, de la Fuente R, Ruiz-Santa-Quiteria JA, Blanco JE, Blanco M, Mora A, Dahbi G, Lopez C, Puentes B, Alonso MP, Blanco J and Orden JA, 2012. Comparison of ruminant and human attaching and effacing *Escherichia coli* (AEEC) strains. Veterinary Microbiology, 155, 341–348. https://doi.org/10.1016/j.vetmic.2011.08.034

Iguchi A, Iyoda S, Kikuchi T, Ogura Y, Katsura K, Ohnishi M, Hayashi T and Thomson NR, 2015. A complete view of the genetic diversity of the *Escherichia coli* O-antigen biosynthesis gene cluster. DNA Research, 22, 101–107. https://doi.org/10.1093/dnares/dsu043

Imamovic L and Muniesa M, 2011. Quantification and evaluation of infectivity of shiga toxin-encoding bacteriophages in beef and salad. Applied and Environment Microbiology, 77, 3536–3540. https://doi.org/10.1128/aem.02703-10

Imamovic L, Tozzoli R, Michelacci V, Minelli F, Marziano ML, Caprioli A and Morabito S, 2010. OI-57, a genomic island of *Escherichia coli* O157, is present in other seropathotypes of Shiga toxin-producing *E. coli* associated with severe human disease. Infection and Immunity, 78, 4697–4704. https://doi.org/10.1128/iai.00512-10

Inward CD, Milford DV and Taylor CM, 1993. Differing outcomes of *Escherichia coli* O157 colitis in identical twins. Pediatric Nephrology, 7, 771–772.

ISO (International Organization for Standardization), 2001. EN ISO 16654:2001. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Escherichia coli* O157 (ISO 16654:2001). International Organization for Standardization.

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

ISO (International Organization for Standardization), 2017. EN ISO 16654:2001/A1:2017. Microbiology of food and animal feeding stuffs - Horizontal method for the detection of Escherichia coli O157 - Amendment 1: Annex B: Result of interlaboratory studies (ISO 16654:2001/Amd 1:2017). International Organization for Standardization.

Iyoda S, Tamura K, Itoh K, Izumiya H, Ueno N, Nagata K, Togo M, Terajima J and Watanabe H, 2000. Inducible stx2 phages are lysogenized in the enteroaggregative and other phenotypic *Escherichia coli* O86:HNM isolated from patients. FEMS Microbiology Letters, 191, 7–10. https://doi.org/10.1111/j.1574-6968.2000.tb09311.x

Jenkins C, Perry NT, Cheasty T, Shaw DJ, Frankel G, Dougan G, Gunn GJ, Smith HR, Paton AW and Paton JC, 2003. Distribution of the saa gene in strains of Shiga toxin-producing *Escherichia coli* of human and bovine origins. Journal of Clinical Microbiology, 41, 1775–1778. https://doi.org/10.1128/jcm.41.4.1775-1778.2003

Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM and Aarestrup FM, 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. Journal of Clinical Microbiology, 52, 1501–1510. https://doi.org/10.1128/jcm.03617-13

Kaper JB, Nataro JP and Mobley HL, 2004. Pathogenic *Escherichia coli*. Nature Reviews, Microbiology, 2, 123–140. https://doi.org/10.1038/nrmicro818

Karch H, Meyer T, Russmann H and Heesemann J, 1992. Frequent loss of Shiga-like toxin genes in clinical isolates of *Escherichia coli* upon subcultivation. Infection and Immunity, 60, 3464–3467.

Karch H, Bielaszewska M, Bitzan M and Schmidt H, 1999. Epidemiology and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Diagnostic Microbiology and Infectious Disease, 34, 229–243.

Karmali MA, Steele BT, Petric M and Lim C, 1983. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. Lancet, 1, 619–620.

Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, Isaac-Renton J, Clark C, Rahn K and Kaper JB, 2003. Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. Journal of Clinical Microbiology, 41, 4930–4940.

Khalil RK, Skinner C, Patfield S and He X, 2016. Phage-mediated Shiga toxin (Stx) horizontal gene transfer and expression in non-Shiga toxigenic *Enterobacter* and *Escherichia coli* strains. Pathogenic Diseases, 74, https://doi.org/10.1093/femspd/ftw037

Kim J, Nietfeldt J, Ju J, Wise J, Fegan N, Desmarchelier P and Benson AK, 2001. Ancestral divergence, genome diversification, and phylogeographic variation in subpopulations of sorbitol-negative, beta-glucuronidase-negative enterohemorrhagic *Escherichia coli* O157. Journal of Bacteriology, 183, 6885–6897. https://doi.org/10.1128/jb.183.23.6885-6897.2001

Kirchner M, Sayers E, Cawthraw S, Duggett N, Gosling R, Jenkins C, Dallman TJ, Mueller-Doblies D and Anjum MF, 2019. A sensitive method for the recovery of *Escherichia coli* serogroup O55 including Shiga toxin-producing variants for potential use in outbreaks. Journal of Applied Microbiology, 127, 889–896. https://doi.org/10.1111/jam.14345

Klapproth JM and Meyer F, 2009. Multitalented lymphostatin. Deutsche Medizinische Wochenschrift, 134, 417–420. https://doi.org/10.1055/s-0029-1208066

Klapproth JM, Scaletsky IC, McNamara BP, Lai LC, Malstrom C, James SP and Donnenberg MS, 2000. A large toxin from pathogenic *Escherichia coli* strains that inhibits lymphocyte activation. Infection and Immunity, 68, 2148–2155.

Konczy P, Ziebell K, Mascarenhas M, Choi A, Michaud C, Kropinski AM, Whittam TS, Wickham M, Finlay B and Karmali MA, 2008. Genomic O island 122, locus for enterocyte effacement, and the evolution of virulent verocytotoxin-producing *Escherichia coli*. Journal of Bacteriology, 190, 5832–5840. https://doi.org/10.1128/jb.00480-08

Kosmider RD, Nally P, Simons RR, Brouwer A, Cheung S, Snary EL and Wooldridge M, 2010. Attribution of human VTEC O157 infection from meat products: a quantitative risk assessment approach. Risk Analysis, 30, 753–765. https://doi.org/10.1111/j.1539-6924.2009.01317.x

Kumar A, Taneja N, Kumar Y and Sharma M, 2012. Detection of Shiga toxin variants among Shiga toxin-forming *Escherichia coli* isolates from animal stool, meat and human stool samples in India. Journal of Applied Microbiology, 113, 1208–1216. https://doi.org/10.1111/j.1365-2672.2012.05415.x

Lacher DW, Gangiredla J, Patel I, Elkins CA and Feng PC, 2016. Use of the *Escherichia coli* identification microarray for characterizing the health risks of shiga toxin-producing *Escherichia coli* isolated from foods. Journal of Food Protection, 79, 1656–1662. https://doi.org/10.4315/0362-028x.jfp-16-176

Lampel KA, 2012. *Shigella* species. In: Lampel KA, Al-Khaldi S and Cahill SM (eds.). Bad bug book, 2nd Edition. Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC. pp. 25–28.

Lathem WW, Grys TE, Witowski SE, Torres AG, Kaper JB, Tarr PI and Welch RA, 2002. StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. Molecular Microbiology, 45, 277–288.

Launders N, Byrne L, Jenkins C, Harker K, Charlett A and Adak GK, 2016. Disease severity of Shiga toxin-producing *E. coli* O157 and factors influencing the development of typical haemolytic uraemic syndrome: a retrospective cohort study, 2009–2012. British Medical Journal Open, 6, e009933. https://doi.org/10.1136/bmjopen-2015-009933

Le Bouguénec C and Servin AL, 2006. Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. FEMS Microbiology Letters, 256, 185–194. https://doi.org/10.1111/j.1574-6968.2006.00144.x

Lee CH, Oon JS, Lee KC and Ling MH, 2012. *Escherichia coli* ATCC 8739 adapts to the presence of sodium chloride, monosodium glutamate, and benzoic acid after extended culture. ISRN Microbiology, 2012, 965356. https://doi.org/10.5402/2012/965356

Lienemann T, Salo E, Rimhanen-Finne R, Ronnholm K, Taimisto M, Hirvonen JJ, Tarkka E, Kuusi M and Siitonen A, 2012. Shiga toxin-producing *Escherichia coli* serotype O78:H(-) in family, Finland, 2009. Emerging Infectious Diseases, 18, 577–581. https://doi.org/10.3201/eid1804.111310

Luna-Gierke RE, Wymore K, Sadlowski J, Clogher P, Gierke RW, Tobin-D'Angelo M, Palmer A, Medus C, Nicholson C, McGuire S, Martin H, Garman K, Griffin PM and Mody RK, 2014. Multiple-aetiology enteric infections involving non-O157 Shiga toxin-producing *Escherichia coli* - FoodNet, 2001–2010. Zoonoses and Public Health, 61, 492–498. https://doi.org/10.1111/zph.12098

Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, Mladonicky JM, Somsel P, Rudrik JT, Dietrich SE, Zhang W, Swaminathan B, Alland D and Whittam TS, 2008. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. Proceedings of the National Academy of Sciences of the United States of America, 105, 4868–4873. https://doi.org/10.1073/pnas.0710834105

Marejková M, Bláhová K, Janda J, Fruth A and Petráš P, 2013. Enterohemorrhagic *Escherichia coli* as causes of hemolytic uremic syndrome in the Czech Republic. PLoS ONE, 8, e73927. https://doi.org/10.1371/journal.pone.0073927

Mariani-Kurkdjian P, Lemaitre C, Bidet P, Perez D, Boggini L, Kwon T and Bonacorsi S, 2014. Haemolytic-uraemic syndrome with bacteraemia caused by a new hybrid *Escherichia coli* pathotype. New Microbes New Infectious, 2, 127–131. https://doi.org/10.1002/nmi2.49

Martin A and Beutin L, 2011. Characteristics of Shiga toxin-producing *Escherichia coli* from meat and milk products of different origins and association with food producing animals as main contamination sources. International Journal of Food Microbiology, 146, 99–104. https://doi.org/10.1016/j.ijfoodmicro.2011.01.041

Martínez-Castillo A and Muniesa M, 2014. Implications of free Shiga toxin-converting bacteriophages occurring outside bacteria for the evolution and the detection of Shiga toxin-producing Escherichia coli. Frontiers in Cellular and Infection Microbiology, 4, 46.

Martinez-Castillo A, Quiros P, Navarro F, Miro E and Muniesa M, 2013. Shiga toxin 2-encoding bacteriophages in human fecal samples from healthy individuals. Applied and Environment Microbiology, 79, 4862–4868. https://doi.org/10.1128/aem.01158-13

Martinez-Medina M and Garcia-Gil LJ, 2014. *Escherichia coli* in chronic inflammatory bowel diseases: an update on adherent invasive *Escherichia coli* pathogenicity. World Journal of Gastrointestinal Pathophysiology, 5, 213–227. https://doi.org/10.4291/wjgp.v5.i3.213

Mellmann A, Bielaszewska M, Kock R, Friedrich AW, Fruth A, Middendorf B, Harmsen D, Schmidt MA and Karch H, 2008. Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic *Escherichia coli*. Emerging Infectious Diseases, 14, 1287–1290. https://doi.org/10.3201/eid1408.071082

Mellmann A, Fruth A, Friedrich AW, Wieler LH, Harmsen D, Werber D, Middendorf B, Bielaszewska M and Karch H, 2009. Phylogeny and disease association of Shiga toxin-producing *Escherichia coli* O91. Emerging Infectious Diseases, 15, 1474–1477. https://doi.org/10.3201/eid1509.090161

Melton-Celsa AR, Darnell SC and O'Brien AD, 1996. Activation of Shiga-like toxins by mouse and human intestinal mucus correlates with virulence of enterohemorrhagic *Escherichia coli* O91:H21 isolates in orally infected, streptomycin-treated mice. Infection and Immunity, 64, 1569–1576.

Michelacci V, Maugliani A, Tozzoli R, Corteselli G, Chiani P, Minelli F, Gigliucci F, Arancia S, Conedera G, Targhetta C, Pierasco A, Collini L, Parisi A, Scavia G and Morabito S, 2018. Characterization of a novel plasmid encoding F4-like fimbriae present in a Shiga-toxin producing enterotoxigenic *Escherichia coli* isolated during the investigation on a case of hemolytic-uremic syndrome. International Journal of Medical Microbiology, 308, 947–955. https://doi.org/10.1016/j.ijmm.2018.07.002

Mobley HL, Island MD and Hausinger RP, 1995. Molecular biology of microbial ureases. Microbiological Reviews, 59, 451–480.

Monaghan A, Byrne B, Fanning S, Sweeney T, McDowell D and Bolton DJ, 2011. Serotypes and virulence profiles of non-O157 Shiga toxin-producing *Escherichia coli* isolates from bovine farms. Applied and Environment Microbiology, 77, 8662–8668. https://doi.org/10.1128/aem.06190-11

Montero DA, Velasco J, Del Canto F, Puente JL, Padola NL, Rasko DA, Farfan M, Salazar JC and Vidal R, 2017. Locus of Adhesion and Autoaggregation (LAA), a pathogenicity island present in emerging Shiga Toxin-producing *Escherichia coli* strains. Scientific Reports, 7, 7011. https://doi.org/10.1038/s41598-017-06999-y

Mora A, Lopez C, Dhabi G, Lopez-Beceiro AM, Fidalgo LE, Diaz EA, Martinez-Carrasco C, Mamani R, Herrera A, Blanco JE, Blanco M and Blanco J, 2012. Seropathotypes, Phylogroups, Stx subtypes, and intimin types of wildlife-carried, shiga toxin-producing *Escherichia coli* strains with the same characteristics as human-pathogenic isolates. Applied and Environment Microbiology, 78, 2578–2585. https://doi.org/10.1128/aem.07520-11

Morabito S, Karch H, Mariani-Kurkdjian P, Schmidt H, Minelli F, Bingen E and Caprioli A, 1998. Enteroaggregative, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. Journal of Clinical Microbiology, 36, 840–842.

Morabito S, Tozzoli R, Oswald E and Caprioli A, 2003. A mosaic pathogenicity island made up of the locus of enterocyte effacement and a pathogenicity island of *Escherichia coli* O157:H7 is frequently present in attaching and effacing *E. coli*. Infection and Immunity, 71, 3343–3348.

Mughini-Gras L, van Pelt W, van der Voort M, Heck M, Friesema I and Franz E, 2018. Attribution of human infections with Shiga toxin-producing *Escherichia coli* (STEC) to livestock sources and identification of source-specific risk factors, The Netherlands (2010–2014). Zoonoses and Public Health, 65, e8–e22. https://doi.org/10.1111/zph.12403

Muniesa M and Jofre J, 2004. Abundance in sewage of bacteriophages infecting *Escherichia coli* O157:H7. Methods in Molecular Biology, 268, 79–88. https://doi.org/10.1385/1-59259-766-1:079

Naseer U, Løbersli I, Hindrum M, Bruvik T and Brandal LT, 2017. Virulence factors of Shiga toxin-producing *Escherichia coli* and the risk of developing haemolytic uraemic syndrome in Norway, 1992–2013. European Journal of Clinical Microbiology & Infectious Diseases, 36, 1613–1620. https://doi.org/10.1007/s10096-017-2974-z

Nataro JP and Kaper JB, 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews, 11, 142–201.

Nataro JP, Mai V, Johnson J, Blackwelder WC, Heimer R, Tirrell S, Edberg SC, Braden CR, Glenn Morris Jr J and Hirshon JM, 2006. Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. Clinical Infectious Diseases, 43, 402–407. https://doi.org/10.1086/505867

Neupane M, Abu-Ali GS, Mitra A, Lacher DW, Manning SD and Riordan JT, 2011. Shiga toxin 2 overexpression in *Escherichia coli* O157:H7 strains associated with severe human disease. Microbial Pathogenesis, 51, 466–470. https://doi.org/10.1016/j.micpath.2011.07.009

Newton HJ, Sloan J, Bulach DM, Seemann T, Allison CC, Tauschek M, Robins-Browne RM, Paton JC, Whittam TS, Paton AW and Hartland EL, 2009. Shiga toxin-producing *Escherichia coli* strains negative for locus of enterocyte effacement. Emerging Infectious Diseases, 15, 372–380. https://doi.org/10.3201/eid1503.080631

Nicholls L, Grant TH and Robins-Browne RM, 2000. Identification of a novel genetic locus that is required for *in vitro* adhesion of a clinical isolate of enterohaemorrhagic *Escherichia coli* to epithelial cells. Molecular Microbiology, 35, 275–288.

Njage PMK, Leekitcharoenphon P and Hald T, 2019. Improving hazard characterization in microbial risk assessment using next generation sequencing data and machine learning: predicting clinical outcomes in shigatoxigenic Escherichia coli. International Journal of Food Microbiology, 292, 72–82. https://doi.org/10.1016/j.ijfoodmicro.2018.11.016

Nyholm O, Heinikainen S, Pelkonen S, Hallanvuo S, Haukka K and Siitonen A, 2015. Hybrids of shigatoxigenic and enterotoxigenic *Escherichia coli* (STEC/ETEC) among human and animal isolates in Finland. Zoonoses and Public Health, 62, 518–524. https://doi.org/10.1111/zph.12177

Ogura Y, Mondal SI, Islam MR, Mako T, Arisawa K, Katsura K, Ooka T, Gotoh Y, Murase K, Ohnishi M and Hayashi T, 2015. The Shiga toxin 2 production level in enterohemorrhagic *Escherichia coli* O157:H7 is correlated with the subtypes of toxin-encoding phage. Scientific Reports, 5, 16663. https://doi.org/10.1038/srep16663

Oh KH, Shin E, Jung SM, Im J, Cho SH, Hong S, Yoo CK and Chung GT, 2017. First isolation of a hybrid shigatoxigenic and enterotoxigenic *Escherichia coli* Strain Harboring the *stx2* and *elt* Genes in Korea. Japanese Journal of Infectious Diseases, 70, 347–348. https://doi.org/10.7883/yoken.JJID.2016.237

OIE terrestrial manual, 2016. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018. Chapter 3.9.10. Verocytotoxigenic *Escherichia Coli*. Available online: http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.09.10_VERO_E_COLI.pdf. Place.

Okeke IN, Scaletsky IC, Soars EH, Macfarlane LR and Torres AG, 2004. Molecular epidemiology of the iron utilization genes of enteroaggregative *Escherichia coli*. Journal of Clinical Microbiology, 42, 36–44.

Ooka T, Seto K, Kawano K, Kobayashi H, Etoh Y, Ichihara S, Kaneko A, Isobe J, Yamaguchi K, Horikawa K, Gomes TA, Linden A, Bardiau M, Mainil JG, Beutin L, Ogura Y and Hayashi T, 2012. Clinical significance of *Escherichia albertii*. Emerging Infectious Diseases, 18, 488–492. https://doi.org/10.3201/eid1803.111401

Ooka T, Tokuoka E, Furukawa M, Nagamura T, Ogura Y, Arisawa K, Harada S and Hayashi T, 2013. Human gastroenteritis outbreak associated with *Escherichia albertii*, Japan. Emerging Infectious Diseases, 19, 144–146. https://doi.org/10.3201/eid1901.120646

Parsons BD, Zelyas N, Berenger BM and Chui L, 2016. Detection, characterization, and typing of shiga toxin-producing *Escherichia coli*. Frontiers in Microbiology, 7, 478. https://doi.org/10.3389/fmicb.2016.00478

Paton AW, Ratcliff RM, Doyle RM, Seymour-Murray J, Davos D, Lanser JA and Paton JC, 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. Journal of Clinical Microbiology, 34, 1622–1627.

Paton AW, Srimanote P, Woodrow MC and Paton JC, 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic E*scherichia coli* strains that are virulent for humans. Infection and Immunity, 69, 6999–7009. https://doi.org/10.1128/iai.69.11.6999-7009.2001

Peiffer I, Guignot J, Barbat A, Carnoy C, Moseley SL, Nowicki BJ, Servin AL and Bernet-Camard MF, 2000. Structural and functional lesions in brush border of human polarized intestinal Caco-2/TC7 cells infected by members of the Afa/Dr diffusely adhering family of *Escherichia coli*. Infection and Immunity, 68, 5979–5990. https://doi.org/10.1128/iai.68.10.5979-5990.2000

Perna NT, Plunkett G III, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Posfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA and Blattner FR, 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature, 409, 529–533. https://doi.org/10.1038/35054089

Persson S, Olsen KE, Ethelberg S and Scheutz F, 2007. Subtyping method for *Escherichia coli* shiga toxin (verocytotoxin) 2 variants and correlations to clinical manifestations. Journal of Clinical Microbiology, 45, 2020–2024. https://doi.org/10.1128/jcm.02591-06

Pires SM, 2013. Assessing the applicability of currently available methods for attributing foodborne disease to sources, including food and food commodities. Foodborne Pathogenic Diseases, 10, 206–213. https://doi.org/10.1089/fpd.2012.1134

Pires SM, Evers EG, van Pelt W, Ayers T, Scallan E, Angulo FJ, Havelaar A and Hald T, 2009. Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathogenic Diseases, 6, 417–424. https://doi.org/10.1089/fpd.2008.0208

Pires SM, Vigre H, Makela P and Hald T, 2010. Using outbreak data for source attribution of human salmonellosis and campylobacteriosis in Europe. Foodborne Pathogenic Diseases, 7, 1351–1361. https://doi.org/10.1089/fpd.2010.0564

Pires SM, Vieira AR, Perez E, Lo Fo Wong D and Hald T, 2012. Attributing human foodborne illness to food sources and water in Latin America and the Caribbean using data from outbreak investigations. International Journal of Food Microbiology, 152, 129–138. https://doi.org/10.1016/j.ijfoodmicro.2011.04.018

Pires SM, Majowicz S, Gill A and Devleesschauwer B, 2019. Global and regional source attribution of Shiga toxin-producing *Escherichia coli* infections using analysis of outbreak surveillance data. Epidemiology and Infection, 147, e236. https://doi.org/10.1017/S095026881900116X

Prager R, Fruth A, Siewert U, Strutz U and Tschape H, 2009. *Escherichia coli* encoding Shiga toxin 2f as an emerging human pathogen. International Journal of Medical Microbiology, 299, 343–353. https://doi.org/10.1016/j.ijmm.2008.10.008

Prager R, Fruth A, Busch U and Tietze E, 2011. Comparative analysis of virulence genes, genetic diversity, and phylogeny of Shiga toxin 2g and heat-stable enterotoxin STIa encoding *Escherichia coli* isolates from humans, animals, and environmental sources. International Journal of Medical Microbiology, 301, 181–191. https://doi.org/10.1016/j.ijmm.2010.06.003

Probert WS, McQuaid C and Schrader K, 2014. Isolation and identification of an *Enterobacter cloacae* strain producing a novel subtype of Shiga toxin type 1. Journal of Clinical Microbiology, 52, 2346–2351. https://doi.org/10.1128/jcm.00338-14

Quirós P, Martinez-Castillo A and Muniesa M, 2015. Improving detection of Shiga toxin-producing *Escherichia coli* by molecular methods by reducing the interference of free Shiga toxin-encoding bacteriophages. Applied and Environment Microbiology, 81, 415–421. https://doi.org/10.1128/aem.02941-14

Rasko DA, Webster DR, Sahl JW, Bashir A, Boisen N, Scheutz F, Paxinos EE, Sebra R, Chin CS, Iliopoulos D, Klammer A, Peluso P, Lee L, Kislyuk AO, Bullard J, Kasarskis A, Wang S, Eid J, Rank D, Redman JC, Steyert SR, Frimodt-Moller J, Struve C, Petersen AM, Krogfelt KA, Nataro JP, Schadt EE and Waldor MK, 2011. Origins of the *E. coli* strain causing an outbreak of hemolytic-uremic syndrome in Germany. New England Journal of Medicine, 365, 709–717. https://doi.org/10.1056/NEJMoa1106920

Riordan JT, Viswanath SB, Manning SD and Whittam TS, 2008. Genetic differentiation of *Escherichia coli* O157:H7 clades associated with human disease by real-time PCR. Journal of Clinical Microbiology, 46, 2070–2073. https://doi.org/10.1128/jcm.00203-08

Russo LM, Abdeltawab NF, O'Brien AD, Kotb M and Melton-Celsa AR, 2015. Mapping of genetic loci that modulate differential colonization by *Escherichia coli* O157:H7 TUV86-2 in advanced recombinant inbred BXD mice. BMC Genomics, 16, 947. https://doi.org/10.1186/s12864-015-2127-7

Sánchez S, Llorente MT, Herrera-Leon L, Ramiro R, Nebreda S, Remacha MA and Herrera-Leon S, 2017. Mucus-activatable shiga toxin genotype stx2d in *Escherichia coli* O157:H7. Emerging Infectious Diseases, 23, 1431–1433. https://doi.org/10.3201/eid2308.170570

Scavia G, Staffolani M, Fisichella S, Striano G, Colletta S, Ferri G, Escher M, Minelli F and Caprioli A, 2008. Enteroaggregative *Escherichia coli* associated with a foodborne outbreak of gastroenteritis. Journal of Medical Microbiology, 57, 1141–1146. https://doi.org/10.1099/jmm.0.2008/001362-0

Scheutz F, Teel LD, Beutin L, Pierard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S and O'Brien AD, 2012. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. Journal of Clinical Microbiology, 50, 2951–2963. https://doi.org/10.1128/jcm.00860-12

Schmidt H, Scheef J, Huppertz HI, Frosch M and Karch H, 1999. *Escherichia coli* O157:H7 and O157:H(-) strains that do not produce Shiga toxin: phenotypic and genetic characterization of isolates associated with diarrhea and hemolytic-uremic syndrome. Journal of Clinical Microbiology, 37, 3491–3496.

Schmidt H, Scheef J, Morabito S, Caprioli A, Wieler LH and Karch H, 2000. A new Shiga toxin 2 variant (Stx2f) from *Escherichia coli* isolated from pigeons. Applied and Environment Microbiology, 66, 1205–1208.

Servin AL, 2005. Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. Clinical Microbiology Reviews, 18, 264–292. https://doi.org/10.1128/cmr.18.2.264-292.2005

Silvestro L, Caputo M, Blancato S, Decastelli L, Fioravanti A, Tozzoli R, Morabito S and Caprioli A, 2004. Asymptomatic carriage of verocytotoxin-producing *Escherichia coli* O157 in farm workers in Northern Italy. Epidemiology and Infection, 132, 915–919.

Singh N, Lapierre P, Quinlan TM, Halse TA, Wirth S, Dickinson MC, Lasek-Nesselquist E and Musser KA, 2019. Whole-genome single-nucleotide polymorphism (SNP) analysis applied directly to stool for genotyping shiga toxin-producing *Escherichia coli*: an advanced molecular detection method for foodborne disease surveillance and outbreak tracking. Journal of Clinical Microbiology, 57, https://doi.org/10.1128/jcm.00307-19

Skinner C, Patfield S, Stanker L and He X, 2013. Development of monoclonal antibodies and immunoassays for sensitive and specific detection of Shiga toxin Stx2f. PLoS ONE, 8, e76563. https://doi.org/10.1371/journal.pone.0076563

Snedeker KG, Shaw DJ, Locking ME and Prescott RJ, 2009. Primary and secondary cases in *Escherichia coli* O157 outbreaks: a statistical analysis. BMC Infectious Diseases, 9, 144. https://doi.org/10.1186/1471-2334-9-144

Sonntag A-K, Prager R, Bielaszewska M, Zhang W, Fruth A, Tschäpe H and Karch H, 2004. Phenotypic and genotypic analyses of enterohemorrhagic Escherichia coli O145 strains from patients in Germany. Journal of Clinical Microbiology, 42, 954–962. https://doi.org/10.1128/jcm.42.3.954-962.2004

Sonntag AK, Zenner E, Karch H and Bielaszewska M, 2005. Pigeons as a possible reservoir of Shiga toxin 2f-producing *Escherichia coli* pathogenic to humans. Berliner und Munchener Tierarztliche Wochenschrift, 118, 464–470.

Soysal N, Mariani-Kurkdjian P, Smail Y, Liguori S, Gouali M, Loukiadis E, Fach P, Bruyand M, Blanco J, Bidet P and Bonacorsi S, 2016. Enterohemorrhagic *Escherichia coli* hybrid pathotype O80:H2 as a new therapeutic challenge. Emerging Infectious Diseases, 22, 1604–1612. https://doi.org/10.3201/eid2209.160304

Stephan R and Untermann F, 1999. Virulence factors and phenotypical traits of verotoxin-producing *Escherichia coli* strains isolated from asymptomatic human carriers. Journal of Clinical Microbiology, 37, 1570–1572.

Stephan R, Ragettli S and Untermann F, 2000. Prevalence and characteristics of verotoxin-producing *Escherichia coli* (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. Journal of Applied Microbiology, 88, 335–341.

Stephens PJ and Joynson JA, 1998. Direct inoculation into media containing bile salts and antibiotics is unsuitable for the detection of acid/salt stressed *Escherichia coli* O157:H7. Letters in Applied Microbiology, 27, 147–151.

Stevens MP, van Diemen PM, Frankel G, Phillips AD and Wallis TS, 2002. Efa1 influences colonization of the bovine intestine by shiga toxin-producing *Escherichia coli* serotypes O5 and O111. Infection and Immunity, 70, 5158–5166.
Strachan NJ, Fenlon DR and Ogden ID, 2001. Modelling the vector pathway and infection of humans in an environmental outbreak of *Escherichia coli* O157. FEMS Microbiology Letters, 203, 69–73.
Stritt A, Tschumi S, Kottanattu L, Bucher BS, Steinmann M, von Steiger N, Stephan R, Hachler H and Simonetti GD, 2013. Neonatal hemolytic uremic syndrome after mother-to-child transmission of a low-pathogenic stx2b harboring shiga toxin-producing *Escherichia coli*. Clinical Infectious Diseases, 56, 114–116. https://doi.org/10.1093/cid/cis851
Szakál DD, Schneider G and Pál T, 2003. A colony blot immune assay to identify enteroinvasive *Escherichia coli* and *Shigella* in stool samples. Diagnostic Microbiology and Infectious Disease, 45, 165–171.
Teunis P, Takumi K and Shinagawa K, 2004. Dose response for infection by *Escherichia coli* O157:H7 from outbreak data. Risk Analysis, 24, 401–407. https://doi.org/10.1111/j.0272-4332.2004.00441.x
Thomas A, Cheasty T, Chart H and Rowe B, 1994. Isolation of Vero cytotoxin-producing *Escherichia coli* serotypes O9ab:H- and O101:H-carrying VT2 variant gene sequences from a patient with haemolytic uraemic syndrome. European Journal of Clinical Microbiology & Infectious Diseases, 13, 1074–1076.
Tilden Jr J, Young W, McNamara AM, Custer C, Boesel B, Lambert-Fair MA, Majkowski J, Vugia D, Werner SB, Hollingsworth J and Morris Jr JG, 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. American Journal of Public Health, 86, 1142–1145.
Torres AG and Payne SM, 1997. Haem iron-transport system in enterohaemorrhagic *Escherichia coli* O157:H7. Molecular Microbiology, 23, 825–833. https://doi.org/10.1046/j.1365-2958.1997.2641628.x
Torres AG, Perna NT, Burland V, Ruknudin A, Blattner FR and Kaper JB, 2002. Characterization of Cah, a calcium-binding and heat-extractable autotransporter protein of enterohaemorrhagic *Escherichia coli*. Molecular Microbiology, 45, 951–966.
Tozzoli R, Caprioli A and Morabito S, 2005. Detection of toxB, a plasmid virulence gene of *Escherichia coli* O157, in enterohemorrhagic and enteropathogenic *E. coli*. Journal of Clinical Microbiology, 43, 4052–4056. https://doi.org/10.1128/jcm.43.8.4052-4056.2005
Tozzoli R, Grande L, Michelacci V, Ranieri P, Maugliani A, Caprioli A and Morabito S, 2014. Shiga toxin-converting phages and the emergence of new pathogenic *Escherichia coli*: a world in motion. Frontiers in Cellular and Infection Microbiology, 4, 80. https://doi.org/10.3389/fcimb.2014.00080
Tuttle J, Gomez T, Doyle MP, Wells JG, Zhao T, Tauxe RV and Griffin PM, 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiology and Infection, 122, 185–192.
Tzschoppe M, Martin A and Beutin L, 2012. A rapid procedure for the detection and isolation of enterohaemorrhagic Escherichia coli (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. International Journal of Food Microbiology, 152, 19–30. https://doi.org/10.1016/j.ijfoodmicro.2011.10.009
USDA (United States Department of Agriculture), 2012. Risk profile for pathogenic non-O157 Shiga toxin-producing *Escherichia coli* (non-O157 STEC). Office of Public Health Science. Office of Policy and Program Development. Food Safety and Inspection Service.
USDA (United States Department of Agriculture), 2019. MLG 5C.00. Detection, isolation and identification of top seven shiga toxin-producing Escherichia coli (STECs) from meat products and carcass and environmental sponges. United States Department of Agriculture. Food Safety and Inspection Service, Office of Public Health Science.
Wagner PL, Acheson DW and Waldor MK, 2001. Human neutrophils and their products induce Shiga toxin production by enterohemorrhagic *Escherichia coli*. Infection and Immunity, 69, 1934–1937. https://doi.org/10.1128/iai.69.3.1934-1937.2001
Wagner PL, Livny J, Neely MN, Acheson DW, Friedman DI and Waldor MK, 2002. Bacteriophage control of Shiga toxin 1 production and release by *Escherichia coli*. Molecular Microbiology, 44, 957–970. https://doi.org/10.1046/j.1365-2958.2002.02950.x
Walley P, 1996. Inferences from multinomial data: learning about a bag of marbles. Journal of the Royal Statistical Society: Series B (Methodological), 58, 3–34. https://doi.org/10.1111/j.2517-6161.1996.tb02065.x
Wang G, Clark CG and Rodgers FG, 2002. Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. Journal of Clinical Microbiology, 40, 3613–3619. https://doi.org/10.1128/jcm.40.10.3613-3619.2002
Weeratna RD and Doyle MP, 1991. Detection and production of verotoxin 1 of *Escherichia coli* O157:H7 in food. Applied and Environment Microbiology, 57, 2951–2955.
Wennerås C and Erling V, 2004. Prevalence of enterotoxigenic *Escherichia coli*-associated diarrhoea and carrier state in the developing world. Journal of Health, Population, and Nutrition, 22, 370–382.
Xicohtencatl-Cortes J, Monteiro-Neto V, Ledesma MA, Jordan DM, Francetic O, Kaper JB, Puente JL and Giron JA, 2007. Intestinal adherence associated with type IV pili of enterohemorrhagic *Escherichia coli* O157:H7. Journal of Clinical Investigation, 117, 3519–3529. https://doi.org/10.1172/jci30727

Yang Z, Kovar J, Kim J, Nietfeldt J, Smith DR, Moxley RA, Olson ME, Fey PD and Benson AK, 2004. Identification of common subpopulations of non-sorbitol-fermenting, beta-glucuronidase-negative *Escherichia coli* O157:H7 from bovine production environments and human clinical samples. Applied and Environment Microbiology, 70, 6846–6854. https://doi.org/10.1128/aem.70.11.6846-6854.2004

Yin S, Jensen MA, Bai J, Debroy C, Barrangou R and Dudley EG, 2013. The evolutionary divergence of Shiga toxin-producing *Escherichia coli* is reflected in clustered regularly interspaced short palindromic repeat (CRISPR) spacer composition. Applied and Environment Microbiology, 79, 5710–5720. https://doi.org/10.1128/aem.00950-13

Yoon JW, Lim JY, Park YH and Hovde CJ, 2005. Involvement of the *Escherichia coli* O157:H7(pO157) ecf operon and lipid A myristoyl transferase activity in bacterial survival in the bovine gastrointestinal tract and bacterial persistence in farm water troughs. Infection and Immunity, 73, 2367–2378. https://doi.org/10.1128/iai.73.4.2367-2378.2005

Zhang W, Mellmann A, Sonntag AK, Wieler L, Bielaszewska M, Tschape H, Karch H and Friedrich AW, 2007. Structural and functional differences between disease-associated genes of enterohaemorrhagic *Escherichia coli* O111. International Journal of Medical Microbiology, 297, 17–26. https://doi.org/10.1016/j.ijmm.2006.10.004

Glossary

Bloody diarrhoea (BD)	Any report from patient or treating medical doctor or general practitioner of blood in the stool. Will cover anything from scanty blood to all blood and no stool. Therefore not clearly defined in Smany papers
Cross-pathotype strains	strain harbouring pathogenicity genes associated with more than one pathovar/pathotype, for example, STEC carrying *eae* associated with EPEC and *stx* genes
Haemolytic uremic syndrome (HUS)	In medicine, haemolytic-uremic syndrome, abbreviated (HUS) is a disease characterised by acute microangiopathic haemolytic anaemia (Hb < 10g/dl with microscopic evidence of fragmented blood cells), low platelet count (thrombocytopenia) (Platelets < 100–150,000 $\times$ 10^9/L) and acute renal failure (Oliguria or anuria with elevated serum urea and creatinine above the upper limit for age)
Haemorrhagic colitis (HC)	Most often will include abdominal cramps, copious bloody diarrhoea described as 'all blood and no stool', unaccompanied by faecal leukocytes, and variable fever
HUS-associated *E. coli* (HUSEC)	STEC isolated from HUS cases or belonging to serotypes or sequence types or virulotypes known to be associated with HUS cases
Pathogenicity	the ability of an organism to cause disease (*i.e.* harm the host) or otherwise induce pathological change in a susceptible host
Pathotype	an *E. coli* population characterised by the presence a common set of virulence factors. Specific pathotypes such as EPEC or ETEC are often linked to a common presentation of clinical symptoms such as persistent diarrhoea in children caused by EPEC or short term self-limiting travellers' diarrhoea by ETEC. This concept has been further refined for STEC to help assess the clinical and public health risks associated with different STEC strains
Seropathotype	an empirical classification scheme used to classify STEC serotypes into five groups (A through E) according to the reported association of serotypes and virulence genes assets with human intestinal disease, outbreaks, and haemorrhagic colitis and haemolytic-uremic syndrome (HUS) (Karmali et al., 2003)
Serotype	a designation of the somatic O antigen and flagellar H antigen separated with an ':' e.g. O157:H7 or O104:H4 in a given strain. The full serotype may include acidic capsular antigen K and fimbrial antigen F, which is primarily used in the serotyping of ExPEC or UPEC strains e.g. O25:K2:H2; F16 or O6:K15:H31; F536
Serogroup	often used to refer to the somatic O antigen e.g. O157 or O104. The correct designation of the O antigen should be O group as there are serological variants e.g. O28ab and O28ac. Different LPS patterns of O157 are often linked to specific H types

Species (in this case *Escherichia coli*)	a taxonomic subdivision of a genus (in this case *Escherichia*). A group of closely related and morphologically similar organisms that, actually or potentially, interbreed. The concept of a bacterial species has traditionally been based on a number of biochemical reactions. Chromosomal DNA-DNA cross-hybridisation has been used to define a species with a general cut off value of more than 70% identity. Similarity of the ribosomal 16S rRNA gene has also been used to define bacterial species. The taxonomic classification of bacteria is described in Bergey's Manual of Systematic Bacteriology, 2005
Strain	a particular variety of a bacterial species, in this case *Escherichia coli*. Usually well characterised in terms of phenotypic, biochemical and/or genetic features pertaining to a given species. An isolate is a yet uncharacterised single strain isolated from a mixed matrix such as food, faeces, urine or blood etc
Virulence	is the ability of STEC to harm the host. It may include the colonization of a specific body compartment, accomplished either by invading the tissues and multiplying in the host or by attaching and causing lesions to the mucosa or other tissues. Additionally, it may be related with the production of toxins (e.g. Shiga toxins, Subtilase, Enterohaemolysin for STEC). STEC can exhibit different degrees of virulence depending on particular characteristics of different strains, i.e. their pool of virulence genes
Virulence factor	proteins encoded by genes generally present on mobile genetic elements end specifying factors involved in the pathogenesis of the STEC-induced disease

Abbreviations

AAF	aggregative adherence fimbriae
A/E	attaching and effacing (bacteria)
aEPEC	atypical EPEC
Afa	afimbrial adhesins
AIDA-I	adhesin involved in diffuse adherence
AIEC	adherent invasive *E. coli*
AMR	antimicrobial resistance
BD	bloody diarrhoea
Bfp	bundle forming pili
CA	competent authority
CFA	colonisation factor antigens
CFU	colony forming units
CI	confidence interval
cgMLST	core genome MLST
D	diarrhoea
DAEC	dffusely adherent *E. coli*
DAF	decay-accelerating factor
DEC	diarrhoeagenic *E. coli*
Dr	Drori antigen
EAEC	enteroaggregative *E. coli*
EAF	EPEC adherence factor
EAST1	enteroaggregative heat stable toxin
EHEC	enterohaemorrhagic *E. coli*
EIEC	enteroinvasive *E. coli*
EPEC	enteropathogenic *E. coli*
ETEC	enterotoxigenic *E. coli*
ExPEC	extra-intestinal pathogenic *E. coli*
EU-FORS	European Union Food-borne Reporting System
EUR	European region
FBO	food-borne outbreak(s)

FWD	food- and waterborne disease
GI	genomic island
HC	haemorhagic colitis
Hosp	hospitalised
HUS	haemolytic uremic syndrome
HUSEC	HUS-associated *E.coli*
Ipa	invasion plasmid antigen
IS	insertion sequence
ivi	in vivo-induced
KatP	catalase peroxidase
LAA	locus of adhesion and autoaggregation
LAMP	loop mediated isothermal amplification
LEE	locus of enterocyte effacement
LT	heat labile
MGE	mobile genetic element
MLST	multilocus sequence typing
MLVA	multilocus variable number tandem repeat analysis
MPN	most probable number
MS	Member State
PAI	pathogenicity island
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
SLT	Shiga like toxin
SNP	single nucleotide polymorphism
ST	heat stable
STEC	Shiga toxin-producing *E. coli*
Stx	Shiga toxin
tEPEC	typical EPEC
TESSy	The European Surveillance System
TTSS	type III secretion system
UI	uncertainty interval
UPEC	uropathogenic *Escherichia coli*
UTI	urinary tract infection
VNTR	variable number tandem repeat
VCA	Vero cell assay
VT	Verocytoxins
VTEC	verotoxin-producing *E. coli*
UI	uncertainty interval
UTI	urinary tract infection
VNTR	variable number tandem repeat
wgMLST	whole genome MLST
WGS	whole genome sequencing
WHO	World Health Organization
WHO-FERG	World Health Organization's foodborne disease burden epidemiology reference group
WPR	Western Pacific Region

Appendix A – *E. coli* pathotypes

Enteropathogenic *E. coli* (EPEC)

Enteropathogenic *E. coli* (EPEC) carry *eae*, but are *stx* negative, and thus belong to the group of bacteria known as attaching and effacing (A/E) pathogens, forming A/E lesions in the small intestine. EPEC are subdivided into typical (tEPEC) and atypical (aEPEC) strains depending on the presence (or absence) of the EPEC Adherence Factor (EAF) plasmid which includes the bundle forming pili (Bfp) operon encoding the pili required for localised adherence on epithelial cells. In general, EPEC are non-invasive and do not produce heat-labile (LT) or heat-stable (ST) enterotoxins. EPEC infection is characterised by watery or bloody diarrhoea with the occurrence caused by tEPEC decreasing with age due to the loss of specific EPEC receptors and/or the development of immunity (Nataro and Kaper, 1998). aEPEC infections, once considered to predominate in developed countries, are now known to exceed those caused by tEPEC throughout the world (Hernandes et al., 2009).

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* (ETEC) are a major cause of traveller's diarrhoea and are endemic in most developing countries with significant mortality rates in children (Snedeker et al., 2009). They are a diverse group of many different serotypes. ETEC cells adhere to the epithelium of the small intestine via one or more colonisation factor antigens (CFA) followed by the expression of heat labile (LT) or heat stable (ST) enterotoxins. The diarrhoea may be accompanied by cramps, nausea and headaches but fever is usually absent. In a study published in 2004, Wennerås and colleagues (Wennerås and Erling, 2004) estimated that there were approximately 840 million cases of ETEC annually in developing countries with 280 million of these being in children less than 4 years of age. ETEC are usually transmitted via contaminated water and food.

Enteroinvasive *E. coli* (EIEC) and Shigella spp.

Enteroinvasive *E. coli* (EIEC) and *Shigella* spp. are facultative intracellular pathogens that cause a mild form of dysentery, characterised by the appearance of blood and mucus in the faeces. The early stage of this infection is usually characterised by mild watery diarrhoea, fatigue, malaise, fever and anorexia but as the infection develops the patient may also suffer abdominal cramps, tenesmus and scanty stools often accompanied by blood and mucus. In the absence of medical attention, the patient may also show signs of dehydration. Most cases are self-limiting although severe life-threatening complications may occur, especially in developing countries where the host may be malnourished, immune-compromised and without access to adequate treatment. There are 21 major serotypes of EIEC, the majority of which are non-motile and lacking the H antigen. *Shigella* includes 49 sero- and subserotypes clustered into 4 species including *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei.* EIEC and *Shigella* spp. carry a 220 kb virulence associated invasion plasmid including the invasion plasmid antigen (Ipa) proteins encoded on the *ipa* operon, which confers an ability to enter and disseminate between intestinal epithelial cells. Thus, these bacteria are highly invasive. Transmission is usually mediated by contaminated food and/or water via the faecal-oral route, but direct person-to-person transmission has also been reported (Lampel, 2012).

Enteroaggregative *E. coli* (EAEC)

Enteroaggregative *E. coli* (EAEC) are characterised by their ability to aggregatively adhere to tissue culture cells in a distinct 'stacked brick-like' manner which is usually mediated by aggregative adherence fimbriae (AAF) encoded by the *aggR* genes. However, not all EAEC strains are *aggR* positive which has resulted in a general classification of typical (*aggR* positive) and atypical (*aggR* negative) groups (Harrington et al., 2006). They also produce an enteroaggregative heat stable toxin (EAST1) encoded by the *astA* genes. Symptoms include acute or persistent diarrhoea, often with mucus and the patient may also have nausea, vomiting, a low grade fever and occasionally bloody stools (Croxen et al., 2013). EAEC infect both children in adults and are responsible for worldwide endemic and epidemic diarrhoeal diseases (Nataro et al., 2006).

Diffusely Adherent *E. coli* (DAEC)

The diffusely adherent *E. coli* (DAEC) are comprised of a heterogenous group of *E. coli* strains with variable virulence and that do not display the patterns of adherence observed with other *E. coli* pathotypes. They are identified by their adherence to HEp-2 as well as HeLa cells in a diffuse pattern

and are divided into two classes. The first class carry afimbrial adhesins (Afa) or Drori antigen (Dr) adhesins and have been found to be associated with urinary tract infections (UTIs) (pyelonephritis, cystitis and asymptomatic bacteriuria) and with various enteric infections (Servin, 2005). In Afa/Dr DAEC, the F1845 and DR adhesins bind to the brush border-associated decay-accelerating factor (DAF) molecule, common on the surface of polarised epithelial cells, destroying or rearranging the microvilli and forming brush border lesions (Peiffer et al., 2000). This manifests as watery diarrhoea that may be persistent and severe in young children (Kaper et al., 2004). Adults may be asymptomatic, but carriage may lead to chronic inflammatory intestinal diseases such as Crohn's disease (Le Bouguénec and Servin, 2006). The second class of DAEC strains includes *E. coli* strains that express an adhesin involved in diffuse adherence (AIDA-I) (Benz and Schmidt, 1989, 1993), which is a potential cause of infantile diarrhoea.

Adherent invasive *E. coli* (AIEC)

This pathotype is defined by the ability to: 1) adhere to differentiated Caco-2 and/or undifferentiated I-407 intestinal epithelial cells; 2) invade I-407 cells; 3) effect host cell action polymerisation and microtubule recruitment in bacterial uptake and 4) survive and replicate within J774-A1 macrophages. Invasive determinants have yet to be detected consistently in all AIEC (Martinez-Medina and Garcia-Gil, 2014). To date, they are the most likely candidate associated with the development of Crohn's disease in genetically susceptible patients (Martinez-Medina and Garcia-Gil, 2014).

The main virulence factors and mechanisms of pathogenicity for each of the seven *E. coli* pathotypes are summarised in Table A.1. Any of the pathotypes mentioned above may be able to acquire the *stx* genes. For example, EPEC, EAEC and *Shigella* spp. have been shown to acquire *stx* genes and cause disease similar to that of STEC.

Table A.1: Intestinal pathogenic *E. coli* pathotypes defined on the basis of the presence of specific virulence genes (genetic identifiers), virulence characteristics and symptoms/illness/disease

Pathotype	Adhesion	Defining virulence genes/factors	Colonisation site	Virulence characteristics	Symptoms/illness/disease[(a)]
Shiga toxin-producing *E. coli* (STEC)	Attaching and effacing (only for LEE$^+$ STEC), AAFI-V fimbriae, LAA	*stx*$^+$	Distal, ileum, colon	Phage encoded Shiga-toxins. A range of other virulence factors may also be present	Mild to severe bloody diarrhoea through to HC, HUS and thrombocytopaenia
Enteropathogenic *E. coli* (EPEC)Typical (tEPEC)	Attaching and effacing	*eae*$^+$, *bfpA*$^+$	Small intestine	Carry both LEE encoded intimin (*eae*) and the bundle forming pili in the EPEC adherence factor (EAF) plasmid	Profuse watery diarrhoea especially in children < 5 years old
Atypical (aEPEC)	Attaching and effacing	*eae*$^+$	Small intestine	Presence of intimin	Profuse watery diarrhoea especially in children < 5 years old
Enterotoxigenic *E. coli* (ETEC)	CF mediated	CFAs, LT, ST	Small intestine	Carry genes encoding thermo labile and/or thermostable toxins and Cytolysin A	Acute watery diarrhoea (< 5 years old)Traveller's diarrhoea
Enteroinvasive *E. coli* (EIEC)(*Shigella*)	NA (invasive)	*Ial*$^+$, *IpaH*$^+$	Colon	Presence of the invasion-associated locus (IAL) of the invasion plasmid antigens (ipa)	Shigellosis/bacillary dysentery
Enteroaggregative *E. coli* (EAEC)	Stacked brick and/or invasive	*aatA*$^+$, *aggR*$^{+,}$	Small intestine and/or colon	Carries the plasmid-encoded AggR master regulon that controls the genes associated with aggregative adherence. Also carries EAEC heat stable enterotoxin 1(EAST1), *Shigella* enterotoxin 1(ShET) and haemolysin (HlyE)	Persistent diarrhoeaTraveller's diarrhoea
Diffusely adherent *E. coli* (DAEC)	Diffusely adherent and/or invasive	*afaC*$^+$	Intestine	Presence of surface afimbrial adhesins including AfaE-1 and AfaE-III and/or fimbrial (Dr) adhesins encoded on the Afa/dr/daa operon	Acute watery diarrhoea in children. Speculated to contribute to Crohn's disease in adults
Adherent invasive *E. coli* (AIEC)	NA (invasive)	Uncharacterised	Small intestine	Uncharacterised	Speculated to contribute to Crohn's disease in adults

(a): Adapted from Croxen et al. (2013); EFSA BIOHAZ Panel (2013b, 2015). *E. coli* strains possessing virulence genes typical of more than one pathotype are increasingly common making it difficult to determine the exact pathotype.

Appendix B – Questionnaire on monitoring for STEC in humans

Questionnaire on diagnostics and characterisation of STEC in humans

Fields marked with * are mandatory.

Questionnaire on diagnostics and characterisation of STEC in humans

Many thanks for collaborating with the BIOHAZ Working Group on the pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC (EFSA-Q-2018-00293) by providing answers to the questions indicated below.

Please check the relevant multiple choice options and fill in this questionnaire according to the available information in your country.

This questionnaire on human samples is divided into two parts: **PART A** and **PART B.**

PART A concerns the **PRIMARY STEC diagnostics in humans** *i.e.* the laboratory performing the initial screening and diagnosis of STEC. In some Countries this may be performed in a variety of different laboratories using different methods. We ask you to answer in general terms so that an average level of performance at the primary diagnostic laboratories is reflected in your choices. **PART B** concerns the **STEC diagnostics in humans at National Reference Laboratory (NRL) level**.

* Is all STEC diagnostics in humans in your country performed at National Reference Laboratory (NRL) level?

- ○ Yes
- ○ No

PART A. Primary STEC diagnostics in humans

PART A. PRIMARY STEC diagnostics in humans

1. Please specify your contact point details

	Affiliation (Competent Authorities, Official Laboratories, etc.)	Address	Reporting Country	Name of the contact person	Email
*1					

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* 2. Before checking the multiple choice options, please give a short description of how primary STEC diagnostics in humans is undertaken in your country. The description can include the number of primary diagnostic laboratories, an estimate of how many are regional/federal, private/public and differences in the clinical criteria for the diagnostics of STEC etc.

2000 character(s) maximum

* 3. Are there National guidelines/guidance for the detection of STEC in humans in your Country?

- Yes
- No
- Don't know

* If yes, please specify:

1000 character(s) maximum

* 4. Is notification of STEC in humans mandatory in your Country?

- Yes
- No
- Don't know

* 5. Is notification of Hemolytic-Uremic Syndrome (HUS) associated with STEC infection mandatory in your country?

- Yes
- No
- Don't know

* 6. What methods are used to detect STEC in human faecal samples at the primary diagnostic level (local /regional hospital level)? Please, tick all that apply.

- Culture-based method with selective and/or differential media
- PCR
- Other methods (e.g. ELISA, toxin detection)
- Don't know

* If PCR, please specify whether:

- DNA is extracted from faeces
- DNA is extracted from bacteria culture

* If PCR, please specify the amplified genes

150 character(s) maximum

* If other methods, please specify which

1000 character(s) maximum

Please, provide details in case different primary diagnostic laboratories at regional level in your country use different approaches regarding the methods used to detect STEC in human fecal specimens.

2000 character(s) maximum

*7. Are you only looking for specific O groups (e.g. O157)?

- Yes
- No
- Don't know

*If Yes, please specify which O group(s)

1000 character(s) maximum

Please, provide details in case different primary diagnostic laboratories at regional level in your country follow different strategies when looking for O groups.

2000 character(s) maximum

*8. Are (some) human fecal samples or human isolates sent to the National Reference Laboratory (NRL)?

- Yes
- No
- Don't know

*If only a sub-set of fecal samples or human isolates is sent to the NRL, what are the criteria to do so?

1000 character(s) maximum

Please provide the estimates of the percentages (%) of faecal samples and/or isolates sent to the NRL. If these aren't available please indicate NA (not available) below.

	Faecal samples	STEC isolates
*%		

*9. Do you have a national collection for the isolated STEC strains?

- Yes
- No
- Don't know

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10. Do you look for STEC in faecal specimens from:

	Yes	No	Don't know
* All cases reporting symptoms of diarrhoea	○	○	○
* Only cases reporting bloody diarrhoea	○	○	○
* Only cases reporting HUS	○	○	○
* Specific age groups	○	○	○
* Cases reporting symptoms of persistent diarrhoea	○	○	○
* Travel-associated cases	○	○	○
* Immunocompromised	○	○	○
* Suspicion of outbreak	○	○	○
* Contacts to patients with symptoms	○	○	○
* Other cases	○	○	○

* If you look for STEC in fecal specimens from other cases, please specify in which cases:

1000 character(s) maximum

Please, provide details in case different primary diagnostic laboratories at regional level in your country follow different strategies in the selection of cases when looking for STEC in fecal specimens.

2000 character(s) maximum

* 11. Do you characterize the virulence gene set (e.g. *eae, aggR, stx ...)* and *stx* subtyping of STEC isolates?

- ○ Yes
- ○ No
- ○ Don't know

* If Yes, please specify which ones

- ☐ *eae*
- ☐ *stx* 1
- ☐ *stx* 1 subtypes (please specify)
- ☐ *stx* 2
- ☐ *stx* 2 subtypes (please specify)
- ☐ *aggR*
- ☐ Additional genes

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* If *stx* 1 subtypes, please specify which ones

1000 character(s) maximum

* If *stx* 2 subtypes, please specify which ones

1000 character(s) maximum

* If additional genes, please specify which ones

1000 character(s) maximum

Please, provide details in case different primary diagnostic laboratories at regional level in your country follow different strategies in the characterisation of the virulence gene set and *stx* subtyping of STEC isolates.

2000 character(s) maximum

Part B. STEC diagnostics at NRL level in humans

PART B. STEC diagnostics at NRL level in humans.

6

12. Please specify your contact point details

	Affiliation (Competent Authorities, Official Laboratories, etc.)	Address	Reporting Country	Name of the contact person	Email
*1					

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* 13. What methods are used to detect STEC in human faecal samples at the National Reference Laboratory (NRL) level?

- ☐ Culture-based method with selective and/or differential media
- ☐ PCR
- ☐ Other methods
- ☐ Don't know

* If PCR, please specify whether:

- ☐ DNA is extracted from faeces
- ☐ DNA is extracted from bacterial culture

* If PCR, please specify the amplified genes

1000 character(s) maximum

* If other methods, please specify which:

1000 character(s) maximum

* 14. Are you only looking for specific O groups (e.g. O157)?

- ○ Yes
- ○ No
- ○ Don't know

* If yes, please specify which O group(s)

1000 character(s) maximum

15. Do you look for STEC in faecal specimens from:

	Yes	No	Don't know
* All cases reporting symptoms of diarrhoea	○	○	○
* Only cases reporting bloody diarrhoea	○	○	○
* Only cases reporting HUS	○	○	○
* Specific age groups	○	○	○
* Cases reporting symptoms of persistent diarrhoea	○	○	○
* Travel-associated cases	○	○	○
* Immunocompromised	○	○	○

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* Suspicion of outbreak	○	○	○
* Contacts to patients with symptoms	○	○	○
* Other cases	○	○	○

* If you look for STEC in fecal specimens from other cases, please specify in which cases:

1000 character(s) maximum

* 16. Do you characterize the virulence gene set (e.g. eae, aggR, stx ...) and stx subtyping of STEC isolates?

- ○ Yes
- ○ No
- ○ Don't know

* If Yes, please specify which ones:

- ☐ *eae*
- ☐ *stx* 1
- ☐ *stx* 1 subtypes (please specify)
- ☐ *stx* 2
- ☐ *stx* 2 subtypes (please specify)
- ☐ *aggR*
- ☐ Additional genes

* If *stx* 1 subtypes, please specify which ones

1000 character(s) maximum

* If stx 2 subtypes, please specify which ones

1000 character(s) maximum

* If additional genes, please specify which ones:

1000 character(s) maximum

* 17. Do you do this characterisation only for a subgroup(s) of the STEC isolates?

- ○ Yes (only for a subgroup(s))
- ○ No (all STEC isolates are characterised)
- ○ Don't know

* If only a subgroup(s) of STEC isolates is characterized, please specify which subgroup(s) (e.g. HUS cases, etc.)

1000 character(s) maximum

* 18. What methods do you use routinely for typing of human STEC isolates?

- ☐ Phenotypic Serotyping
- ☐ PCR Serotyping
- ☐ *stx* subtyping PCR
- ☐ PFGE
- ☐ MLVA
- ☐ WGS
- ☐ Other methods
- ☐ Don't know

* If other methods, please specify which ones:

1000 character(s) maximum

* 19. Does your country submit the complete set of national STEC typing data that is obtained in the entire country to TESSy?

- ○ Yes
- ○ No
- ○ Don't know

* If No, please give an estimate of the percentage of national STEC typing data that is reported out of the total:

1000 character(s) maximum

* If No, what reasons prevent you from reporting the complete national information?

- ☐ Routine typing data for STEC is not submitted to the national reference laboratory
- ☐ Data is available but is not easily accessible
- ☐ Notification of STEC to the NRL is not mandatory
- ☐ Other reasons

* If other reasons, please specify which ones:

1000 character(s) maximum

* 20. Do you report the virulence gene set and *stx* subtyping of STEC isolates to TESSY?

- ○ Yes
- ○

10

No

Don't know

* If no, what reasons prevent you from reporting the information requested?

- [ ] Routine typing for STEC is not available at my national reference laboratory
- [ ] Data is available but is not easily accessible
- [ ] The request form takes too long to complete
- [ ] Other reasons

* If Other reasons, please specify which ones:

1000 character(s) maximum

Appendix C – Questionnaire on monitoring for STEC in food, feed and animals

Questionnaire on monitoring for STEC in food, feed and animals

Fields marked with * are mandatory.

Questionnaire on monitoring for STEC in food, feed and animals

Many thanks for collaborating with the BIOHAZ Working Group on ***the pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC*** *(EFSA-Q-2018-00293) by providing answers to the questions indicated below.*

Please note that this questionnaire has already been distributed to the VTEC/STEC National Reference Laboratory contact points which were provided by the VTEC EURL. Therefore we would kindly ask you to please forward this questionnaire only to other official laboratories and/or competent authorities which have not been identified as VTEC/STEC National Reference Laboratory contact point(s) in your country.

Please check the relevant multiple choice options and fill in this questionnaire according to the available information in your country.

1

1. Please specify your contact point details

	Affiliation (Competent Authorities, Official Laboratories, etc.)	Address	Reporting Country	Email
*1				

2

* 2. Which method have you implemented for the detection of STEC in food/feed/animal samples? Please, tick all that apply.

- ☐ Culture-based method with selective and differential media
- ☐ ISO/TS 13136:2012
- ☐ Other PCR-based methods
- ☐ Cell cultures
- ☐ Immunological-based methods
- ☐ Other methods

* If ISO/TS 13136:2012, please specify amplified genes

1000 character(s) maximum

* If other methods, please specify which:

1000 character(s) maximum

* 3. Do you have accredited a method for the detection of STEC in food /feed /animal samples?

- ☐ European Reference Laboratory (EURL) method (available here)
- ☐ ISO/TS 13136:2012
- ☐ Other methods
- ☐ Don't know

* If other methods, please specify which:

1000 character(s) maximum

* 4. Do you carry out virulence gene profiling e.g. *stx* subtypes, other virulence genes markers (*eae*, *aggR*, *aaiC*, etc.)

- ○ Yes
- ○ No
- ○ Don't know

* If yes, please specify the method:

- ☐ EURL method (available here)
- ☐ Other methods

* If other methods, please specify which:

1500 character(s) maximum

3

* 5. If appropriate, do you perform any additional characterisation of STEC isolates in food/feed/animals (e. g. serotype, antimicrobial resistance (AMR) profile, whole genome sequencing (WGS), etc.)?

- Yes
- No

* If Yes, please specify which type of characterisation:

2000 character(s) maximum

* 6. Does your country have a sampling strategy for routine testing of STEC in food/feed and animals?

- Yes
- No
- Don't know

* If yes, please describe it:

2000 character(s) maximum

* 7. Does your country submit the complete set of STEC typing data that is obtained at NRL level or official laboratory level to the EFSA zoonoses database?

- Yes
- No
- Don't know

* If no, what reasons prevent you from completing the submission of the information requested?

- Typing data for STEC are not available at the data provider level
- Data is available but is not easily accessible
- The request form takes too long to complete
- Other reasons

* If other reasons, please specify which:

Appendix D – Implicated vehicle categories of the food- and waterborne outbreaks data model from the EFSA's zoonoses database

Table D.1: Categories described for the implicated vehicle in the food- and waterborne outbreaks data model of EFSA's zoonoses database

Food category	Description
Bakery products	Bakery products include bread and ordinary bakery wares (all types of non-sweet bakery products and bread-derived products) and sweet, salty and savoury fine bakery wares (ready-to-eat products as well as mixes for preparing fine baked goods) Bread and ordinary bakery wares: The category bread contains the main subcategories white bread, brown bread and wholemeal bread Crackers, excluding sweet crackers: the term 'cracker' refers to a thin, crisp wafer, usually of unsweetened dough. Flavoured crackers (e.g. cheese flavoured). Examples include: soda crackers, rye crisps and matzahs Other ordinary bakery products: includes all other ordinary bakery wares, such as bagels, pita, English muffins, cornbread and biscuits. The term 'biscuit' in this category refers to a small cake of shortened bread, leavened with baking powder or baking soda. It does not refer to the British 'biscuit,' which is a 'cookie' or 'sweet cracker' included in the category Cakes, cookies and pies Bread-type products, including bread stuffing and bread crumbs: includes bread-based products, such as croutons, bread stuffing and stuffing mixes and prepared doughs (e.g. for biscuits) Fine bakery wares (sweet, salty and savoury) and mixes: Cakes, cookies and pies (e.g. fruit-filled or custard types): the term 'sweet cracker' or 'sweet biscuit' used in this category refers to a cookie-like product that may be eaten as a dessert. Examples include: butter cake, cheesecake, fruit-filled cereal bars, pound cake, moist cake, western cakes, moon cakes, sponge cake, fruit-filled pies (e.g. apple pie), oatmeal cookies, sugar cookies and British 'biscuits' (cookies or sweet crackers) Other fine bakery products: includes products that may be eaten as a dessert or as breakfast. Examples include: doughnuts, sweet rolls, scones, muffins, pancakes, waffles, filled sweet buns, Danish pastries, wafers or cones for ice cream, flour confectionery and trifles. This category also includes tiramisu Please specify the subcategory (e.g. fine bakery wares, pies) and, if available, the filling (e.g. fruit, custard, raw eggs) in the free text data element (e.g. 'fine bakery product containing pasteurised dairy products and raw eggs, tiramisu')
Bovine meat and products thereof	Bovine meat is defined as edible parts of domestic bovine animals (including *Bubalus* and *Bison* species), including blood (Regulation (EC) No 853/2004[(a)]). Please also note the following related definitions in the EU legislation, which can help to specify the foodstuff implicated in the free text data element Fresh meat is meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere (Regulation (EC) No 853/2004[(a)]) Meat preparations are defined as fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat (Regulation (EC) No 853/2004[(a)]) Minced meat is boned meat that has been minced into fragments and contains less than 1% salt (Regulation (EC) No 853/2004[(a)]), e.g. steak tartare Meat products are defined as processed products resulting from the processing of meat or from further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (Regulation (EC) No 853/2004[(a)]) Examples of bovine meat and products thereof include: beef steak, stewing steak, grilled liver, roast beef, sausages and steak tartare

Food category	Description
Broiler meat (*Gallus gallus*) and products thereof	Broiler meat is defined as edible parts of domestic chicken (*Gallus gallus*), including blood. For the definition of meat products, please see 'Bovine meat and products thereof'
Buffet meals	A buffet meal is a meal at which guests serve themselves from various dishes displayed on a large table
Canned food products	Food preserved by canning; the process of preserving food by sterilisation and cooking in a sealed metal can, which destroys bacteria and protects against recontamination Please specify the canned food product (e.g. meat, fish, vegetable) in the free text data element (e.g. 'baked beans, canned')
Cereal products including rice and seeds/ pulses (nuts, almonds)	Cereal is grass whose starchy grains are used as food, e.g. wheat, rice, rye, oats, maize, buckwheat, millet and grain Foodstuff prepared from the starchy grains of cereal grasses is also referred to as cereal Please specify the cereal products (e.g. plant species) and treatment (cut, pre-cut, cooked) in the free text data element
Cheese	Cheese is the ripened or unripened soft, semi-soft, hard or extra-hard product of milk, which may be coated, and in which the whey protein/casein ratio does not exceed that of milk, obtained by coagulating of milk or protein of milk and/or products obtained from milk which give an end product with similar characteristics Please specify the species of origin of the milk (such as cow, goat, sheep) as well as whether the cheese was made from raw, low-heat-treated milk or pasteurised milk In addition, the main type of cheese (hard, semi-soft or soft) and, if possible, also a detailed type (e.g. Camembert), if possible, in the free text data element. For example, 'soft cheese made from raw goats' milk'
Crustaceans, shellfish, molluscs and products thereof	Shellfish is a broad term for all aquatic animals that have a shell of some kind. Shellfish are separated into two basic categories: crustaceans and molluscs. However, the EU definition of shellfish includes only bivalve and gastropod molluscs (Council Directive 79/923/EEC[(b)]). Examples of edible shellfish are sea cucumber and sea urchin Crustaceans are one of two main classifications of shellfish (the other being mollusc). Crustaceans have elongated bodies and jointed, soft (crust-like) shells. Examples of edible crustaceans include shrimp (e.g. Atlantic white shrimp), prawn (e.g. giant river prawn), lobster (e.g. European lobster), crayfish (e.g. European crayfish) and crab (e.g. edible crab) Molluscs are animals with a soft body, internal or external shell, muscular foot and/or tentacles. Molluscs are divided into three groups: gastropods (also called univalves), bivalves (including live bivalve molluscs) and cephalopods. Examples of edible molluscs include abalone (sea ear), snail (e.g. vineyard snail) and clam. Examples of edible bivalve molluscs include mussels and oysters. Please specify the species as well as the treatment (e.g. live, cooked) in the free text data element (e.g. 'deep-fried shrimps')
Dairy products (other than cheeses)	Dairy products are defined as processed products resulting from the processing of raw milk or from the further processing of such processed products (Regulation (EC) No 853/2004[(a)]). Examples for dairy products are cream, buttermilk, milk powder, butter, yoghurt, ice cream and puddings made from milk

Food category	Description
Drinks, including bottled water	Drinks are any liquids suitable for drinking, also called beverages. Juice drinks are drinks made from fruit juice plus other ingredients, such as water, flavourings, artificial sweeteners, colourings and preservatives. Fruit juice drink can contain as little as 5% juice Soft drinks are non-alcoholic, flavoured, carbonated beverages, usually commercially prepared and sold in bottles or cans Alcoholic drinks are made by fermenting fruit juices, sugars and fermentable carbohydrates with yeast to form alcohol. These include beer, cider and perry, 4–6% alcohol by volume; wines, 9–13% alcohol; spirits (e.g. brandy, gin, rum, vodka, whisky) made by distilling fermented liquor, 38–45% alcohol; liqueurs made from distilled spirits, sweetened and flavoured, 20–40% alcohol; and fortified wines (aperitif wines, Madeira, port, sherry) made by adding spirit to wine, 18–25% alcohol In the context of the food-borne outbreak system, the category 'Drinks, including bottled water' does not include milk, fruit juice, fruit nectar, vegetable juice and tap water, but it includes fruit-flavoured drinks and juice drinks The definition of drinks also includes hot drinks such as coffee and tea Bottled water is sold for human consumption. It is sealed in a sanitary container and must meet all regulations for drinking water. Bottled water contains no sweeteners or chemical additives and must be calorie and sugar-free 'Natural mineral water' means microbiologically wholesome water originating in an underground water table or deposit and emerging from a spring tapped at one or more natural or bore exits. Before water is recognised as a natural mineral water, it has to be demonstrated that it: is obtained from an underground source, has a stable composition, is protected from all sources of pollution, meets chemical and microbiological safety standards, is not subject to treatment which affects its characteristic properties. Natural mineral water is bottled at source and is sold under one trade description. The name of the source and its place of exploitation are stated on the label together with a statement of the analytical composition (EC Directives 80/777[(c)], 96/70[(d)] and 80/778[(e)]). In contrast, recognition of a spring water underground source is not required Spring water meets the same chemical and microbiological standards as tap water and, currently, can be subject to treatment. However, like natural mineral water, spring water is bottled at source, sold under one trade description and the name of the source and its place of exploitation are included in labelling (EC Directives 80/777[(c)], 96/70[(d)] and 80/778[(e)]) Bottled drinking water, which is not restricted to a particular type of source, comprises bottled water, other than natural mineral water and spring water, and includes water referred to as 'table water'. Bottled drinking water is required to comply with the same compositional and microbiological standards as tap water (EC Directives 80/777[(c)], 96/70[(d)] and 80/778[(e)])
Eggs and egg products	Eggs are defined as eggs in shell that are produced by farmed birds and are fit for direct human consumption or for the preparation of egg products Egg products are processed products resulting from the processing of eggs or of various components or mixtures of eggs or from the further processing of such processed products (Regulation (EC) No 853/2004[(a)]). Examples of egg products include: Liquid egg products: the purified whole egg, egg yolk or egg white is pasteurised and chemically preserved (e.g. by addition of salt) Frozen egg products: the purified whole egg, egg yolk or egg white is pasteurised and frozen Dried and/or heat-coagulated egg products: sugars are removed from the purified whole egg, egg yolk or egg white, which is then pasteurised and dried Preserved eggs, including alkaline, salted and canned eggs: includes traditional Oriental preserved products, such as salt-cured duck eggs and alkaline treated 'thousand-year-old-eggs' Egg-based desserts: includes ready-to-eat products and products to be prepared from a dry mix. Examples include: flan and egg custard. This also includes custard fillings for fine bakery wares (e.g. pies)

Food category	Description
Fish and fish products	Fish, as a food, describes the edible parts of water-dwelling, cold-blooded vertebrates with gills. Examples of edible fish include salmon, trout, tuna, eel, silver carp and anchovy Examples of fish products include frozen fish fingers, roe and smoked salmon Please specify the fish species (e.g. salmon, herring) as well as the treatment (e.g. smoked, raw, cooked, etc.) in the free text data element (e.g. 'cold-smoked salmon')
Fruit, berries and juices and other products thereof	Fruit is defined as all fruit. Tomatoes are not regarded as fruit (Council Directive 2001/112/EC[(f)]) Fruit purée is defined as the fermentable but unfermented product obtained by sieving the edible part of whole or peeled fruit without removing the juice (Council Directive 2001/112/EC[(f)]) Fruit juice is defined as: The fermentable but unfermented product obtained from fruit which is sound and ripe, fresh or preserved by chilling, of one or more kinds mixed together, having the characteristic colour, flavour and taste typical of the juice of the fruit from which it comes. Flavour, pulp and cells from the juice which are separated during processing may be restored to the same juice. In the case of citrus fruits, the fruit juice must come from the endocarp. Lime juice, however, may be obtained from the whole fruit, by suitable production processes whereby the proportion of constituents of the outer part of the fruit is reduced to a minimum. The product obtained from concentrated fruit juice by – replacing, in the concentrated fruit juice, water extracted from that juice during concentration, restoring the flavours and, if appropriate, pulp and cells lost from the juice but recovered during the process of producing the fruit juice in question or of fruit juice of the same kind. The water added must display appropriate characteristics, particularly from the chemical, microbiological and organoleptic viewpoints, in such a way as to guarantee the essential qualities of the juice. The product thus obtained must display organoleptic and analytical characteristics at least equivalent to those of an average type of juice obtained from fruits of the same kind within the meaning of (a) (Council Directive 2001/112/EC[(f)]). Concentrated fruit juice is the product obtained from fruit juice of one or more kinds by the physical removal of a specific proportion of the water content. Where the product is intended for direct consumption that removal will be of at least 50% (Council Directive 2001/112/EC[(f)]) Fruit nectar is the fermentable but unfermented product obtained by adding water and sugars and/or honey to the products to fruit purée or to a mixture of those products, that meet the requirements of Annex IV (Council Directive 2001/112/EC[(f)])
Herbs and spices	Herbs are the aromatic leaves of plants without woody stems that grow in temperate zones Spices are seasonings obtained from the bark, buds, fruit or flower parts, roots, seeds or stems of various aromatic plants and trees Herbs and spices are usually derived from botanical sources, which may be dehydrated and are either ground or whole Examples of herbs include basil, oregano and thyme. Examples of spices include cumin and caraway seeds. Spices may also be found as blends in powder or paste form. Examples of spice blends include chilli seasoning, chilli paste, curry paste, curry roux and dry cures or rubs that are applied to external surfaces of meat or fish
Meat and meat products	Fresh meat is meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere (Regulation (EC) No 853/2004[(a)]) Meat preparations are defined as fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat (Regulation (EC) No 853/2004[(a)]) Minced meat is boned meat that has been minced into fragments and contains less than 1% salt (Regulation (EC) No 853/2004[(a)]), e.g. steak tartare Meat products are defined as processed products resulting from the processing of meat or from further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (Regulation (EC) No 853/2004[(a)])

Food category	Description
Milk	The following products shall be considered as drinking milk: raw milk intended for direct human consumption: milk, which has not been heated above 40°C or subjected to treatment having equivalent effect and intended to consumed raw; pasteurised milk: milk heat treated to destroy disease-causing bacteria; ultra-high temperature (UHT) milk: milk heated for a short time, around 1–2 seconds, at a temperature exceeding 135°C, which is the temperature required to kill spores in milk Please specify the species of origin of the milk (such as cow, goat, sheep) as well as the treatment of the milk in the free text data element (e.g. 'raw goat milk', 'pasteurised cow milk' or 'UHT milk')
Mixed food	Mixed meals are meals composed of various foods, e.g. paella, risotto, curries and nasi goreng. This category also includes miscellaneous foodstuffs served on one plate Please also select this category if it was not possible to narrow the suspected food down to an individual food or ingredient during the investigation of the food-borne outbreak
Other foods	This category should be chosen if the implicated food is none of those mentioned above. In this case, it should be specified in the free text data element
Other or mixed red meat and products thereof	Other red meat is meat from species, other than birds, that are not mentioned above, such as wild game and farmed game Wild game includes wild ungulates and lagomorphs, as well as other land mammals that are hunted for human consumption. Meat from wild boars is classified in this category; it would be desirable to indicate in the comment section that the meat pertains to wild boar Farmed game includes farmed ratites and farmed land mammals other than those referred to as 'Domestic ungulates' (Regulation (EC) No 853/2004[(a)]) Mixed red meat is a mix of red meat from different species, e.g. meatballs consisting of bovine and pig meat. For the definition of meat products please see 'Bovine meat and products thereof'
Other, mixed or unspecified poultry meat and products thereof	Other poultry meat is defined as edible parts of 'poultry', including blood 'Poultry' are farmed birds, including birds that are not considered as domestic but which are farmed as domestic animals, with the exception of ratites, which are considered 'farmed game' (Regulation (EC) No 853/2004[(a)]). In this case, all poultry EXCEPT Gallus and turkey. Examples in this category include duck, goose, pheasant, guinea fowl and ostrich For the definition of meat products, please see 'Bovine meat and products thereof'
Pig meat and products thereof	Pig meat is defined as edible parts of domestic porcine animals, including blood (Regulation (EC) No 853/2004[(a)]) For the definition of meat products, please see 'Bovine meat and products thereof'
Sheep meat and products thereof	Sheep meat is defined as edible parts of domestic ovine animals, including blood (Regulation (EC) No 853/2004[(a)])
Sweets and chocolate	Sweets are foods, such as candy, milk-free puddings or preserves, which are high in sugar content and milk-free puddings Candy, specifically sugar candy, is a confection made from a concentrated solution of sugar in water, to which a variety of flavourings and colorants is added. Some candy, like marshmallows and gummy bears, may contain gelatine Preserves refer to fruit, or vegetables, that have been prepared, canned or jarred for long-term storage. Examples for preserves are jam and jelly Chocolate is obtained by an adequate manufacturing process from cocoa materials which may be combined with milk products, sugars and/or sweeteners and other additives. Other edible foodstuffs, excluding added flour and starch and animal fats other than milk fat, may be added to form various chocolate products (Directive 2000/36/EC[(g)])

Food category	Description
Tap water, including well water	Tap water or ordinary drinking water refers to: all water, either in its original state or after treatment, intended for drinking, cooking, food preparation or other domestic purposes, regardless of its origin and whether it is supplied from a distribution network, from a tanker or in bottles or containers (Council Directive 98/83/EC[h]) In this context, tap water, including well water, does not include water in bottles. Water in bottles is included in the item 'Drinks, including bottled water' Please specify in the free text data element whether the water was treated or untreated
Turkey meat and products thereof	Turkey meat is defined as edible parts of domestic turkeys, including bloodFor the definition of meat products please see 'Bovine meat and products thereof'
Unknown	This category should be chosen if the implicated food is not identified
Vegetables and juices and other products thereof	Vegetables are plants or parts of plants cultivated for food Some foods that are botanically fruits, such as tomatoes and cucumbers, and seeds, such as peas and beans, are included with the vegetables; some plants, such as rhubarb, are classed as fruit, although they are not botanically fruits. The distinction in popular usage depends on whether they are eaten as savoury (vegetables) or sweet (fruit) dishes Examples of vegetables include cauliflower, broccoli, pea, cucumber, lentil, avocado and garlic. 'Sea vegetables' like sea lettuce and seaweed are also part of this group Vegetable juice is the juice obtained from vegetables and usually made from carrots, beets, pumpkin or tomatoes. Please specify the plant species or cultivar group as well as the treatment (e.g. raw, cooked juice) in the free text data element (e.g. 'raw iceberg lettuce')

(a): Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin OJ L 139, 30.4.2004, p. 55–205.
(b): Council Directive 79/923/EEC of 30 October 1979 on the quality required of shellfish waters. OJ L 281, 10.11.1979, p. 47–52.
(c): Council Directive 80/777/EEC of 15 July 1980 on the approximation of the laws of the Member States relating to the exploitation and marketing of natural mineral waters. OJ L 229, 30.8.1980, p. 1–10.
(d): Directive 96/70/EC of the European Parliament and of the Council of 28 October 1996 amending Council Directive 80/777/EEC on the approximation of the laws of the Member States relating to the exploitation and marketing of natural mineral waters. OJ L 299, 23.11.1996, p. 26–28.
(e): Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption. OJ L 229, 30.8.1980, p. 11–29.
(f): Council Directive 2001/112/EC of 20 December 2001 relating to fruit juices and certain similar products intended for human consumption. OJ L 10, 12.1.2002, p. 58–66.
(g): Directive 2000/36/EC of the European Parliament and of the Council of 23 June 2000 relating to cocoa and chocolate products intended for human consumption. OJ L 197, 3.8.2000, p. 19–25.
(h): Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 32–54.

Appendix E – R code for the source attribution model and associated estimation of uncertainty

The R code for the source attribution model and the input data used are available as downloadable files through the Knowledge Junction under the https://doi.org/10.5281/zenodo.3601875

Appendix F – Uncertainty analysis

The sources of uncertainty associated with the available data have been summarised in tabular format (Table F.1), describing the nature or cause of the uncertainties. Additional considerations about the uncertainties in the assessment and their impact on the conclusions are described below.

Table F.1: Sources of uncertainty identified in the assessment

Source or location of uncertainty	Nature or cause of uncertainty as described by the experts and impact of uncertainties on the conclusions
A systematic literature review was not undertaken (All ToRs)	• The topics were addressed by non-systematic searches, expert knowledge, footnote chasing, questionnaires, project reports and experiences in the Member States represented by the working group members and the members of the BIOHAZ panel. • Systematic appraisal analysis for the quality of the studies was also not performed. • The uncertainties related to the evidence obtained from literature used in this opinion will affect all of the ToRs but the impact on the conclusions is expected to be relatively low because of the expertise and experience of the Working Group members and the BIOHAZ panel members.
Current STEC surveillance and monitoring data (ToR1)	• There are a range of methods (agar culture, cell culture, immunological and molecular-based methods) available for the detection of STEC, but their effectiveness may be limited by issues such as sensitivity and selectivity. • Testing methods may be more effective at detecting specific serogroups, such as O157. This will result in bias in terms of the serogroups reported from all sample types. • Mild human infections are less likely to be reported, thus serogroups and/or virulence gene profiles associated with diarrhoea only are unlikely to be identifiable. • Asymptomatic human cases are not routinely detected and/or reported. • The impact of these uncertainties on the answer to ToR1 is considered to be low.
Current STEC characterisation data (ToR1)	• The strain characterisation data currently available to inform pathogenicity assessment is limited as relatively few human, food, feed or animal isolates have been tested for key virulence genes. • Only confirmed reported human cases were used in this analysis and the data set characterising these isolates is incomplete. • Moreover, the data that are available are mostly limited to serogroup, Stx toxin, gene subtype and the presence/absence of *eae*. Hence, there may be, as yet undetected, virulence markers associated with different clinical outcomes. • The impact of these uncertainties on the answer to ToR1 is also considered to be low.
Pathogenicity assessment based on strain characteristics (ToR1)	• Several studies have shown that the expression of virulence genes in STEC is dependent of environmental factors, including the presence of other bacteria. • Human factors such as age, immune status and therapeutic history also impact on clinical outcome. Pathogenicity assessment based solely on strain characteristics will therefore be uncertain as other key influencing factors are being ignored. • *stx2a* genes may have been incorrectly identified as *stx2c* resulting in HUS cases being incorrectly associated with the latter. • It is not defined what proportion of cases with a given *stx* subtype must be associated with HUS before this should be considered as indicative of 'having potential for severe disease'. • Current STEC isolation methods are limited in terms of; (1) sensitivity; (2) selectivity and (3) human error resulting in false positives or negatives. Moreover, as most of the current methods were developed for O157, other STEC serogroups may not be detected when present. The data provided may be unreliable resulting in overemphasis on strains which carry more frequently investigated combinations of specific virulence genes. • The conclusions have been drafted taking these uncertainties into account, these uncertainties are therefore likely to have a low impact.

Source or location of uncertainty	Nature or cause of uncertainty as described by the experts and impact of uncertainties on the conclusions
Source attribution data (ToR3)	• Monitoring of food-borne outbreaks and annual reporting is mandatory under Directive 2003/99/EC[7]; however, virulence gene information is usually not reported to EFSA. • Only using outbreak data from strong evidence outbreaks resulted in a small data set that is not necessarily representative of the whole of the EU/EEA. • Not all countries investigate and report food-borne outbreaks equally, thus, the data set may be unrepresentative of the entire population of the EU.
Uncertainties in the ranking of food commodities (ToR3)	• Some foods are more likely to cause outbreaks than others, especially large outbreaks; thus, the relative importance of sources of outbreak-associated cases may not be representative of the overall contribution of sources for the total burden of disease. • The estimated relative contribution of each food type is dependent upon the probability that the food is involved in outbreaks that are identified and successfully investigated. For example, cases of severe illness or illness in children tend to be more frequently notified, and cases of young adults less frequently (Franklin et al., 2015) and this may also be true for outbreaks. Thus, certain risk groups within the larger population and smaller outbreaks may be underrepresented in the available data. • The foods identified in outbreak investigations may not be representative of foods responsible for sporadic disease. Although a study found that outbreak and sporadic infections caused by four priority pathogens (*Salmonella*, *Campylobacter*, STEC O157 and *Listeria monocytogenes*) were similar in the US, a number of published studies have noted that the food sources for some pathogens can vary substantially. For STEC, potential differences are relevant for sources that are frequently involved in outbreaks (raw produce, unpasteurised dairy products), but are less likely to cause sporadic cases, either because contamination events are rare (even if with a large impact) or because they are not consumed frequently by the general population, but at high frequency among specific risk groups. • In the majority of outbreaks in the data set studied, the source was not identified, and the resources invested may be dependent on the size, severity, geographical spread and publicity associated with the outbreak. • The lack of a comprehensive database of food items associated with STEC outbreaks, representative for the EU as a whole, has a negative impact on the food ranking analysis and may result in the true ranking of food commodities based on public health risks being different from the one obtained. • The conclusions have been drafted taking these uncertainties into account, these uncertainties are therefore likely to have a low impact on the answer to ToR3.

Appendix G – Analysis of the TESSy STEC data (2012 to 2017)

The TESSy data (2012–2017, inclusive) were analysed to determine associations between *stx* subtypes, the presence/absence of *eae* and severe illness expressed as HUS (Table G.1), hospitalizations (Table G.2) and bloody diarrhoea (BD) (Table G.3).

Table G.1: *Stx* subtyping and HUS

***stx* types**	***stx* subtypes**	***eae* positive**				***eae* negative**			
		No HUS	**HUS**	**Unknown HUS result**	**Total number of cases**	**No HUS**	**HUS**	**Unknown HUS result**	**Total number of human cases**
stx1 total		511	6	98	615	315	1	86	402
stx2 total		744	160	336	1,240	355	10	47	412
stx1 + *stx2* total		639	40	176	855	352	5	61	418
stx1	*stx1a*	506	6	96	608	139	0	28	167
	stx1c	5	0	2	7	158	1	58	217
	stx1d	0	0	0	0	17	0	0	17
	stx1a+stx1c	0	0	0	0	1	0	0	1
stx2	*stx2a*	324	122	196	642	43	5	7	55
	stx2b	5	0	0	5	205	1	30	236
	stx2c	154	7	91	252	19	1	1	21
	stx2d	10	1	2	13	26	3	5	34
	stx2e	0	0	0	0	29	0	3	32
	stx2f [(a)]	200	8	45	253	10	0	0	10
	stx2g	1	0	0	1	21	0	1	22
	stx2a+stx2c	44	18	1	63	0	0	0	0
	stx2a+stx2b	0	0	0	0	1	0	0	1
	stx2a+stx2g	1	0	0	1	1	0	0	1
	stx2a+stx2c+stx2d	5	4	1	10	0	0	0	0
stx1+*stx2*	*stx1a+stx2a*	126	33	24	183	21	1	1	23
	stx1a+stx2b	2	0		2	108	0	26	134
	stx1a+stx2c	481	4	150	635	2	0	1	3
	stx1a+stx2d	1	1	0	2	10	1	0	11
	stx1a+stx2a+stx2c	14	0	0	14	0	0	0	0
	stx1a+stx2a+stx2c+stx2d	10	2	0	12	0	0	0	0
	stx1a+stx2a+stx2d	0	0	0	0	3	1	0	4

stx types	*stx* subtypes	*eae* positive				*eae* negative			
		No HUS	HUS	Unknown HUS result	Total number of cases	No HUS	HUS	Unknown HUS result	Total number of human cases
	stx1c+stx2a	0	0	0	0	4	0	1	5
	stx1c+stx2b	4	0	2	6	200	2	32	234
	stx1c+stx2c	1	0	0	1	3	0	0	3
	stx1c+stx2d	0	0		0	1	0		1
ALL	Overall total	1,894	206	610	2,710	1,022	16	194	1,232

Note: Table shows *eae*-positive and *eae*-negative HUS-positive and HUS-negative human STEC cases per *stx* subtype as well as the total number of reported human cases per *stx* subtype with unknown HUS result (Data in the EU/EEA from TESSy (ECDC)).

(a): In cases reported with combination of virulence genes: '*eae* = positive, *stx1* = not applicable (NA), *stx2* = positive, *stx1*-subtype = unknown, *stx2*-subtype = Stx2f', 'NA' was interpreted as *stx1*=negative and *stx2f* positive. Stx2f has never been seen together with Stx1 and some laboratories have a separate procedure for the detection of *stx2f* because it is not detectable with the generic *stx2* PCR primers and/or DNA dot blots.

Table G.2: *Stx* subtyping and hospitalised human STEC cases

stx types	*stx* subtypes	*eae* positive				*eae* negative			
		No hospitalisation	Hospitalisation	Unknown hospitalisation information	Total number of cases	No hospitalisation	Hospitalisation	Unknown hospitalisation information	Total number of cases
stx1 total		231	87	297	615	165	42	195	402
stx2 total		434	314	492	1,240	131	42	239	412
stx1+*stx2* total		366	203	286	855	210	38	170	418
stx1	*stx1a*	226	86	296	608	65	17	85	167
	stx1c	5	1	1	7	99	23	95	217
	stx1d	0	0	0	0	1	1	15	17
	stx1a+stx1c	0	0	0	0	0	1	0	1
stx2	*stx2a*	176	228	238	642	17	8	30	55
	stx2b	1	0	4	5	74	20	142	236
	stx2c	138	34	80	252	5	3	13	21
	stx2d	6	3	4	13	14	7	13	34
	stx2e	0	0	0	0	14	2	16	32
	stx2f[(a)]	94	25	134	253	1	0	9	10
	stx2g	0	0	1	1	5	1	16	22

stx types	*stx* subtypes	*eae* positive				*eae* negative			
		No hospitalisation	Hospitalisation	Unknown hospitalisation information	Total number of cases	No hospitalisation	Hospitalisation	Unknown hospitalisation information	Total number of cases
	stx2a+stx2c	18	24	21	63	0	0	0	0
	stx2a+stx2b	0	0	0	0	1	0	0	1
	stx2a+stx2g	1	0		1	0	1	0	1
	stx2a+stx2c+stx2d	0	0	10	10	0	0	0	0
stx1+stx2	*stx1a+stx2a*	33	48	102	183	7	8	8	23
	stx1a+stx2b	1	1		2	73	5	56	134
	stx1a+stx2c	320	150	165	635	0	0	3	3
	stx1a+stx2d	0	0	2	2	4	2	5	11
	stx1a+stx2a+stx2c	7	4	3	14	0	0	0	0
	stx1a+stx2a+stx2c+ stx2d	0	0	12	12	0	0	0	0
	stx1a+stx2a+stx2d	0	0		0	1	1	2	4
	stx1c+stx2a	0	0	0	0	1	0	4	5
	stx1c+stx2b	5	0	1	6	123	21	90	234
	stx1c+stx2c	0	0	1	1	0	1	2	3
	stx1c+stx2d	0	0		0	1	0	0	1
All	Overall total	1,031	604	1,075	2,710	506	122	604	1,232

Note: Table shows *eae*-positive and *eae*-negative human STEC cases with and without hospitalisation per *stx* subtype as well as the total number of reported human cases per *stx* subtype with unknown information about hospitalisation (Data in the EU/EEA from TESSy (ECDC)).

(a): In cases reported with combination of virulence genes: '*eae* = positive, *stx1* = not applicable (NA), *stx2* = positive, *stx1*-subtype = unknown, *stx2*-subtype= Stx2f', 'NA' was interpreted as *stx1* = negative and *stx2f* positive. Stx2f has never been seen together with Stx1 and some laboratories have a separate procedure for the detection of *stx2f* because it is not detectable with the generic *stx2* PCR primers and/or DNA dot blots.

Table G.3: *Stx* subtyping and bloody diarrhoea (BD)

stx types	*stx* subtypes	*eae* positive				*eae* negative			
		Diarrhoea	Bloody diarrhoea	Neurological, asymptomatic, unknown or other clinical outcome	Total number of cases	Diarrhoea	Bloody diarrhoea	Neurological, asymptomatic, unknown or other clinical outcome	Total number of cases
stx1 Total		317	119	179	615	219	36	147	402
stx2 Total		544	366	330	1,240	277	48	87	412
stx1 + *stx2* Total		238	438	179	855	228	55	135	418
stx1	*stx1a*	314	118	176	608	103	9	55	167
	stx1c	3	1	3	7	103	25	89	217
	stx1d	0	0	0	0	12	2	3	17
	stx1a+stx1c	0	0	0	0	1	0	0	1
stx2	*stx2a*	198	278	166	642	28	10	17	55
	stx2b	5	0	0	5	170	20	46	236
	stx2c	143	45	64	252	15	3	3	21
	stx2d	5	4	4	13	21	4	9	34
	stx2e	0	0	0	0	15	7	10	32
	stx2f [(a)]	179	17	57	253	8	2	0	10
	stx2g	0	1	0	1	18	2	2	22
	stx2a+stx2c	10	19	34	63	0	0	0	0
	stx2a+stx2b	0	0	0	0	1	0	0	1
	stx2a+stx2g	1	0	0	1	1	0	0	1
	stx2a+stx2c+stx2d	3	2	5	10	0	0	0	0
stx1+stx2	*stx1a+stx2a*	59	77	47	183	10	8	5	23
	stx1a+stx2b	2	0	0	2	75	15	44	134
	stx1a+stx2c	166	344	125	635	2	0	1	3
	stx1a+stx2d	2	0	0	2	5	2	4	11
	stx1a+stx2a+stx2c	1	10	3	14	0	0	0	0
	stx1a+stx2a+stx2c+stx2d	6	4	2	12	0	0	0	0
	stx1a+stx2a+stx2d	0	0	0	0	2	0	2	4
	stx1c+stx2a	0	0	0	0	0	1	4	5
	stx1c+stx2b	1	3	2	6	131	29	74	234

stx types	*stx* subtypes	*eae* positive				*eae* negative			
		Diarrhoea	Bloody diarrhoea	Neurological, asymptomatic, unknown or other clinical outcome	Total number of cases	Diarrhoea	Bloody diarrhoea	Neurological, asymptomatic, unknown or other clinical outcome	Total number of cases
	stx1c+stx2c	1	0	0	1	3	0	0	3
	stx1c+stx2d	0	0	0	0	0	0	1	1
All	Overall total	1,099	923	688	2,710	724	139	369	1,232

Note: Table shows *eae*-positive and *eae*-negative human STEC cases per *stx* subtype with diarrhoea or bloody diarrhoea as well as the total number of reported human cases per *stx* subtype with either unknown, asymptomatic, neurological or other clinical outcome (Data in the EU/EEA from TESSy (ECDC)).

(a): In cases reported with combination of virulence genes: '*eae* = positive, *stx1* = not applicable (NA), *stx2* = positive, *stx1*-subtype = unknown, *stx2*-subtype = Stx2f', 'NA' was interpreted as *stx1* = negative and *stx2f* positive. Stx2f has never been seen together with Stx1 and some laboratories have a separate procedure for the detection of *stx2f* because it is not detectable with the generic *stx2* PCR primers and/or DNA dot blots.

Appendix H – Data reported in the Zoonoses database on occurrence of 'strong evidence' STEC outbreaks from 2012 to 2017

Table H.1: Detailed information, as reported for strong evidence STEC outbreaks from 2012 to 2017 in the EU/EEA in accordance with Directive 2003/99/EC[7]

Year	Reporting country	Causative agent	Food vehicle implicated	Type of evidence	Number of human cases	Number of hospitalisations	Number of deaths
2012	Belgium	STEC O157	Bovine meat and products thereof	Analytical epidemiological evidence	3	1	0
2012	Belgium	STEC O157	Bovine meat and products thereof	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	25	16	0
2012	Belgium	STEC O157	Bovine meat and products thereof	Descriptive epidemiological evidence	2	2	0
2012	Denmark	STEC O157	Minced beef	Descriptive epidemiological evidence	14	9	0
2012	United Kingdom	STEC O157	Beef burgers	Descriptive epidemiological evidence	10	2	0
2012	United Kingdom	STEC O157	Beef burgers	Descriptive epidemiological evidence	2	0	0
2013	United Kingdom	STEC O157	Bovine meat and products thereof - beef burgers	Descriptive epidemiological evidence	3	0	0
2013	France	STEC	Bovine meat and products thereof	Detection of causative agent in food vehicle or its component – Symptoms and onset of illness pathognomonic to causative agent	3	3	0
2013	France	STEC	Bovine meat and products thereof	Descriptive epidemiological evidence	5	4	0
2013	Belgium	STEC O157	Bovine meat and products thereof	Descriptive epidemiological evidence	18	16	0
2016	Ireland	STEC O157; STEC O182:H rough; STEC O26	Bovine meat and products thereof	Descriptive epidemiological evidence	10	5	0
2016	Sweden	STEC O157	Minced meat	Product-tracing investigations Detection of causative agent in food chain or its environment – Detection of indistinguishable causative agent in humans	26	0	0
2017	Finland	STEC O157	Home-made ground beef steak	Product-tracing investigations Descriptive environmental evidence Descriptive epidemiological evidence	3	2	0

Year	Reporting country	Causative agent	Food vehicle implicated	Type of evidence	Number of human cases	Number of hospitalisations	Number of deaths
2017	Belgium	STEC O157	Bovine meat and products thereof	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	8	8	0
2017	United Kingdom	STEC O157	Beef burgers	Analytical epidemiological evidence	11	8	0
Bovine meat and products thereof (total number of cases, hospitalisations and deaths)					**143**	**76**	**0**
2012	Finland	STEC O157	Raw milk	Detection of causative agent in food chain or its environment – Detection of indistinguishable causative agent in humans Descriptive epidemiological evidence Analytical epidemiological evidence	8	6	0
2013	Ireland	STEC O157	Unpasteurised cheese	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	2	1	0
2013	Sweden	STEC O26; STEC O174	Cheese	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	10	0	0
2014	United Kingdom	STEC O157	Raw cows drinking milk	Descriptive epidemiological evidence	9	2	0
2014	Germany	STEC	Raw milk	Detection of causative agent in food chain or its environment – Detection of indistinguishable causative agent in humans	5	0	0
2014	Spain	STEC	Milk	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans Analytical epidemiological evidence	2	2	0
2015	Ireland	STEC	Cheese	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	2	0	0
2016	United Kingdom	STEC O157	Unpasteurised soft blue cheese	Analytical epidemiological evidence	26	17	1

Year	Reporting country	Causative agent	Food vehicle implicated	Type of evidence	Number of human cases	Number of hospitalisations	Number of deaths
2016	Germany	STEC other than O157 O26 O103 O111 O145	Raw milk	Product-tracing investigations Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans Descriptive epidemiological evidence	3	3	0
2016	Belgium	STEC O157	Cheese	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	3	1	0
2017	Italy	STEC O111	Cheese – ingestion of ricotta, mozzarella and handcrafted ice cream	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	3	3	1
2017	United Kingdom	STEC O157	Raw drinking milk	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans Descriptive epidemiological evidence	7	3	0
2017	Germany	STEC; *Campylobacter* spp.	Raw milk	Descriptive epidemiological evidence	12	3	0
2017	Sweden	STEC O157	Milk	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	2	2	0
Milk and dairy products (total number of cases, hospitalisations and deaths)					**94**	**43**	**2**
2012	Ireland	STEC O157	Private water supply	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	2	0	0
2012	Ireland	STEC O157	Well, untreated ground water	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	6	0	0
2012	Ireland	STEC O157	Treated well water	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	27	0	0

Year	Reporting country	Causative agent	Food vehicle implicated	Type of evidence	Number of human cases	Number of hospitalisations	Number of deaths
2012	Ireland	STEC O26	Well, ground water	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	2	0	0
2013	Austria	STEC O128	Tap water, including well water	Descriptive epidemiological evidence	2	1	0
2014	Finland	STEC O103; *C. jejuni*	Well water	Descriptive epidemiological evidence	9	1	0
2015	Ireland	STEC	Tap water, including well water	Descriptive epidemiological evidence	5	0	0
2015	United Kingdom	STEC O157	Private water supply – spring water	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans Descriptive epidemiological evidence	22	5	0
Tap water, including well water (total number of cases, hospitalisations and deaths)					**75**	**7**	**0**
2013	United Kingdom	STEC O157	Pre-packed watercress	Detection of causative agent in food vehicle or its component – Symptoms and onset of illness pathognomonic to causative agent	6	0	0
2013	United Kingdom	STEC O157	Pre-packed watercress	Analytical epidemiological evidence	22	8	0
2013	Sweden	STEC O157	Salad	Analytical epidemiological evidence	28	0	0
2014	United Kingdom	STEC O157	Bagged ready to eat salad	Analytical epidemiological evidence	102	0	0
2014	United Kingdom	STEC O157	Bagged rocket leaves	Descriptive epidemiological evidence	10	2	0
2016	Finland	STEC; EPEC	Rucola	Product-tracing investigations Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans Descriptive epidemiological evidence Analytical epidemiological evidence	237	0	0
2016	United Kingdom	STEC O157	Salad leaves	Analytical epidemiological evidence	170	63	2
Vegetables, fruits and products thereof (total number of cases, hospitalisations and deaths)					**575**	**73**	**2**

Year	Reporting country	Causative agent	Food vehicle implicated	Type of evidence	Number of human cases	Number of hospitalisations	Number of deaths
2012	United Kingdom	STEC O157	Roast pork (roast hog) – main meal	Descriptive epidemiological evidence	3	1	0
2012	United Kingdom	STEC O157	Roast pork (main meal) and Cold meats	Descriptive epidemiological evidence	3	1	0
Pig meat and products thereof (total number of cases, hospitalisations and deaths)					**6**	**2**	**0**
2012	Austria	STEC O113	Other or mixed red meat and products thereof	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans	3	0	0
2016	United Kingdom	STEC O157	Venison	Descriptive epidemiological evidence	7	0	0
Other or mixed red meat and products thereof (total number of cases, hospitalisations and deaths)					**10**	**0**	**0**
2016	United Kingdom	STEC O157	Other or mixed red meat and products thereof – minced lamb	Detection of causative agent in food vehicle or its component – Detection of indistinguishable causative agent in humans Analytical epidemiological evidence	27	9	0
Sheep meat and products thereof (total number of cases, hospitalisations and deaths)					**27**	**9**	**0**
2017	Spain	STEC	Meat and meat products	Analytical epidemiological evidence	2	1	0
Unspecified meat (total number of cases, hospitalisations and deaths)					**2**	**1**	**0**
2013	France	STEC	Fish and fish products	Detection of causative agent in food vehicle or its component – Symptoms and onset of illness pathognomonic to causative agent	5	0	0
Fish and seafood (total number of cases, hospitalisations and deaths)					**5**	**0**	**0**
2013	Portugal	STEC	Parsley (vegetable fresh, intended to eaten raw)	Detection of causative agent in food vehicle or its component – Symptoms and onset of illness pathognomonic to causative agent	50	3	0
Herbs and spices (total number of cases, hospitalisations and deaths)					**50**	**3**	**0**

Appendix I – Analysis of the results of the consultation to Member States (questionnaire on STEC surveillance in humans)

Main testing strategy/main diagnostic tool(s) used in routine diagnostic laboratories with regard to STEC in different EU/EEA MS

The following information is based on the questionnaire on diagnostics and characterisation of STEC strains from human cases in 29 EU/EEA MS (Section 2.1.4.1, Appendix B). The most common approach on primary and NRL level to detect STEC human cases is screening isolates for virulence genes (*stx*1 and *stx2*, *eae*). However, there is no uniform approach regarding the methods used to detect STEC in different laboratories in different MSs. Few information is available about the clinical criteria of STEC sampling and diagnostic capacity for STEC on primary level. Detection of STEC cases strongly varies between the laboratories on local level, as almost two-thirds of the MS do not have national guidelines. In five MSs (5/29; 17%), all STEC diagnostics is done on primary level as there is a) no NRL (three countries), b) no STEC diagnostics is done on NRL level (two countries). In addition, in one MS detection in NRL is only performed in case of bloody diarrhoea and HUS. In six MSs (21%), all STEC diagnostics is done on NRL level. Generally, these countries are reporting low number of STEC cases to TESSy (ECDC) annually.

The suspected outbreak-related cases are detected most frequently both on primary and on NRL level. On the primary level, more severe cases (such as HUS and bloody diarrhoea) are more likely to be detected for STEC than diarrhoea in general/persistent diarrhoea or e.g. diarrhoea associated to travel. Six laboratories (6/23; 26%) on primary level and nine laboratories (9/24; 38%) on NRL level notified that all diarrhoea cases are detected for STEC. All countries report case-based data to TESSy except Bulgaria, which reported aggregated data. Aggregated reporting does not allow reporting any detailed information on laboratory variables (e.g. virulence genes). Data reported to TESSy do not separate sporadic and outbreak-related cases. Outbreaks are separately reported to EFSA. In 2017, 5% of the domestic STEC cases were reported as outbreak related (EFSA and ECDC, 2018).

Summary of analysis of the results of questionnaire on monitoring for STEC in humans (Appendix B)

The answers to the questionnaire distributed using the EU survey tool to the national contact points of the public health national reference laboratories in 30 countries (28 EU MSs, Iceland and Norway) in the FWD and Zoonoses network are summarised below.

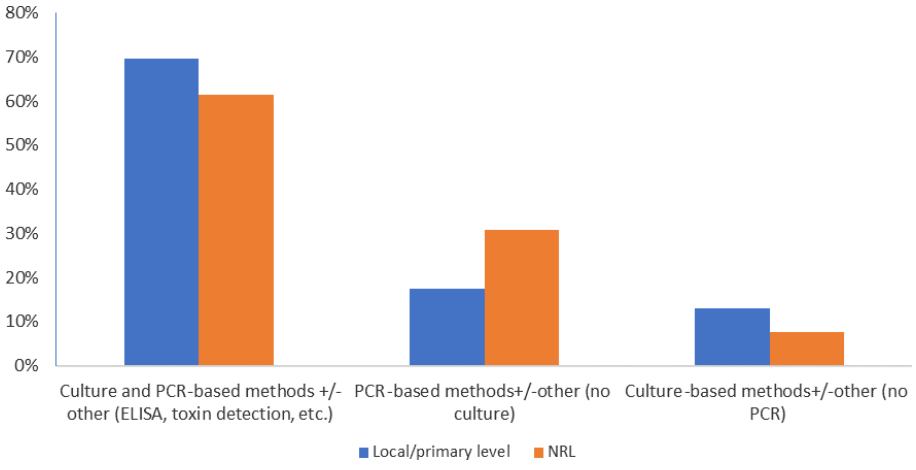

Figure I.1: Methods used for detection of STEC in human samples

The most common approach on primary and NRL level to detect STEC is screening for virulence genes (*stx1* and *stx2*, *eae*) by PCR (Figure I.1). Positive samples are further tested with culture-based methods and/or other methods (e.g. ELISA, toxin detection). However, there is no uniform approach regarding the methods used to detect STEC in different laboratories. In many countries, little information is available about the clinical criteria of STEC sampling and diagnostic capacity for STEC on primary level.

In five MSs (5/29; 17%), all STEC diagnostics is done on primary level as there is a) no NRL (three countries) b) no STEC diagnostics is done on NRL level (two countries). In addition, in one MS, detection in NRL is only performed in case of bloody diarrhoea and HUS.

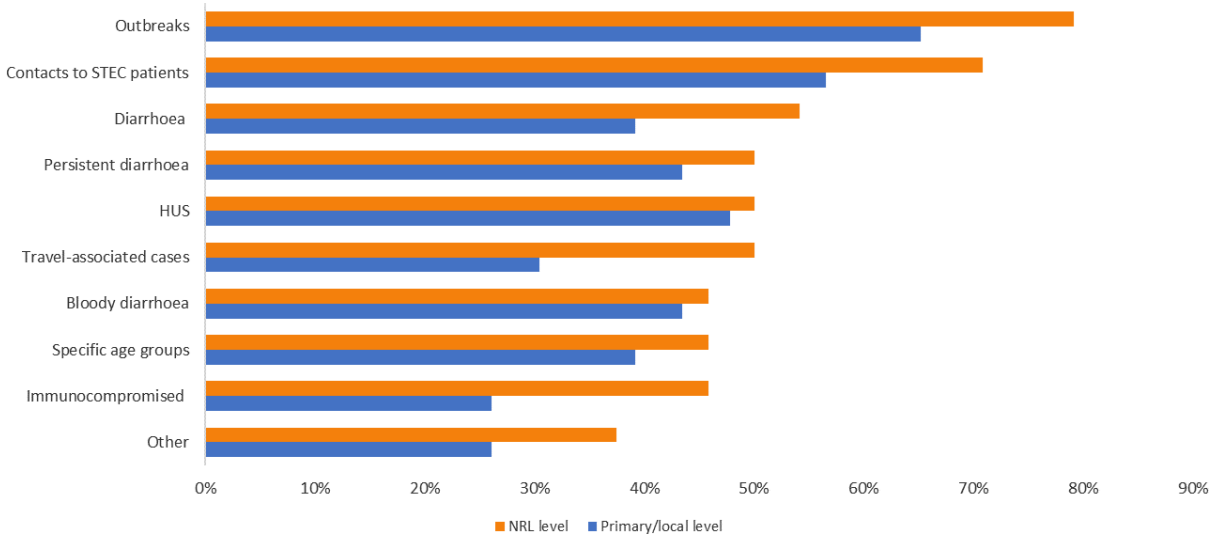

Figure I.2: Detection for the presence of STEC in human samples of different cases or specific situations

The detection methods used for STEC vary between the laboratories at the local level (Figure I.1), as almost two-thirds of the MSs do not have national guidelines/a national sampling strategy (Figure I.6). On the primary level, more severe cases (such as HUS and bloody diarrhoea) are more likely to be checked for the presence of STEC than diarrhoea in general or diarrhoea associated with travel. Nine laboratories (9/23; 39%) at the primary level and 13 laboratories (13/24; 54%) on NRL level notified that all diarrhoea cases are investigated for STEC. Contact to STEC patients (not specified, if these also/mainly include asymptomatic cases) are sampled second most frequently following detection of suspected STEC outbreak cases – both on primary and on NRL level. The 'Other' category includes cases without clinical symptoms (i.e. persons of specific profession as handling with food or working in community facilities after contact with patients with symptoms) and confirmation of clearance of STEC carriage (Figure I.2).

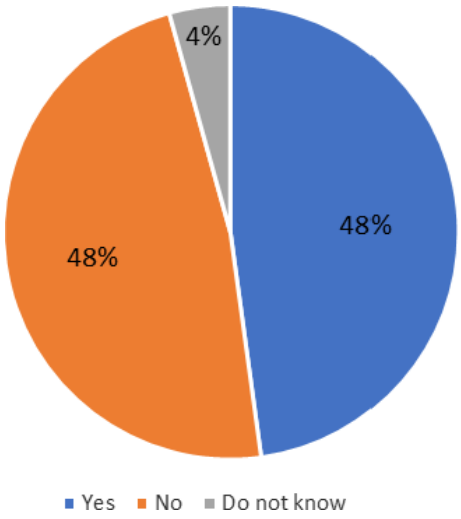

Figure I.3: Virulence gene profiling at the primary level

Almost half (48%; 11/23) of the primary level laboratories are doing virulence profiling (Figure I.3) including at least *stx1*, *stx2* and/or *eae*. About half of them (45%; 5/11) also include subtyping of *stx1* and *stx2* and additional virulence genes detection (e.g. *aaiC*, *aggR*, e*hxA*, *ipaH* and *aatA*).

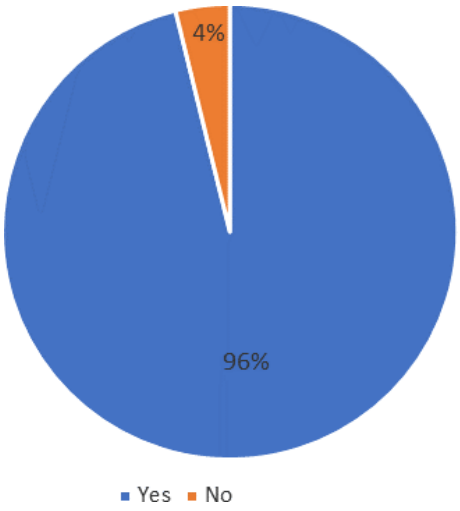

Figure I.4: Virulence gene profiling at the NRL level

At the NRL level, virulence gene profiling is usually done for all STEC isolates including subtyping of s*tx1* and *stx2* (Figure I.4). Only one reference laboratory informed that there is no capacity for typing of STEC (possible isolates sent to another country for characterisation). At the NRL level, 16 (62%) laboratories also type for one or several additional virulence genes (e.g. *aaiC, aggR, ehxA, ipaH, aatA, sfpA, esp, kutp, hlyA, hilA, estA, eltA*) and when WGS based typing is in place, the whole array of virulence genes is detected (> 50 more genes).

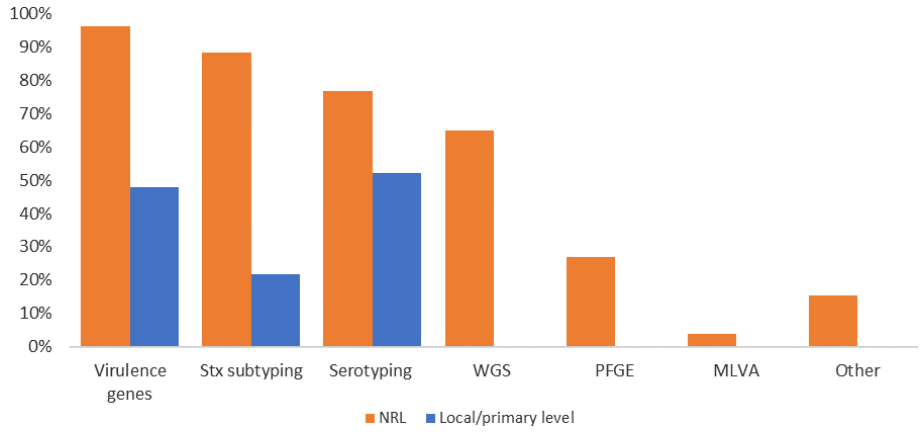

Figure I.5: Additional characterisation methods of human STEC isolates

Serotyping was the most commonly mentioned characterisation method on the primary level for STEC isolates (Figure I.5). This does not however mean than all primary laboratories serotype their isolates. In addition, the focus is mostly to detect serogroup O157. In general, countries had difficulties to conclude, which different methods are routinely used on primary level laboratories. In some countries, primary level laboratories can do virulence gene profiling with *stx*-subtyping and additional virulence genes and in some countries, all suspected faeces samples are sent to NRL for confirmation.

At the NRL level, WGS is used routinely together with other methods in 17 MSs (65%) and four countries only use WGS. In addition, two countries mentioned that WGS is not routinely used but it is available if needed.

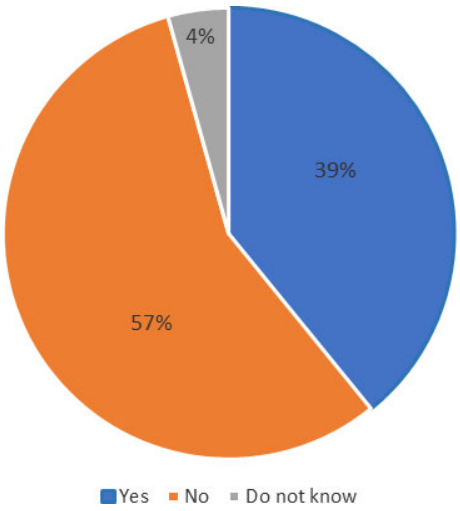

Figure I.6: Sampling strategy for routine testing (national guidelines)

Slightly over one-third (39%; 9/23) of the MSs have national sampling strategy/national guidelines for STEC detection (Figure I.6). Among countries having national guidelines, 33% had a strategy to sample all cases reporting symptoms of diarrhoea for STEC, and 44% had a strategy to sample specific groups (e.g. children less than 7 years old with diarrhoea, patients with bloody stools or persistent diarrhoea associated to travel). Among the countries not having national guidelines 38% reported to routinely sample all faecal specimens submitted to laboratories for STEC.

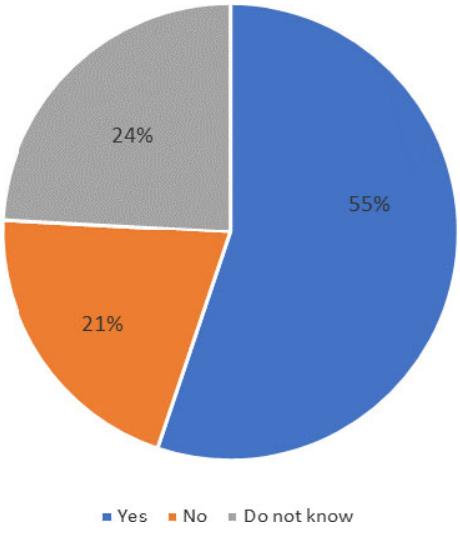

Figure I.7: Submission of STEC data to ECDC database

Just less than 60% of the MSs submit a complete set of STEC (sub)typing data to TESSy annually (Figure I.7). In TESSy these data, were available for 64% and 60% of the isolates reported annually for *stx* and *eae,* respectively (referring above to the most common detection and characterisation methods) in 2012–2017. *Stx*-subtyping data is reported 19% and 24% of the *stx1* and *stx2* positive cases, respectively, during the same period and less than 4% for other virulence genes (*aggR* and *aaiC*). This is much less than what countries responded for the use of the virulence gene profiling particularly on the NRL level. As more data most likely is available nationally, countries should be encouraged to report these data to TESSy.

Several reasons were mentioned not to report the full set of STEC data to TESSy: (1) no permission/no nomination to report, (2) no STEC isolates detected in the country, (3) financial and human resource problems, (4) no national reporting protocol, (5) isolates not sent to NRL, (6) not all data is transferred from laboratory databases to national infectious disease register, which is data source for TESSy (7) the set of virulence genes tested have been changing as well as the methods for retrieving the virulence data. Some countries mentioned that they report all other than WGS data and the WGS-based data still needs validation before it can be reported to TESSy.

Appendix J – Analysis of the results of the consultation to Member States (questionnaire on STEC monitoring in food, feed and animals)

Criteria for reporting STEC contamination of food, feed and animals

Data regarding STEC in foods are provided by the Member States as a result of the reporting obligations described in the Directive 2003/99/EC[7], which indicates that the presence of STEC should be investigated at the most appropriate stage of the food chain. It has to be stressed that this directive does not prescribe exact reporting details and does not include any microbiological criterion for specific food types or animal species, which makes the reporting not harmonised either in terms of sampling strategy or in terms of methodology to be used for testing the samples.

Sprouts are the exception to this rule. In the aftermath of the major outbreak of STEC O104:H4 infections in Europe (mainly Germany and France) in 2011, the European Commission issued an amendment of the Commission Regulation (EU) No 2073/2005[8] including a specific microbiological criterion for STEC in this food commodity (Regulation (EU) No 209/2013[11]). The regulation includes rules for both the testing of seeds intended for sprouting as well as for the spent irrigation water. Additionally, the international standard ISO TSs 13136:2012 (ISO, 2012) is indicated as a reference method to be used for verifying the absence of STEC in the food commodity.

National strategies for surveillance of STEC in food, feed and animals in different European countries

The lack of a regulatory framework for testing food and animals for the presence of STEC has resulted in a fragmented approach towards this activity in the different Member States. Some MS have issued their own rules in their national sampling plans, while others restrict the sampling strategies to specific epidemiologic investigations during outbreaks or sporadic cases or on the occasion of national surveys on specific food types or animal species. Notwithstanding this situation it has to be observed that, although fragmented, data on the monitoring of STEC in food were provided by 25 MSs in 2017, on more than 21,000 food samples suggesting a general awareness and concern of the STEC issue. In contrast, there was a decrease in the testing of animal samples. Since 2016, the number of animal samples tested by the EU MSs decreased to less than half when compared to the numbers tested up until 2015.

Stages of the food chain (at farm level, at the abattoir, at food processing)

In spite of the lack of specific directives for sampling, the data reported to EFSA cover almost all the sampling stages including the farm, the abattoir, the food processing plants and retail level. Unfortunately, these are unevenly spread among the different MS making it difficult to compare the contamination levels in different countries.

Summary of analysis of the results of questionnaire on surveillance for STEC in food, feed and animals (Appendix C)

The answers to the questionnaire distributed using the EU survey tool to the national contact points of the National Reference Laboratories (NRLs) of 28 MSs, Iceland, Norway and Switzerland are summarised below.

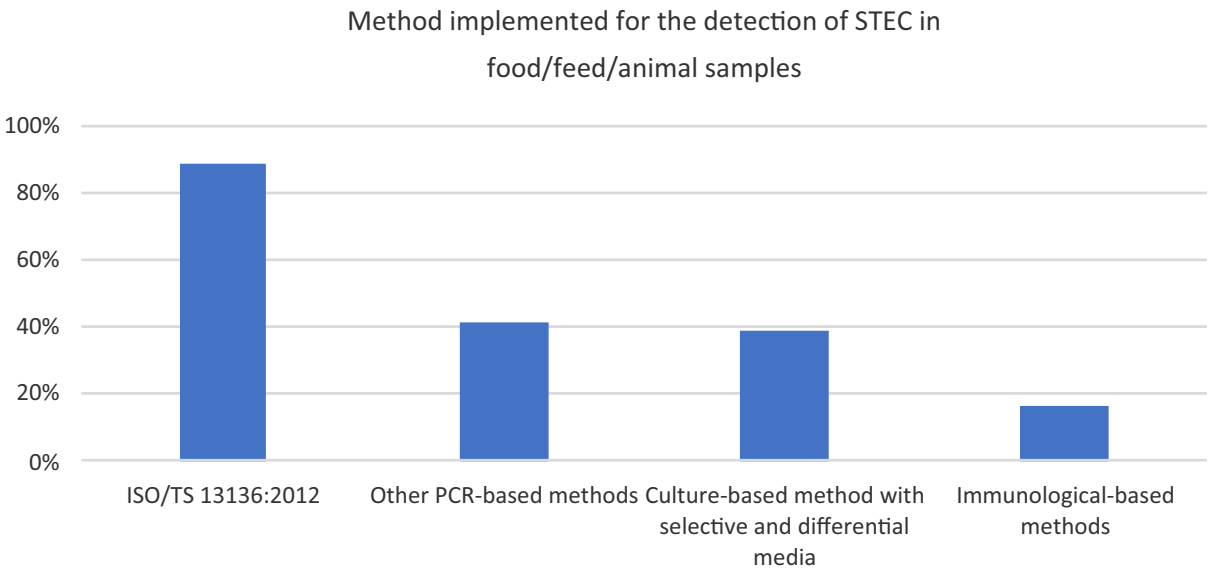

Figure J.1: Methods implemented for the detection of STEC in food/feed/animals samples by NRLs

Even though these answers concern the NRLs only, Figure J.1 reflects what we have also seen during the analysis of the EU STEC monitoring data from 2017 to 2018. There is substantial harmonisation with respect to the analysis for STEC detection in food in the EU either at the NRL or at the OL level. It is important to specify that the other PCR-based methods for STEC detection are developed on the ISO TS 13136 procedure (ISO, 2012) although they may use different primers. This should bring the column of the ISO TS 13136 methods closer to 100%. However, different NRLs declared to use more than one type of method to confirm the isolated colonies.

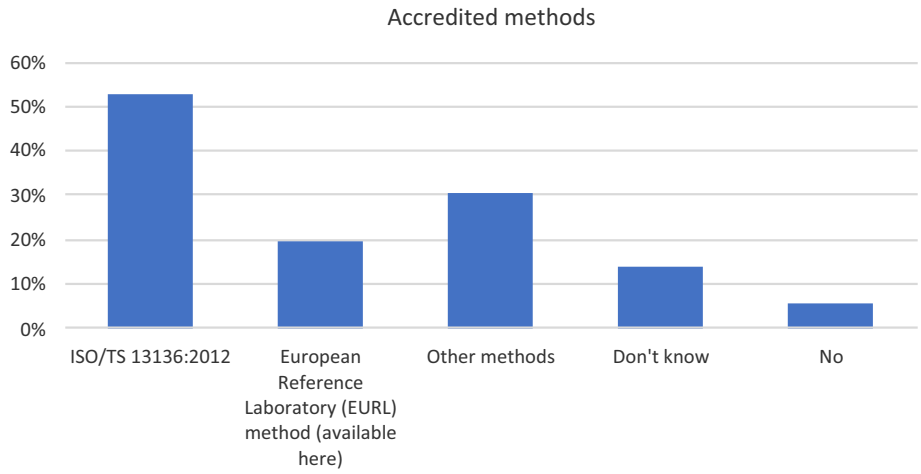

Figure J.2: Accredited methods in the STEC NRLs

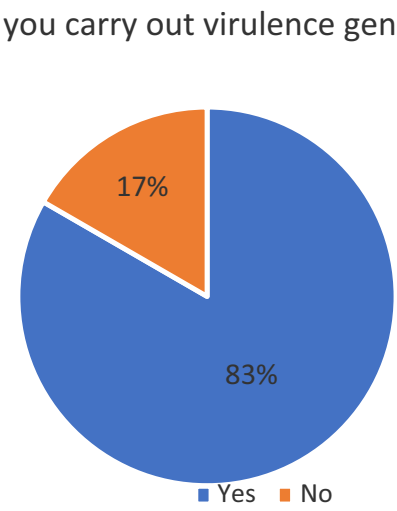

Figure J.3: Virulence gene profiling at the NRL level

Figure J.3 shows how much the ability to carry out virulence genes profiling has been represented by the survey. The data are interesting and were expected. The NRLs for *E. coli* are perfectly capable to perform virulence genes profiling at the deepest level (e.g. Shiga toxin genes subtyping). Differently, the official laboratories generally do not do so with this level of detail and this activity is not assigned in all MS to the NRLs. This would explain why only one-third of the STEC isolates from food, feed and animals (roughly) in the EFSA data collection are provided with the information on the presence of *stx1*, *stx2* and *eae* genes. At the same time, since almost the totality of the food samples are assayed using the CEN ISO/TS 13136 standard (ISO, 2012; EFSA and ECDC, 2018), the reporting of this information for one-third of the isolates is not totally convincing. As a matter of fact, the method gives back at least part of the virulence genes profiles (namely *stx1*, *stx2* and *eae* genes) but this information is not reported to EFSA, highlighting that part of the problem resides in the reporting level.

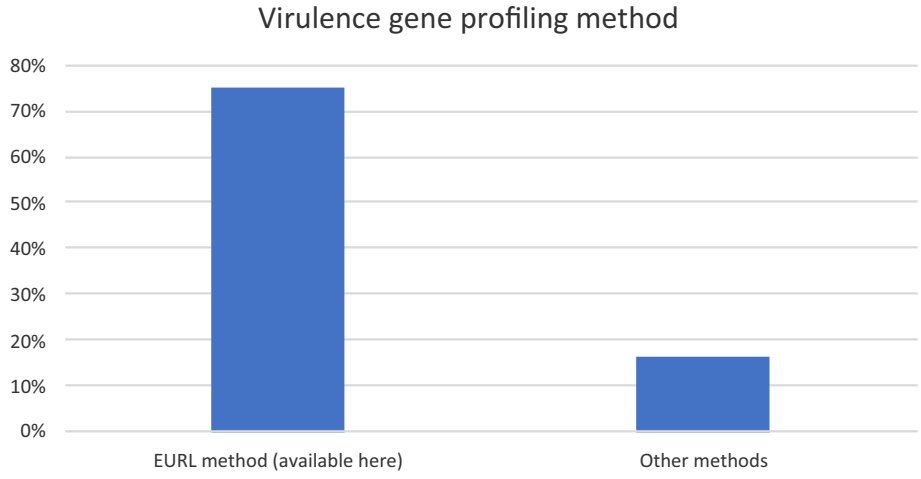

Figure J.4: Virulence gene profiling methods used in NRLs

It is not surprising that most of the NRLs refer to the EURL method (http://old.iss.it/binary/vtec/cont/EU_RL_VTEC_Method_06_Rev_1.pdf) for assays that are not covered by an international standard (e.g. ISO) (Figure J.4). Most probably, those NRLs referring 'other methods' for virulence gene profiling use WGS or use in-house methods for specific subsets of genes. This is confirmed by the reply to the next question.

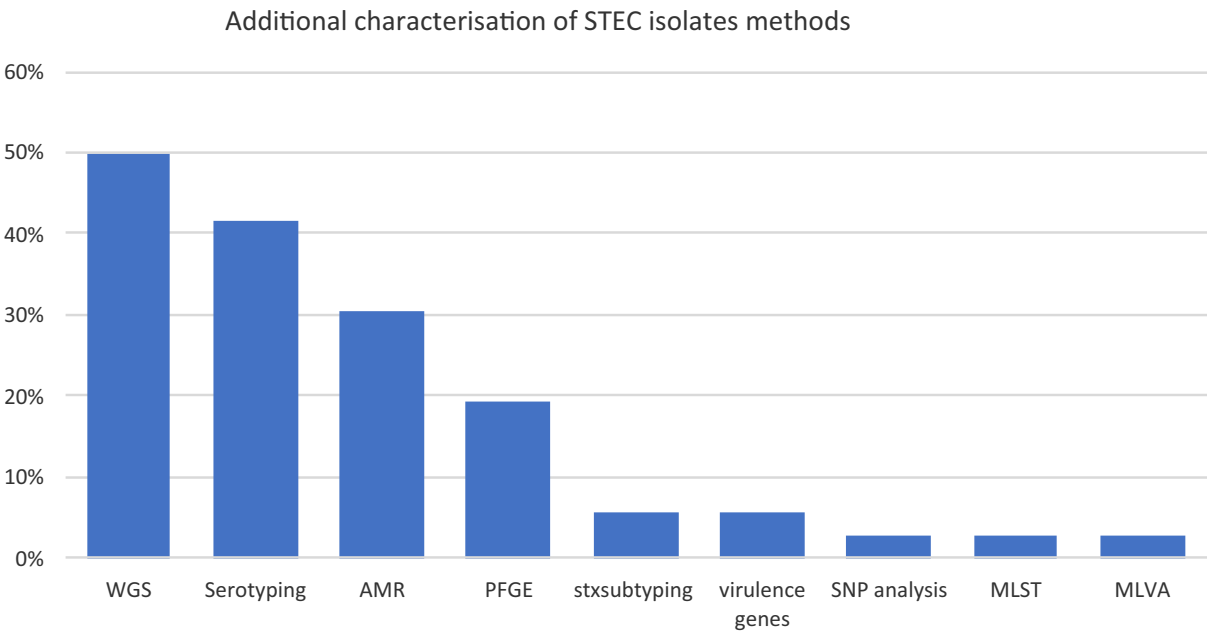

Figure J.5: Additional characterisation methods of food/feed animal STEC isolates

Approximately half of the NRLS are already sequencing (WGS) STEC isolates from food, feed and animal samples (Figure J.5). This figure is higher than that suggested by the EFSA survey in 2016 (EFSA, 2018).

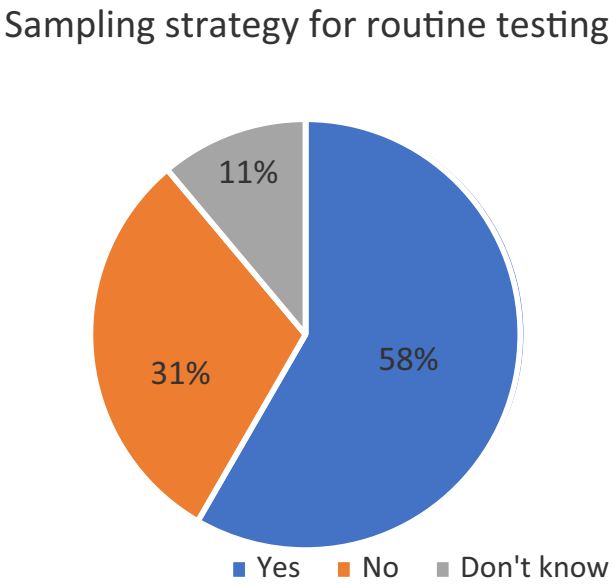

Figure J.6: Sampling strategy for routine testing (national guidelines)

From the replies to the survey, more than half of the MS reported a national sampling strategy for routine STEC testing in food, feed or animal samples (Figure J.6). These strategies concern mainly meat, milk and sprouts and do not cover the entire spectrum of food commodities. It is of note that the remaining half of the MS do not have a national sampling strategy to test food for the presence of STEC or this information was not available to the respondent NRL.

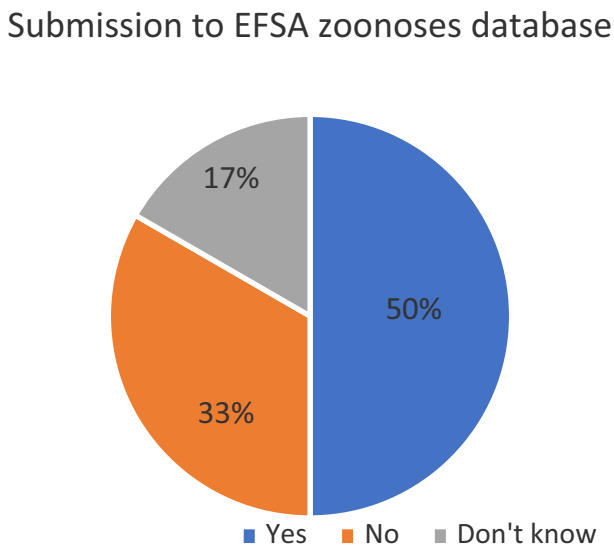

Figure J.7: Submission of STEC data to EFSA zoonoses database

Apparently, half of the Member States submit a complete set of STEC typing data that is obtained at NRL level or official laboratory level to the EFSA zoonoses database annually (Figure J.7). However, we know that these data are only available for one-third of the reported isolates annually. Apparently, it would mean that the MSs that report these data are those reporting less isolates. Additionally, some of the responders have declared that the reporting is cumbersome and the typing data are not always available at the data provider level. A key point is the use of WGS. A couple of MSs declared that they use WGS and that this info is made public and not sent to EFSA (one MS), while another reported that is currently setting up WGS for further typing.

According to the EUSRs in the last 2 years (EFSA and ECDC, 2017, 2018), there has been a large increase in the adoption of the international standard ISO TS 13136:2012 (ISO, 2012), which targets all STEC and not just a selection of the more common serogroups. In 2017, 97.4% of the food samples were tested using this method (EFSA and ECDC, 2018). However, only about half of the animal samples tested in 2017 were analysed using the ISO TS 13136:2012 method, while the remaining samples were tested using the ISO 16654:2001 (ISO, 2001) or equivalent methods, which specifies the detection of *E. coli* O157 only.