EFSA Journal 2011;9(6):2274

SCIENTIFIC REPORT OF EFSA

Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables[1]

European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 10 August 2011, replaces the earlier version published on 9 June 2011[*]

KEY WORDS

VTEC, STEC, *E. coli*, produce, vegetables, O104:H4.

---

[1] On request from the European Commission, Question No EFSA-Q-2011-00774, issued on 8 June 2011.

[2] Correspondence: BIOHAZ@efsa.europa.eu

[3] EFSA wishes to thank EFSA Staff: M.T. Da Silva Felicio, L. Vivas-Alegre, E. Liébana and M. Hugas from the Biological Hazards (BIOHAZ) Unit for the preparatory work on this scientific output, and also wishes to thank the staff of the Biological Monitoring (BIOMO), Emerging Risks (EMRISK) and Dietary and Chemical Monitoring (DCM) Units for the data support provided to this scientific output. In addition, EFSA wishes to thank the BIOHAZ Panel Members C. Nguyen-The and J. McLauchlin, and the Head of the *E. coli* Reference Laboratory, A. Caprioli, for review of the document.

[*] Editorial amendment on page 7 of the reporting year in "Several differences are found to date between the 2011 outbreak strain and the previously reported O104:H4 strain in 2001 in Germany …" and on page 17 of the following citations of references: Franz et al., 2007 and Franz et al., 2005.

Suggested citation: European Food Safety Authority; Urgent advice on the public health risk of Shiga-toxin producing *Escherichia coli* in fresh vegetables. EFSA Journal 2011; 9(6):2274. [50 pp.] doi:10.2903/j.efsa.2011.2274. Available online: www.efsa.europa.eu/efsajournal

SUMMARY

The scope of this Scientific Report of EFSA is to provide a fast-track assessment of the exposure of the consumer to STEC through consumption of raw vegetables, and to suggest possible mitigation options.

The German outbreak strain seems to share virulence characteristics of STEC and EAEC strains. STEC strains usually have an animal reservoir, while EAEC have a human reservoir. Infections in humans caused by similar strains (same serotype, same phylogroup, same MLST type, and with similar virulence gene array) have been reported in the past, and as such, the strain could not be regarded as "new". However this outbreak strain is rare, and until now it has never been found to be responsible for the rate of infection and severity of disease seen during the current outbreak. Sequence analysis and comparative genomics will be able to show if the outbreak strain is an EAEC that acquired EHEC virulence determinants, or *vice versa*. The antimicrobial resistance genotype of the outbreak strain, and the molecular typing of the *bla*$_{\text{CTX-M-15}}$-containing plasmid, could provide some clues on the epidemiology of this pathogen.

Regarding the **Exposure Assessment**, many different types of foods have been identified as a potential source of STEC. These are usually raw or undercooked foodstuffs contaminated with faeces from ruminants, either during primary production (e.g. slaughtering, milking, fertilised vegetables) or further processing and handling. Data on STEC are reported annually on a mandatory basis by EU Member States to the European Commission and EFSA. When interpreting this data it is important to note that they are not directly comparable due to differences in sampling strategies and applied analytical methods. In the scientific literature, outbreaks of STEC infection are becoming increasingly recognised as associated with vegetables, particularly contaminated sprouting seeds and green leafy salad vegetables. Outbreaks may have more than one exposure route involved. For example, primary human infection may originate from consumption of contaminated food or direct contact with an animal carrying STEC, while secondary infection may occur by the faecal-oral route, after contamination of food through handling by an infected person shedding the bacteria. As a result, especially during the late stages of an outbreak multiple exposure routes are likely. Contamination of fresh produce with STEC is rare but has been linked to some severe outbreaks. In some outbreaks, the origin of contamination was suspected to be contaminated irrigation water and access of farm animals to the immediate environment of fresh-produce. In most outbreaks however, the origin of contamination was not elucidated. Contamination of vegetables with STEC can occur in different steps of the food chain: during primary production; during harvest and post-harvest including handling and processing, at marketing and retail and during catering and in the care of the consumer after sale during transport and in domestic settings. Bacterial contamination of vegetables occurs mostly on the surface of the tissues of the plants but it may also be internal. Although there is no conclusive data, in theory internalisation of STEC would result in increased survival both in pre-harvest and post-harvest due to protection from exposure to UV and desiccation, as well as increased protection to surface decontamination treatments. Pre-harvest contamination can derive from infected farm animals. The possible routes of contamination are irrigation water contaminated with animal waste as well as sewage, application of organic fertilizers of animal and/or human origin and direct contact of animals with fresh produce growing fields. There is considerable debate on the possibility of STEC being present internally in leafy vegetables, particularly if exposed during the pre-harvest phase. It should be noted that internalisation of *E. coli* in plants has been only shown in experimental laboratory conditions specially in the case of root inoculations with very young plants and using high inoculation doses. Processing of vegetables involves many points of contact with people, surfaces, water and the environment (soil, dust) and this represents potential opportunities for contamination with food-borne pathogens. Minimally processed vegetables and sliced fruit exhibit a characteristic high humidity. This fact, together with the high number of cut surfaces, can provide ideal conditions for microbial growth, including that of food-borne pathogens and spoilage micro-organisms. Cutting practices increased the chance of bacterial cross-contamination and can result in increased

susceptibility of bacterial attachment. There is scarce information on the prevalence and quantity of STEC in vegetables both from surveillance and outbreak investigations. It is currently not possible to estimate the relative exposure to humans from pre-harvest or post-harvest contamination of vegetables by STEC. At the same time, scarcity of data also hampers the estimation of the relative significance of surface or internal contamination of vegetables by STEC for human exposure.

Regarding **Mitigation Options**, the use of Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs), and Hazard Analysis and Critical Control Point (HACCP) in the fresh fruit and vegetable industry provide the basic framework for safe products for the consumer. GAPs describe preventive measures implemented in farming operations to reduce product contamination and provide guidance for food-safety practices in the field. Implementing HACCP programs in processing and packaging facilities is a requisite for food safety. Since there is evidence of asymptomatic carriers of STEC in humans, screening of humans involved in food handling is relevant. The monitoring and/or exclusion of STEC carriers from food handling could be considered as a mitigation option. *In reference to Pre-harvest mitigation options*: the application of mitigation strategies reflected in GAPs in line with codes available from international organisations is recommended. In particular, to avoid access of farm animals (in particular ruminants) to the immediate environment of fresh produce; to use of irrigation and of agricultural water which are of adequate microbiological quality; and to control the sourcing, handling and treatment of manure and slurry that are to be used for fertilising fields intended to grow produce for human consumption. *In reference to Post-harvest mitigation options*: current technologies or practices do not effectively eliminate any microbiological hazard acquired during post-harvest processing of fresh vegetables. Therefore the main focus should be on prevention of contamination both during pre-harvest and post-harvest. The only effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating (e.g. cooking or pasteurization) or irradiation. Adhesion of pathogens to surfaces and internalisation of pathogens limits the usefulness of conventional processing and chemical sanitizing methods in preventing transmission from contaminated produce. The application of mitigation strategies reflected in GMPs and GHPs in line with codes available from international organisations is recommended. In particular, the use of water of adequate microbiological quality during further processing; to ensure basic training on food hygiene practices to food handlers; to ensure adequate design and hygiene management of food premises including pest control plans and the correct management of cold chain seems of particular importance for those products processed for ready-to-eat consumption (e.g. cut vegetables, unpasteurised vegetable juices). *In reference to catering and home*: GHPs when preparing food e.g. wash hands before and after preparing foods, wash all fruit and vegetables with potable running water, avoid cross-contamination, keep storage temperatures low for food. Peeling or cooking fruit and vegetables can also remove microbes. Although these measures have been proven to be useful they cannot completely eliminate the risk.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

An increase in the number of human cases of Haemolytic Uremic Syndrome (HUS) was reported by Germany to the European Centre for Disease Prevention and Control (ECDC) on 22 May 2011. Since then, the Commission has been following closely this outbreak caused by Shiga-toxin producing *Escherichia coli*- bacteria (STEC), serotype O104:H4. The STEC outbreak is already responsible for several fatalities in Germany. Other Member States (UK, Sweden, Denmark, the Netherlands and France) have reported human cases as well. All these were associated with travel in Germany.

The epidemiological investigations carried out by German authorities seem to link the current outbreak with raw vegetables. The strain identified in this outbreak had previously not been associated so far with significant health problems in the EU and seems to have a different epidemiology than STEC causing human disease so far in the EU.

Taking into account the characteristics of this new strain STEC O104:H4, questions could be raised on the potential initial source contamination of the food chain and possible resulting food safety risks.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

Therefore, in accordance with Article 31 of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority (EFSA) to provide scientific assistance and urgent advice on the following questions related to the current outbreak caused by STEC:

1. What is the relative exposure of humans by:
    - surface contamination of vegetables;
    - internal contamination of vegetables;
    - routine handling steps such as processing, packaging and distribution in the possible contamination of vegetables;

2. Given the identified risk, to recommend risk mitigation options.

Clarification of the Terms of Reference

The following was further clarified with the European Commission:

- The scope covers all pathogenic STEC strains;
- "Relative exposure" refers to the comparison between exposure to vegetables contaminated in the pre-harvest stage vs. vegetables contaminated in the post-harvest stage as well as differentiation between externally- and internally- contaminated should be made.

ASSESSMENT

1. Introduction

On 21st May 2011, Germany reported an ongoing outbreak of Shiga-toxin producing *Escherichia coli*-bacteria (STEC[4]), serotype O104:H4. Epidemiological information on the outbreak is maintained and periodically updated by the European Centre for Disease Prevention and Control (link: http://ecdc.europa.eu/en/healthtopics/escherichia_coli/epidemiological_data/Pages/Epidemiological_updates.aspx), and the World Health Organisation (link: http://www.euro.who.int/en/what-we-do/health-topics/emergencies/international-health-regulations/ehec-outbreak-in-germany). In the past STEC O104:H4 had been isolated in humans twice in Germany in 2001 (Mellmann et al., 2008) and once in Korea in 2005 (Bae et al., 2006). In addition, according to the information reported to ECDC, a total of 10 persons were infected with STEC O104 in the EU Member States from 2004 to 2009.

Currently available epidemiological information on this STEC outbreak in Germany suggests that STEC-contaminated food is the vehicle of infection. A case control study carried out in Hamburg identified consumption of contaminated raw tomatoes, cucumbers and /or leafy salad as significant risk factors (Frank et al., 2011). However, at this stage the specific food vehicle(s) have not been identified.

The possible involvement of fresh produce as the source of this outbreak led the European Commission to address an urgent request to EFSA in order to provide scientific assistance and urgent advice on human exposure via vegetables and mitigation options.

The scope of this Scientific Report of EFSA is to provide a fast-track assessment of the exposure of the consumer to STEC through consumption of raw vegetables, and to suggest possible mitigation options.

2. Hazard identification and characterisation

2.1. Description of the German outbreak strain

The German outbreak strain is a Shiga toxin producer *Escherichia coli* (STEC) that belongs to serotype **O104:H4**, and has been microbiologically characterized (for details see the link http://www.rki.de/cln_178/nn_217400/EN/Home/EHEC__O104__H4,templateId=raw,property=publicationFile.pdf/EHEC_O104_H4.pdf) as follows:

- Shigatoxin 1 negative
- Shigatoxin 2 (*stx*2a) positive
- Intimin (*eae* gene) negative
- Enterohemolysin negative
- EAEC (enteroaggregative *E. coli*) virulence plasmid:
  - *aat*A positive (ABC-transporter protein gene)
  - *agg*R positive (master regulator gene of Vir-plasmid genes)

---

[4] Also known as Verocytotoxin-producing *Escherichia coli* (VTEC)

    - *aap* positive (secreted protein dispersin gene)
    - *agg* positive (AAF/I-fimbral subunit-gene)
    - *agg*C positive (AAF/I-fimbral operon-gene)
- MLST Sequence Type: ST678 (*adk* 6, *fum*C6, *gyr*B 5, *icd* 136, *mdh* 9, *pur*A 7, *rec*A 7).
- Antimicrobial resistance profile: Resistant to ampicillin, amoxicillin/clavulanic acid, piperacillin/sulbactam, piperacillin/tazobactam, cefuroxime, cefuroxime-axetil, cefoxitin, cefotaxime, cetfazidime, cefpodoxime, streptomycin, nalidixic acid, tetracyclin, trimethoprim/sulfamethoxazol.
- The strain carries plasmid-borne $bla_{\text{CTX-M-15}}$ and a $bla_{\text{TEM-1}}$ genes.

An *E. coli* O104:H4 with a MLST ST678 was previously observed about 10 years ago in Germany in a Haemolytic Uremic Syndrome (HUS) case (Mellmann et al., 2008). This strain belonged to clonal group B1. Like the current outbreak strain, this isolate was *stx*2-positive and *eae*-negative. Several differences are found to date between the 2011 outbreak strain and the previously reported O104:H4 strain in 2001 in Germany (HUSEC 041, further details available at: http://www.ehec.org/index.php?hid=43&lang=de&pid=HUSEC), namely:

- Fimbriae expressed by HUSEC 041 are of type AAF/III[5].
- The macrorestriction-PFGE-pattern (XbaI) of the current EHEC O104:H4 is different compared to HUSEC041 (Robert Koch Institute, Personal Communication).

In conclusion, the STEC O104:H4 outbreak strain shows an unusual combination of virulence factors of STEC and EAEC which has only been reported sporadically in humans before (Morabito et al., 1998).

2.2. *E. coli* characteristics

E. coli is a normal inhabitant of the intestines of most animals, including humans. Some *E. coli* strains can cause a wide variety of intestinal and extra-intestinal diseases, such as diarrhoea, urinary tract infections, septicaemia, and neonatal meningitis.

E. coli is a bacterium, which very easily and frequently exchanges genetic information through horizontal gene transfer (e.g. by conjugation, transformation or transduction) with other related bacteria, such as other *E. coli* strains, *Salmonella, Shigella*. Therefore, *E. coli* strains may exhibit characteristics that have been acquired from a wide variety of sources.

A recent review describes the population structure of commensal *E. coli*, the factors involved in the spread of different strains, how the bacteria can adapt to different niches, and how a commensal lifestyle can evolve into a pathogenic one (Tenaillon et al., 2010).

All humans and animals carry *E. coli* in their intestines as they are part of the normal gut flora and usually harmless. However, there are several types of *E. coli* strains that may cause gastrointestinal illness in humans. These strain types can be divided into several pathogroups: Enteropathogenic

---

[5] Poster by Prager, Fruth and Tschäpe presented at the EHEC Workshop 2007, Bayern, Germany

(EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enterohaemorrhagic (EHEC[6]), and Enteroaggregative (EAEC).

2.3. Enterohaemorrhagic *E. coli* (EHEC)

Some *E. coli* strains are capable of producing toxins, which are very similar to toxins that are produced by *Shigella dysenteriae*. Two types of toxins have been described: Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). These strains are referred to as STEC (Shiga toxin-producing) or also VTEC (verocytotoxin-producing) *E. coli*. STEC causes a spectrum of illnesses in humans from asymptomatic infection, to diarrhoea, severe bloody diarrhoea, hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). Infection can be food-borne as well as transmitted via other humans, direct or indirect contact with animals (particularly ruminants) or through contaminated water. EFSA's BIOHAZ Panel issued a Scientific Opinion addressing the monitoring of STEC and the identification of human STEC types (EFSA, 2007).

Humans can act as a reservoir for infection by asymptomatically carrying the bacteria in the intestinal tract (Silvestro et al., 2004; Stephan et al., 2000; Vincent et al., 2010). Nevertheless, ruminants are recognised as the main natural reservoir for STEC: cattle being the animal source which contributes with most of the STEC that are virulent to humans (Caprioli et al., 2005). Both domestic and wild animals are rarely diseased by the bacteria, and thus they act as asymptomatic carriers. It can be concluded that STEC strains have most commonly an animal reservoir.

Pigs have been found as carriers of STEC, but less frequently (Bonardi et al., 2003; Borie et al., 1997; Heuvelink et al., 1999; Johnsen et al., 2001). Prevalence of STEC in European-reared poultry seems to be also low compared to ruminants (Dipineto et al., 2006; Schouten et al., 2005). Some studies in Europe and elsewhere have reported findings in other animal species, including turkeys, rodents, birds, insects and molluscs (Doane et al., 2007; Literak et al., 2009).

STEC strains are a good example of the evolution and emergence of pathogenic *E. coli*. Unknown before the late 1970s, these bacteria are a major cause of hemorrhagic colitis and haemolytic uremic syndrome, worldwide. The production of verocytotoxins is the main virulence feature of STEC but cannot be solely responsible for pathogenicity. STEC associated with severe human disease are usually capable of colonizing the intestinal mucosa with a characteristic attaching-and-effacing mechanism, genetically governed by the locus of enterocyte effacement (LEE), and possess other mobile genetic elements carrying additional virulence genes such as plasmids, phages, and pathogenicity islands (e.g., O-I 122). An overview of the STEC virulence factors, including their genetic basis is provided by Bolton (Bolton, 2010).

The major virulence factors of STEC strains are the Shiga toxins, products of the locus of enterocyte effacement, and products encoded by the EHEC-hemolysin plasmid. Molecular analysis shows that STEC acquired the majority of these virulence factors by horizontal transfer of genetic material. In the case of Shiga toxins, phages encoding them are probably responsible for this transfer. For the locus of enterocyte effacement, however, it is not clear how often this transfer took place and which parts of the locus were involved in this transfer. The large EHEC-hemolysin plasmid is clearly a mosaic structure, which arose from multiple recombination events with foreign DNA (Boerlin, 1999).

---

[6] EHEC are a subset of STEC consistently associated with severe human disease.

It is not possible at the present time to fully define human pathogenic STEC. However, the concept of seropathotype has evolved which classifies STEC into groups based on empirical knowledge about the typical clinical outcome of STEC infections combined with knowledge about serotype, *stx* subtypes and presence of additional virulence factors. STEC strains have been divided into 5 seropathotypes (Gyles, 2007; Karmali et al., 2003):

A, including the O157 strains that are common causes of outbreaks and HUS in most countries;

B, non-O157 strains that cause occasional outbreaks but are fairly common isolates from sporadic cases and HUS (examples: O26:H11, O103:H2, O111:NM, O121:H19, O145:NM);

C, non-O157 strains associated only with sporadic cases;

D, strains associated with diarrhoea, not more severe symptoms; and

E, strains not associated with human disease.

E. coli O157:H7 is the dominant STEC serotype in humans in many parts of the world. However, multiple reports have shown that other STEC, including serogroups O26, O111, O103, and O118, frequently cause sporadic cases in humans and have been implicated in numerous outbreaks. A detailed review has been produced by Kaspar and colleagues (Kaspar et al., 2009).

A comparison of *E. coli* O157:H7 genomes has revealed the extent and significant impact of horizontal transfer on the evolution of virulence (Hayashi et al., 2001; Perna et al., 2001). Furthermore, microarray comparisons have shown divergence in gene content among closely related O157 strains (Wick et al., 2005).

STEC are zoonotic pathogens with domestic and wild ruminants representing their main natural reservoir.

2.4. Enteroaggregative *E. coli* (EAEC)

Enteroaggregative *E. coli* (EAEC) are defined as *E. coli* that do not secrete heat-labile or heat-stabile enterotoxins and adhere to mucosal cells in an aggregative pattern (Nataro et al., 1998). EAEC have been implicated as a cause of persistent diarrhea in children and acquired immunodeficiency syndrome−associated diarrhea, as well as acute diarrhea in travelers. Not all EAEC strains have been shown to cause diarrhea in humans. The EAEC are a heterogeneous group of bacteria that display a wide array of virulence factors. The relative contribution to disease for each one of these virulence factors is unclear. Furthermore, the interaction between the host immune response and heterogeneity of virulence factors of EAEC is complex. Outbreaks of diarrhoeal illness due to EAEC have been reported and linked to the ingestion of food which was contaminated by food handlers. In addition, it has been shown that EAEC carriage by humans is possible (Huang et al., 2003; Huang et al., 2006).

Most enteroaggregative *E. coli* (EAEC) strains harbour a 60- to 65-MDa plasmid called pAA which has been shown to encode the aggregative adherence fimbriae AAF/I and AAF/II; the enterotoxin EAST1 and Pet, a serine protease which has been described as causing enterotoxic and cytotoxic effects. Another serine protease denominated Pic, encoded by a chromosomal gene displaying mucinolytic activity, serum resistance, and hemagglutination, has also been associated with EAEC strains (Moon et al., 2005).

EAEC have rarely been identified in animals, suggesting that they are not zoonotic, but exclusive to humans as a pathogen (Cassar et al., 2004).

2.5. Typing of *E. coli*

The purpose of microbial typing is to determine characteristics of isolates at the subspecies level in order to identify relatedness and/or to allow source tracking/attribution. For these purposes several methods are available. The principles on which these methods are based on, and their discriminatory power, are very different, which greatly influences the choice of method according to the intended use. Phenotypic tests (e.g. serotyping) are relatively easy to perform, but lack discriminatory power. Within serotypes, many different genetically-related clusters of isolates can occur that can be identified by genotyping methods.

Strains of *E. coli* are serotyped by an internationally-recognised and evolving scheme comprising over 180 O-types (lipopolysaccharide) and 56 H-types (flagella). Several of the most recently designated types include STEC strains. Full serotyping is generally performed in national reference laboratories although antisera for some common STEC O-groups are available commercially.

Many STEC strains that cause human illness show specific combinations of O- and H-types (e.g. O157:H7, O26:H11, O91:H21). Data collected by Enter-Net[7] indicate that the distribution of STEC serotypes between countries is not uniform but this may be strongly influenced by the detection methods used and criteria applied for testing. Molecular 'serotyping' methods attempt to avoid the dependency on antisera and make serotype characterisation more widely available. Methods have targeted various genes involved in the biosynthesis of the O antigen by identifying sequences unique to O groups such as O157, O26, O111, O113 and O145 for specific Polymerase Chain Reactions (PCRs). PCR-RFLP (PCR restriction fragment length polymorphism) and PCR combined with sequencing have also been used. Determination of H-type has been directed mainly at the *fli*C gene that is present even if the isolate is non-motile. The large number of O-types of *E. coli* means that in the short to medium term, DNA-based tests are unlikely to replace conventional serotyping in the reference laboratory setting for comprehensive characterisation of isolates.

Over 200 O:H serotypes producing STEC have been identified from all sources (Scheutz and Strockbine, 2005), although many lack the full complement of known virulence factors found in strains that cause serious disease; however over 100 have been associated with disease in humans.

The role of some putative virulence factors is still uncertain and they may be detected as markers of particular strains rather than contributing to the disease process. There are substantial gaps in knowledge about the interaction between STEC and their hosts; some STEC, including O157, may be carried asymptomatically by both adults and children. The definition of pathogenic strains has been based on phenotypic properties and the linkage of certain serotypes to serious illness.

Phylogenetic analysis has shown that *E. coli* is composed of four main phylogenetic groups (A, B1, B2, and D) and that virulent extra-intestinal strains mainly belong to groups B2 and D (Clermont et al., 2000). STEC strains normally belong to phylogenetic groups D, B1 and to a lesser extent A. The majority of STEC O157 belong to group D, while other STEC serotypes belong to groups B1 and A (Ziebell et al., 2008).

Characterisation of STEC with respect to the presence of a range of virulence properties may further identify markers that confer the capacity to cause serious infections and so identify strains with increased risk of causing disease.

Pulsed-filed-gel-electrophoresis (PFGE) is a band-based molecular technique with generally a high discriminatory power. It allows identification of lineages or clusters of epidemiologically related isolates within serotypes. It is intended for tracing outbreaks in a limited time period and less suitable for performing phylogenetic analyses. Multi-locus-sequence typing (MLST) is a sequence-based method targeting 5-7 highly conserved genes. This method has generally less discriminatory power

---

[7] http://www.hpa.org.uk/AboutTheHPA/WhatTheHealthProtectionAgencyDoes/InternationalWork/EnterNet/

than PFGE or MLVA but is the most reliable method to determine genetic relatedness of epidemiologically-unrelated isolates. As an example, *E. coli* are currently assigned by MLST to a certain sequence type (ST), and within those STs diverse clusters can be detected by PFGE.

2.6. Epidemiologically relevant features linked to antimicrobial resistance in the outbreak strain

As mentioned above, the outbreak strain is an ESBL (extended spectrum ß-lactamase) producer and it carries a $bla_{\text{CTX-M-15}}$ gene on a conjugative plasmid.

Since the early 2000s the CTX-M group of genes, named after their ability to produce enzymes capable of hydrolysing cefotaxime, emerged in human isolates. These genes were also located on highly transmissible plasmids, thus facilitating fast and efficient spread of resistance (Bonnet, 2004; Canton and Coque, 2006; Canton et al., 2008; Hunter et al., 2010; Livermore and Hawkey, 2005; Pitout et al., 2005a; Pitout and Laupland, 2008). Bacteria that express CTX-M enzymes are also commonly co-resistant or multiresistant, exhibiting resistance to multiple antimicrobials including quinolones (Jacoby et al., 2006).

In the last decade the epidemiology of ESBLs in humans has changed. Successful international bacterial clones harbouring members of the CTX-M family have emerged and spread globally. As a result the CTX-M-ß-lactamases have become the most prevalent ESBLs in human Enterobacteriaceae worldwide (Livermore et al., 2007; Pitout et al., 2005a; Pitout et al., 2005b). The epidemiology of bacteria that produce CTX-M enzymes has also changed. Since the early 2000s, *E. coli* producing CTX-M enzymes (specifically CTX-M-15) have increasingly been found in the community in uncomplicated and complicated (including bacteraemias) community-acquired urinary tract infections, as well as in serious intra-abdominal and skin and soft-tissue infections (Canton et al., 2008; Livermore et al., 2007; Peirano et al., 2010; Pitout and Laupland, 2008; Rodriguez-Bano et al., 2008; Rodriguez-Bano et al., 2004; Rodriguez-Bano et al., 2006; Woodford et al., 2004).

In the last few years some ESBLs relevant to human medicine have been described in isolates from animals. By far the most common genes associated with this resistance have been those encoding CTX-M enzymes (the most commonly identified ESBL). The ESBL enzymes associated with animals correspond to CTX-M (-1, -2, -9, -14, -32). Sporadic reports of CTX-M-15 in animals also exist.

The knowledge that some plasmid types are prevalent in resistant populations is useful for tracing their global spread among enterobacterial populations from humans, animals and the environment.

Highly transmissible IncFII plasmids or multireplicons of Inc FII associated with Inc FIA and FIB plasmids, and carrying $bla_{\text{CTX-M-15}}$ are of particular interest as they are globally spread among *E. coli* populations from humans and animals (Carattoli, 2009).

A recent study (Mshana et al., 2009) reported on the emergence of a cluster of CTX-M-15 producers in Germany exclusively associated with a conjugative 145.5 kb IncFI replicon-type plasmid in nosocomial settings. This was the first report of a CTX-M-15 occurrence in Germany associated with an exclusive IncFI replicon-type. The study furthermore illustrated that the prevalence of CTX-M-15 is not due to spread of a single clonal type but is associated with the spread of related *E. coli* isolates. Dissemination of the ESBL phenotype was linked to the lateral transfer of highly adapted IncF1 conjugative plasmid.

It is currently unknown what the replicon type and molecular fingerprint of the plasmid/plasmids present in the German outbreak strain are, but this information could give additional molecular markers to trace the outbreak strain.

2.7. Discussion on the possible origin of the strain based on the genetic elements and resistance pattern of the outbreak strain

- The German outbreak strain seems to share virulence characteristics of STEC and EAEC strains. STEC strains usually have an animal reservoir, while EAEC have a human reservoir.

- Both the current outbreak strain and the historical O104:H4 strain (HUSEC 041) belong to phylogenetic group B1. The most common STEC *E. coli* O157:H7, belongs to group D, and many extraintestinal pathogenic *E. coli* (ExPEC) are found to belong to groups B2 or D. Generally, groups B1 and A are more associated with non-O157 STEC and with commensal *E. coli*. Because of the common feature of horizontal gene transfer in *E. coli*, these are just trends, and general interpretations of results should be made with caution.

- Infections in humans caused by similar strains (same serotype, same phylogroup, same MLST type, and with similar virulence gene array) have been reported in the past, and as such the 2011 outbreak strain could not be regarded as "new". However the German outbreak strain is rare, and until now it has never been found to be responsible for the rate of infection and severity of disease seen during the current outbreak.

- The PFGE restriction profiles of the historical O104:H4 strain (HUSEC 041), and that of the 2011 outbreak strain are different. Without a better knowledge of the background genetic diversity that is present among serotype O104 strains, it is difficult to draw definitive conclusions on the significance of such differences.

- Sequence analysis and comparative genomics will be able to show if the German outbreak strain is an EAEC that acquired EHEC virulence determinants, or if it is the other way around.

- Traditionally, STEC strains do not present a high level of resistance to antimicrobials (especially in the case of O157:H7), although there are occasional reports of resistant strains, including ESBL-producers. The antimicrobial resistance genotype of the outbreak strain, and the molecular typing of the $bla_{\text{CTX-M-15}}$–containing plasmid, as well as of any other co-resident plasmids could provide some clues on the epidemiology of this pathogen.

3. Exposure Assessment

3.1. Human exposure routes

Figure 1 shows the four routes through which humans can become exposed to STEC and potentially infected. These have been reviewed by EFSA in a previous Scientific Opinion (EFSA, 2007).

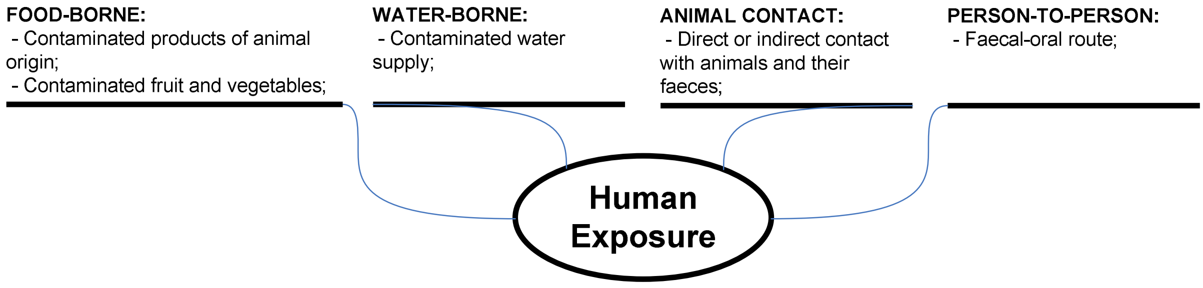

Figure 1: STEC human exposure routes.

Outbreaks may have more than one exposure route involved. For example, primary human infection may originate from consumption of contaminated food or direct contact with an animal carrying STEC, while secondary infection may occur by the faecal-oral route, after contamination of food through handling by an infected person shedding the bacteria. As a result, especially during the late stages of an outbreak multiple exposure routes are likely.

3.1.1. Food sources of STEC and EU monitoring data

Many different types of foods have been identified as a potential source of STEC (Caprioli et al., 2005). It usually involves raw or undercooked foodstuffs contaminated with faeces from ruminants, either during primary production (e.g. slaughtering, milking, fertilised vegetables) or further processing and handling (e.g. cross contamination during processing, human-to-food contamination via food handlers).

Data on STEC are reported annually on a mandatory basis by EU Member States to the European Commission and EFSA based on Zoonoses Directive 2003/99/EC[8]. Most Member States have provided data on their STEC investigations in the past years. When interpreting this data it is important to note that data from different investigations are not directly comparable due to differences in sampling strategies and applied analytical methods. In fact the most widely used analytical method only aims at detecting STEC O157, whereas fewer investigations have been conducted with analytical methods aiming at detecting all or selected serotypes of STEC.

3.1.1.1. Meat, milk, cattle and sheep

Most reported data on STEC are from animals (mainly ruminants) and meat and milk thereof, since these are considered to be main sources of human infections. These data are summarised in the Community and EU Summary Reports on zoonoses and food-borne outbreaks in 2004-2009 (EFSA, 2005, 2006; EFSA and ECDC, 2006, 2007, 2009, 2010, 2011).

During the years 2007-2009, overall 0.3% - 2.3 % of fresh bovine meat samples were found positive for STEC in the reporting Member States, and 0.1% - 0.7% of these samples were positive for STEC O157 (see Table 1). The proportion of positive samples varied widely between the Member States, from 0% to 14.9%. Some data were also reported on fresh sheep meat, where 0.7% - 3.2% of the samples were positive for STEC at EU level and no samples were positive for STEC O157. The proportion of positive sheep meat samples ranged between the Member States from 0% to 10.5%. In addition, STEC was also reported from some samples of raw cow's milk during the years.

In cattle during the years 2007-2009, STEC was reported in animal samples at levels of 2.2% - 6.8 % at EU level, and STEC O157 was found from 0.5% - 2.9 % of these samples. The prevalence of STEC in cattle varied between the Member States from 0% to 48.5%. In sheep overall 0.9% - 20% of the animal samples were found STEC positive in the reporting Member States and 0.3% - 1.6% of these samples STEC O157 positive in 2007-2009. The prevalence of STEC in sheep varied also widely between the Member States from 0% to 70.5%. However, the number of reporting Member States was low in case of sheep. The specimens taken from animals varied between the Member States and included faeces, ear, hide and fleece samples.

---

[8] 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.

Table 1: STEC in fresh bovine and sheep meat, cattle and sheep in EU, 2007-2009 (Community and EU Summary Reports)*

Animal/ food category	**No MSs**	**2007**			**2008**			**2009**		
		N	STEC	STEC O157	N	STEC	STEC O157	N	STEC	STEC O157
Fresh bovine meat	13-14	14,115	0.3%	0.1%	14,598	0.3%	0.1%	9,285	2.3%	0.7%
Fresh sheep meat	4-5	285	1.8%	0%	1,263	0.7%	0%	248	3.2%	0%
Cattle (animals)	9-11	5,154	3.6%	2.9%	5,368	2.2%	0.5%	5,555	6.8%	2.7%
Sheep (animals)	4	533	0.9%	0.4%	671	3.1%	1.6%	324	20%	0.3%

*Only investigations with > 25 samples included; N= number of samples ; No MSs= Number of Member States reporting data

3.1.1.2. Vegetables and fruits

During the years 2004-2009, 14 Member States tested for and reported data on STEC in fruits, vegetables and products thereof (Table 2). In total 5,910 such samples were examined and out of them only 11 were found positive for STEC (0.19%) and 8 of these samples were identified as STEC O157 (0.14%). Most of the positive findings were from vegetables, where 0.50% of the samples tested positive for STEC. In 2008, the STEC positive samples were found from the following investigations carried out by the Member States: five STEC O157 positive samples were reported out of 947 samples of vegetables tested; one STEC non-O157 positive sample was detected from pre-cut ready-to-eat fruits and vegetables from catering and two STEC positive samples were found out of 23 vegetable samples investigated. In 2009, the positive samples were detected from an investigation covering 57 vegetable samples where three STEC O157 positive samples were observed.

Table 2: STEC findings in fruits and vegetables and products thereof as reported by the Member States in accordance with Directive 2003/99/EC in 2004-2009.

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

[a]STEC non-O157

3.1.2. Role of vegetables in the food-borne route

Raw vegetables are an important component of the human diet. Details on production and trade data are presented in Appendix A[9], while consumption data are presented in Appendix B for the up to now identified suspect food vehicles (sprouts, tomatoes, lettuce and cucumber) in the German STEC outbreak.

In the scientific literature, outbreaks of STEC infection are becoming increasingly recognised as associated with vegetable products, particularly contaminated sprouting seeds (Breuer et al., 2001; Ferguson et al., 2005; Michino et al., 1999; Mohle-Boetani et al., 2001), and green leafy salad

---

[9] EUROSTAT data on sprouts were not readily available.

vegetables (Ackers et al., 1998; CDC, 2006; Friesema et al., 2008; Grant et al., 2008; Hilborn et al., 1999; Söderström et al., 2005; Wendel et al., 2009).

The Standing Committee on Veterinary Measures Relating to Public Health issued an Opinion on verotoxigenic *E. coli*, and identified among others fresh produce, in particular sproutes and unpasteurised fruit and vegetable juices as of particular public health concern (SCVPH, 2003). These food categories have also been recently reported in the EFSA STEC-related Scientific Opinion as an important mode of transmission within the food-borne route (EFSA, 2007).

Contamination of vegetables can occur in different steps of the food chain:

1. during primary production including harvest;
2. during post-harvest including handling, transport and processing;
3. at marketing and retail;
4. during catering and in the care of the consumer after sale during transport and in domestic settings.

Taking *E. coli* O157:H7 as an example of STEC, different routes have been identified as possible sources of contamination for fresh produce (Beuchat and Ryu, 1997; FAO and WHO, 2008). These are summarised in Figure 2.

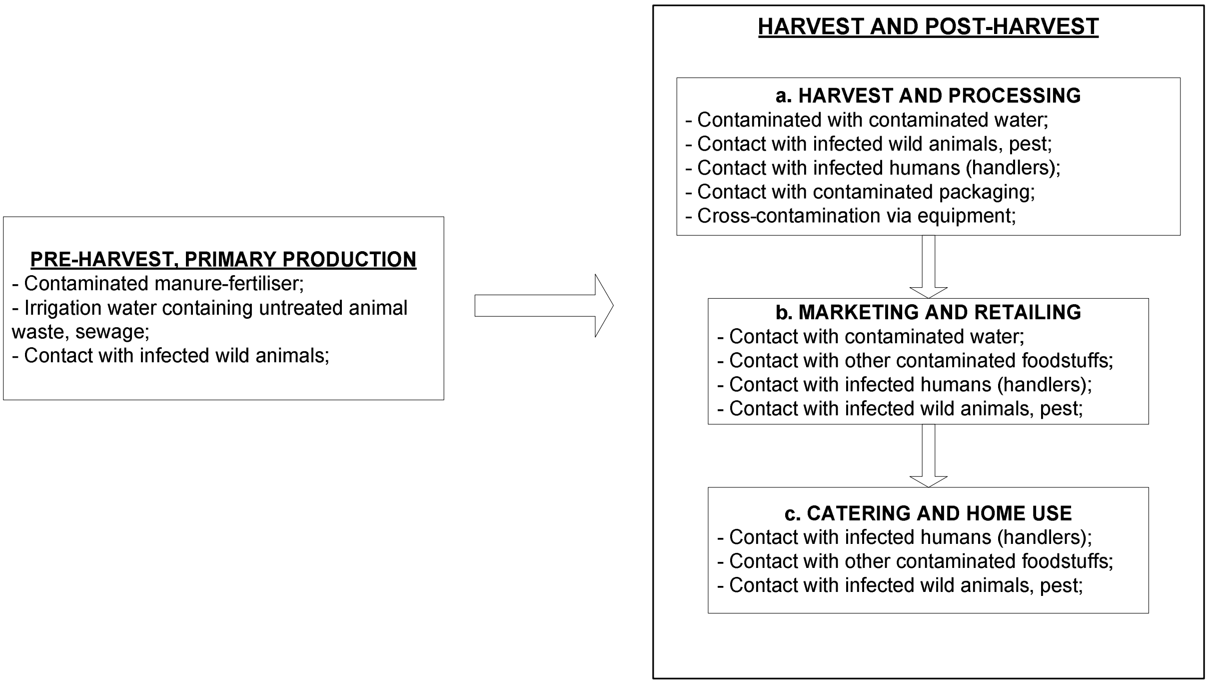

Figure 2: Potential routes involved in the contamination of vegetables at the different steps of the food chain.

3.1.2.1. Pre-harvest contamination

Possible routes of contamination

Pre-harvest contamination can derive from infected farm animals. The possible routes of contamination are: irrigation water contaminated with animal waste as well as sewage, application of organic fertilizers of animal and/or human origin and direct contact of animals (wild or domestic) with fresh produce growing fields. Flooding, if contaminated cattle farms are close to produce fields can also be a potential contamination route. A number of outbreaks have been traced to the use of contaminated water in irrigation. Cases of STEC O157:H7 in Sweden in 2005 were traced back to lettuce irrigated with water from a stream contaminated with cattle faeces (Söderström et al., 2005). An investigation on environmental factors related to irrigation water that may have contributed to the contamination of spinach in the 2006 USA multistate STEC O157:H7 outbreak suggested that depths to groundwater and groundwater-surface water interactions may pose risks to ready-to-eat crops (Gelting et al., 2011). A study of an outbreak of STEC O157:H7 associated with lettuce consumption in Montana, suggested that the most likely sources of contamination were the manure used as a fertilizer, runoff from fields containing cattle faeces, and contaminated irrigation water. These suggestions were not confirmed, however, as the investigators found no evidence of STEC O157:H7 in samples of lettuce, water, manure, or cattle faeces 2 to 3 weeks after the outbreak (Tyrrel et al., 2006).

The route of spinach contamination for the 2006 *E. coli* O157:H7 outbreak linked to baby spinach in USA was considered to be through the transfer of *E. coli* O157:H7 from a cattle ranch near the field via infected wild pigs that found access to the crop through a broken fence. Yet, it is noteworthy that a survey found a high prevalence of *E. coli* O157:H7 within the area of Salinas valley, suggesting the actual route could have been via contaminated irrigation water (Warriner et al., 2009). These findings underline the complexity of the pre-harvest environment and the ease with which plant tissue can become contaminated with food-borne pathogens.

Contamination of vegetables

Bacterial contamination of vegetables occurs mostly on the surface of the tissues of the plants but it may also be internal.

Three basic modes of bacterial interaction with growing plants could be considered: (a) attachment of bacteria to the surface of plants (epiphytes), (b) access of bacteria to the plants through natural openings (stomata) or damaged tissue, and (c) internalisation via root colonization (Warriner et al., 2003).

a) **Attachment of bacteria to the surface of plants.**

Three leaf attachment mechanisms have been described in STEC O157 (Berger et al., 2010). First, STEC O157:H7 adhere strongly to tomato skin, spinach leaves and roots of alfalfa sprouts. Adhesion to these surfaces is mediated by curli (Jeter and Matthysse, 2005). Second, it has been shown that adhesion of STEC O157, as well as the related enteropathogenic *E. coli* (EPEC), to a variety of salad leaves is mediated by the filamentous type III secretion system (T3SS), which is composed of EspA filaments (Knutton, 1995; Shaw et al., 2008). Finally, flagella also play a role in STEC O157 leaf attachment as deletion of fliC encoding flagellin reduced the level of adhesion (Xicohtencatl-Cortes et al., 2009).

b) Internalisation via the leaves.

The study of the internalisation of microorganisms (i.e. bacteria and viruses) in vegetables is not a novel research area as such but rather a specialisation of phytology research (Ryall and Pentzer, 1982). Evidence of experimental penetration of STEC 0157:H7 in growing vegetables, has been extensively reported in literature (Cooley et al., 2003; Itoh et al., 1998; Solomon et al., 2002a; Solomon et al., 2002b; Takeuchi and Frank, 2000)

It has been conclusively proved that human pathogens can enter both stomata and cut edges of fresh produce (Warriner et al., 2009). Like *Salmonella*, STEC O157 can also reach the sub-stomatal cavity and the spongy mesophyll and survive in this environment (Franz et al., 2007; Itoh et al., 1998; Jablasone et al., 2005; Solomon et al., 2002b; Wachtel et al., 2002; Warriner et al., 2003; Xicohtencatl-Cortes et al., 2009). Thus, under appropriate conditions, enteric pathogens, such as STEC O157:H7 will grow on and invade the plant tissue, following contamination of fresh produce.

Plant injury can influence colonization of lettuce by *E. coli* in the field. Mechanically damaged lettuce increased the persistence of generic *E. coli* as well as STEC O157:H7 in the field, and although *E. coli* populations decreased on lettuce in the field, harvested lettuce that was mechanically damaged did support growth of STEC O157:H7. Soft rot also supports the growth of STEC O157:H7. Access to plant nutrients and exudates positively affects the survival and growth of STEC O157:H7 on produce (Critzer et al., 2010).

c) Internalisation via roots.

The possibility of pathogens such as STEC O157 to become internalised into the vascular system of growing plants has received significant attention. The transmission of STEC O157:H7 from manure-contaminated soil and irrigation water to lettuce plants was demonstrated using laser scanning confocal microscopy, epifluorescence microscopy, and recovery of viable cells from the inner tissues of plants.

Root inoculation leads to contamination of the entire plant, indicating that the pathogens are capable of moving on or within the plant in the absence of competition. STEC O157:H7 in contaminated water can enter the vascular system of lettuce and reach the edible parts of the plant (Erickson et al., 2007b; Solomon et al., 2002b). Inoculation with green fluorescent protein-labeled STEC O157:H7 showed invasion of the roots at lateral root junctions in *Arabidopsis thaliana*, a small flowering plant largely used as a model organism in plant biology. Survival of STEC O157:H7 on this soil-grown plant declined as the plants matured, but the pathogen was detectable for at least 21 days (Cooley et al., 2003). Another study indicated that internalisation of STEC O157 via leafy plant roots in the field is rare and when it does occur, STEC O157 does not persist 7 days later (Erickson et al., 2007a).

Even though internalisation of STEC has been reported mainly in leafy plants, an internalisation of *E. coli* O157 in tomatoes was described by Ibarra-Sanchez and colleagues (Ibarra-Sanchez et al., 2004). The presence of viable enterohaemorrhagic *E. coli* O157:H7 was demonstrated not only on the outer surfaces but also in the inner tissues and stomata of cotyledons of radish sprouts grown from seeds experimentally contaminated with the bacterium (Itoh et al., 1998).

Internalisation of *E. coli* O157:H7 and *Salmonella* into the leaf tissue was experimentally demonstrated in seedlings grown in soil amended with inoculated non-composted fresh manure (Franz et al., 2007; Solomon et al., 2002b). *E. coli* O157:H7 was also isolated from young lettuces after experimental exposure to low numbers of this bacteria (Mootian et al., 2009). However, the internal presence of pathogens was never demonstrated in mature leafy vegetables (Jablasone et al., 2005). In fact, other studies reported STEC O157:H7 internalised in cress, lettuce, radish and spinach seedlings but it was not recovered within the tissues of mature plants (Jablasone et al., 2005; Warriner et al., 2003). With a laboratory simulated lettuce production chain, where *E. coli* O157:H7 was allowed to decline to levels of 10^2 cfu/g in manure-amended soil, this pathogen could not be detected on lettuce plants grown in the used substrate (Franz et al., 2005). Similarly, the transmission of *E. coli* O157:H7 from natural and experimentally inoculated manure-amended soil to lettuce could not be demonstrated (Johannessen et al., 2005; Johannessen et al., 2004).

Survival of STEC on vegetables

Despite the varied environmental conditions, some works reported survival for several weeks on plants of STEC (and other enteric pathogens). Survival of STEC O157 has been demonstrated for 177 days when sprayed directly onto parsley (Islam et al., 2004), and for 30 days on lettuce (Solomon et al., 2003). However, these studies typically were conducted with relatively high inoculum densities, which may not be consistent with what would presumably occur in a natural soil contamination scenario. In contrast to the above works, survival for only a few days after irrigation of spinach with water containing a high number of STEC O157 was reported (Ingram et al., 2011). In case of low contamination, STEC O157 was no longer detected 24 h after irrigation (Ingram et al., 2011).

Both pathogen-related and host-related factors leading to the internalisation of the bacteria into plant tissues are still in need of further research, including the likelihood of occurrence under natural conditions (Johannessen et al., 2005) and the role of the plant in human pathogen-plant interactions is currently being noticed as a research need in the US (Barak and Ivey, 2011).

There is considerable debate on the possibility of pathogens being present internally in leafy vegetables. It should be noted that internalisation of *E. coli* in plants has been only shown in experimental laboratory conditions, and in the case of root inoculations with very young plants and using high inoculation doses.

3.1.2.2. Harvest and post-harvest contamination

As described in Figure 2, after the growing and harvesting step there are many different opportunities for STEC to contaminate fresh produce both at pre- and post-harvest, such as the packing, fresh cut processing, storage, distribution to retail, marketing, consumer handling or catering facilities.

Harvest, handling and processing

Leafy vegetables and herbs may be harvested by hand or harvested mechanically. The harvested leaves or plants may be hand sorted, they may undergo some minor processing such as removal of outer wrap leaves or coring, washing or spraying, and they may be placed in bulk containers or packed ready for dispatch in boxes or covered with plastic film. These processes involve many points of contact with people, surfaces, water and the environment (e.g. soil, dust) and represent potential opportunities for contamination with food-borne pathogens. Packing may take place in the open field or in a designated packing house. The location of the packing station or packing house (which may be no more than a shed with a roof and open or limited walls) in or very near the growing fields may result in exposure to dust and wildlife carrying food safety hazards. Other operations in the packing house include sorting, trimming, washing and drying, grading, packing, pre-cooling and storage. The sources of microbiological contamination in the packing house include the raw material, personnel, handling tools and equipment, packing house physical environment and water or ice.

E. coli has been found on transport bins, conveyor belts and cooler surfaces used for processing cabbages in packing houses (Prazak et al., 2002) and in another study the contamination with *E. coli* on cabbages increased from a mean count of 0.7 $\log_{10}$ cfu/g to 0.86 cfu/g as they moved from the conveyor belt to the final box (Johnston et al., 2006).

Water is used widely in packing houses for cleaning (produce, equipment and surfaces), transport, cooling and packing. As leafy vegetables and herbs may be ready-to-eat at this stage or further processing may not remove contamination, the use of potable water is required.

During processing, leafy vegetables and herbs may be exposed to microbial contamination and microorganisms may persist and grow. Of particular concern during processing is the contact between the leafy vegetables and herbs and the multiple surfaces in the factory environment; the microbiological status of water; and the potential for tissue injury during primary preparation. Some processes have the potential to reduce microbial risks (e.g. disinfection), control microbial growth (e.g. chilling) and protect the product from further exposure (e.g. packaging).

Contamination of leafy vegetables during processing after harvesting, including an increase in *E. coli* numbers has been reported (Johnston et al., 2006). Cooling crops to 4ºC or less will slow or prevent the growth of pathogens including *E. coli* O157:H7. Leafy greens are generally cooled under forced air, vacuum cooling may be used, but passive storage under refrigeration is still a widely used method.

Internalisation has been experimentally reported in the literature through practices such as vacuum cooling or modified atmosphere packaging. These can experimentally promote the internalisation of *E. coli* O157:H7 into lettuce, probably favoured by low temperatures (Li et al., 2008; Takeuchi et al., 2001). It has also been shown that *E. coli* O157:H7 can grow on iceberg lettuce held at refrigeration temperatures (Koseki and Isobe, 2005).

Cutting practices increase the chance of bacterial cross-contamination and can result in increased susceptibility of bacterial attachment (Garrood et al., 2004). *E. coli* O157:H7 populations have been shown to increase 4, 4.5 and 11-fold within four hours on romaine lettuce that received mechanical, physiological, and disease-induced lesions respectively whereas a two-fold increase occurred on leaves that were left intact (Brandl and Amundson, 2008). In this study the influence of leaf age was also investigated; the *E. coli* O157:H7 population on young leaves was 27-fold greater than on middle-aged leaves. These findings highlight the potential for low numbers of this pathogen to rapidly increase to levels that present a significant human health concern.

Marketing and retail

Handling practices in distribution facilities as well as during transport could pose an additional risk for introduction of food-borne pathogens in fresh produce (FAO and WHO, 2008). Cross contamination opportunities may also exist during marketing and retail.

Catering and home

Cross-contamination during transport from the retailer to catering establishments and for domestic use can occur. The food-handler or even the consumer can potentially contaminate or cross-contaminate vegetable products at any stage after sale. Contamination may also occur via faecal contact from an asymptomatic infected food handler. Cross contamination may also occur between raw animal products and ready-to-eat vegetables during food preparation for example via food contact surfaces or hands.

3.1.3. Evidence for source-attribution of STEC in the EU

Food-borne infections (including STEC and other zoonotic agents) associated with salad vegetables have been reported in recent years, and were attributed in part to higher consumption rates and to the increases in ready-to-eat vegetable food types (Sivapalasingam et al., 2004; Warriner et al., 2003). Increased processing and handling of produce may increase cross-contamination. Cutting/shredding operations will release plant nutrients which, combined with the high humidity maintained by the packaging, is likely to both increase survival and allow growth of bacteria. Ready-to-eat fresh vegetable and fruits demand has continuously increased over the last decades reflecting the consumer's preference for fresh and healthy foods with minimal preparation. Minimally processed vegetables and some sliced fruit belong to the low-acid foods, and exhibit a characteristic high humidity. These facts, together with the high number of cut surfaces, can provide ideal conditions for microbial growth, including that of food-borne pathogens and spoilage micro-organisms.

A total of 211 food-borne outbreaks and 13 (drinking) water-borne outbreaks caused by pathogenic *E.coli* including STEC was reported by the EU Member States in 2007-2009 (see Table 3). Out of these, detailed data was available from 57 outbreaks and the food vehicle was identified in 40 outbreaks. The implicated food vehicle was meat in 16 outbreaks, dairy product in 9 outbreaks and other food in 15 outbreaks. None of the outbreaks were reported to be caused by fruits or vegetables.

For the years 2004-2006, when the reporting of food-borne outbreaks was not yet harmonised in EU, the data is less comparable and therefore more difficult to interpret. However, for these years, together 195 food-borne outbreaks caused by *E. coli* were reported by the Member States (see Table 3). In three of these outbreaks vegetables and salads were reported as the food vehicle (two outbreaks in Sweden in 2005 and 2006 and one in Portugal in 2006). The Swedish outbreak in 2005 affected 135 persons, the source was lettuce and the location of exposure both restaurants and private households. The other Swedish outbreak in 2006 included 10 persons, was caused by vegetables and took place in a kindergarten[10]. The outbreak in Portugal affected 10 persons and it occurred at an institutional canteen[11]. In addition, one outbreak in Denmark in 2006 was associated with herbs and spices (pesto sauce and imported basil was implicated) and covered 250 human cases and took place in a school.

These data on outbreaks are summarised in the Community and EU Summary Report on zoonoses and food-borne outbreaks in 2004-2009 (EFSA, 2005, 2006, 2007, 2009; EFSA and ECDC, 2010, 2011).

Table 3: Reported food and water-borne *E. coli* outbreaks (EFSA, 2005, 2006, 2009; EFSA and ECDC, 2006, 2007, 2009, 2010, 2011) in accordance with Directive 2003/99/EC, 2005-2009

EHEC/STEC outbreaks	2009	2008	2007	2004-2006
Food-borne outbreaks	75	75	61	195
Water-borne outbreaks	5	4	4	5
Human cases in food-borne outbreaks	595	339	479[a]	2,345[b]
Human cases in water-borne outbreaks	12	22	62	26

[a] Includes only verified outbreaks; [b] Information from some outbreaks missing.

EFSA's BIOHAZ Panel recommended in 2007 that monitoring should concentrate on STEC O157 since this serotype is most frequently associated with severe human infections (including HUS) in the EU. Monitoring should then be extended to other serotypes (e.g. those of O26, O103, O91, O145 and O111) that are identified by periodical analysis of European human disease and epidemiological data as most frequently causing for human infections (EFSA, 2007).

Improved surveillance programmes have resulted in food-borne outbreaks being more readily identified, with fresh produce now increasingly likely to be considered a vehicle of infection and therefore included in any investigation (Heaton and Jones, 2008).

In the European Union an outbreak of STEC O157 occurred in 2007 simultaneously in the Netherlands and Iceland. The most probable cause of this international outbreak was contaminated

---

[10] The Report referred to in Article 9 of Directive 2003/ 99/ EC of Sweden on trends and sources of zoonoses and zoonotic agents in humans, foodstuffs, animals and feedingstuffs in 2006. Available at: http://www.efsa.europa.eu/en/reportingonzoonoses/zoonosescomsumrep.htm

[11] Report referred to in Article 9 of Directive 2003/ 99/ EC of Portugal on trends and sources of zoonoses and zoonotic agents in humans, foodstuffs, animals and feedingstuffs in 2006. Available at: http://www.efsa.europa.eu/en/reportingonzoonoses/zoonosescomsumrep.htm

lettuce, shredded and pre-packed in a Dutch food processing plant. However, the only epidemiological link between the cases in the Netherlands and in Iceland was the implicated Dutch processing plant (Friesema et al., 2008).

In addition, nine confirmed cases of STEC O157 infection have been identified in Aberdeen, Scotland in 2007, where the most likely vehicle of infection based on descriptive epidemiology was the salad component of cold meat salad platters and smoked and poached salmon platters (Webster et al., 2007).

In Sweden in 2005 another outbreak of STEC O157 VT2 infections affected 120 people and was associated with consumption of iceberg lettuce using a case control study (Söderström et al., 2005).

In March 1997, an outbreak of STEC O157 infection occurred amongst holidaymakers returning from Canary Islands staying in four hotels. Three of the four hotels were supplied with water from a private well which appeared to be the probable vehicle of transmission and a case-control study showed illness was associated with consumption of raw vegetables which may have been washed in well water (Pebody et al., 1999).

A retrospective cohort study was also conducted after 2 confirmed and 8 probable cases of STEC 0157 occurred in a school-group from Somerset (England) following a trip to France. Despite its low statistical power, due to small numbers, this study suggested the most likely vehicle of infection was cucumber salad. The cucumbers were purchased from Belgium but it was not possible to trace them back to source (Duffell et al., 2003).

However, it should be noted that the strength of evidence of the above mentioned European outbreaks is limited as it is only based on descriptive epidemiological evidence.

Vegetables and fruits have been incriminated in outbreaks of food-borne diseases inside and outside the EU caused by *Escherichia coli* O157:H7 (Beuchat and Ryu, 1997; Trias et al., 2008). In the USA produce-related outbreaks caused by STEC[12] have been limited to leafy greens, predominantly lettuce and spinach. An outbreak of STEC O157:H7 infections linked to consumption of pre-packaged spinach occurred in 2006 across 26 states in the USA, resulting in 183 confirmed infections and three deaths (Wendel et al., 2009). In this US outbreak of STEC O157 infections associated with pre-packaged spinach, trace-back and environmental investigations determined that one ranch in California's SalinasValley was the likely source of the outbreak. The patterns produced by pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat analysis (MLVA) from the strains involved in the outbreak matched those from isolates recovered from local feral swine and cattle faeces (Jay et al., 2007). However, the manner in which the spinach became contaminated was not determined.

The largest STEC O157 outbreak to date, in 1996 centred in Sakai City, Osaka, Japan, was traced to consumption of white radish sprouts (Michino et al., 1999).

Food items frequently implicated in outbreaks of STEC O157:H7 infections were lettuce and apple cider. In addition, a multistate outbreak of infection with a *E. coli* O6 strain was associated with carrot consumption in Rhode Island and New Hampshire, and an outbreak of STEC O11:H43 infections was associated with pineapple consumption in the US (Sivapalasingam et al., 2004).

There is scarce information on the prevalence and quantity of STEC in vegetables both from surveillance and outbreak investigations. It is currently not possible to estimate the relative exposure to humans from pre-harvest or post-harvest contamination of vegetables by STEC. At the same time,

---

[12] VTEC, STEC and EHEC are frequently used as synonyms to identify this particular group of bacteria. Currently, the accepted nomenclature for reporting of this food-borne pathogen in the EU is VTEC.

scarcity of data also hampers the estimation of the relative significance of surface or internal contamination of vegetables by STEC for human exposure.

In addition, since the food source of the current German outbreak has not yet been determined, it is currently not possible to make conclusions on the human relative exposure to STEC based on the consumption data presented in Appendix B. For illustration purposes this appendix provides data for the food commodities that have up to now been identified as suspect vehicles given the epidemiological investigations of this outbreak.

4. Mitigation options for the vegetable food-borne route

The WHO/FAO (FAO and WHO, 2008) have thoroughly reviewed in a meeting report microbiological hazards occurring in fresh leafy vegetables and herbs, including advice on mitigation options. At the same time, a Code of Hygienic practice for fresh fruits and vegetables has been previously published by the Codex Alimentarius Commission (CAC, 2003).

The use of Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs), and Hazard Analysis and Critical Control Point (HACCP) in the fresh fruit and vegetable industry provide the basic framework for safe products for the consumer. Good Agricultural Practices (GAPs) describe preventive measures implemented in farming operations to reduce product contamination and provide guidance for food-safety practices in the field. Implementing GAPs provides some assurance to the retailer that product is as safe as reasonably possible.

Training and awareness on hygiene practices throughout the food chain is essential. In addition, and since there is evidence of asymptomatic carriers of STEC in humans, screening of humans involved in food handling is relevant (Silvestro et al., 2004; Stephan et al., 2000). The monitoring and/or exclusion of STEC carriers from food handling should be considered as a mitigation option.

Minimal processing of fruits and vegetables presents unique challenges, because cutting and slicing remove the natural protective barriers of the intact plant and thus may increase the risk by creating more favourable conditions for survival and growth of STEC. Thus, implementing HACCP programs in processing and packaging facilities is a requisite for food safety (James, 2006). However it has to be noticed that in particular HACCP may be a risk management tool difficult to implement as no true Critical Control Point for fresh produce can be identified, unless methods permitting an important pathogen reduction on fresh fruits and vegetables such as irradiation (EFSA, 2011) would be used.

In the European Union various Member States have developed guides for the application of GAPs, GMPs and HACCP. In addition to that there are also some guides for hygiene practices for the production of ready-to-eat vegetables or for the application of the HACCP principles, in accordance with Article 9 of Regulation (EC) No 852/2004 of the European Parliament and of the Council on the hygiene of foodstuffs. In line with that, implementation of traceability systems is currently a “must” in the food industry (Regulation (EC) No 178/2002 of the European Parliament and of the Council on laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety); by these means the life of a product can be followed in both ways: backwards and forwards. Under the “precautionary principle” industries should develop all necessary measures to prevent/diminish/avoid the risk of contamination, and so they should have documentation in place to demonstrate compliance with the above mentioned principle (e.g. analysis of produce, of water, preservation of witness samples in the warehouse).

An EU Process Hygiene Criterion for *E. coli* in ready-to-eat vegetables has been established through Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Surveillance is based on a 3-class sampling plan. Sampling frequencies even if not prescribed require appropriate coverage to the nature and size of the food business, providing that the safety of foodstuffs will not be endangered. It applies during the manufacturing process and in case of unsatisfactory results it requires improvements in production hygiene and selection of raw materials. It does neither require typification of the strain(s) involved nor withdrawal of the product from the market.

4.1. Pre-harvest

Traditional means of controlling infectious agents, such as eradication or test and removal of carrier animals, do not appear to be feasible for STECs. Nevertheless, good farm management practices—especially those related to maintenance and multiplication of the agent in feed and water—may provide practical means to substantially reduce the prevalence of these agents in cattle on farms and at slaughter plants.

In general, the application of mitigation strategies reflected in GAPs in line with codes available from international organisations is recommended (CAC, 2003; FAO and WHO, 2008). Of particular importance at this stage may be, to:

- avoid access of farm animals (in particular ruminants) to the immediate environment of fresh produce;
- use irrigation and agricultural water which are of adequate microbiological quality;
- control the sourcing, handling and treatment of manure and slurry that are to be used for fertilising fields intended to grow produce for human consumption;

4.2. Post-harvest

4.2.1. Harvest and processing

Poor hygiene practices during handling has been suspected as source of contamination in some investigations of food-borne outbreaks associated with leafy vegetables and herbs (FAO and WHO, 2008).

Susceptibility of non-O157 STEC to various intervention techniques is probably similar to that of other *E. coli*, although there are known differences among strains in acid tolerance and sensitivity to some other agents. Current technologies or practices do not effectively eliminate any microbiological hazard acquired during post-harvest processing of fresh vegetables. Therefore the main focus should be on prevention of contamination both during pre-harvest and post-harvest (FAO and WHO, 2008).

The only effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating (e.g. cooking or pasteurization) or irradiation.

Although various disinfectants can be used to reduce the microbial load on fruits and vegetables, their efficacy is variable and none are able to ensure elimination of pathogens (WHO, 1998). The inner tissues of fruits and vegetables are usually regarded as sterile. However, bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures and if these waters are contaminated with human pathogens these may also be introduced (European Commission, 2002). Adhesion of pathogens to surfaces and internalisation of pathogens limits the usefulness of conventional processing and chemical sanitizing methods in preventing transmission from contaminated produce.

It has to be considered that washing may also aid the spread of contamination and internalisation of bacteria (WHO, 1998). Irradiation has been reported as a possible alternative to chemical disinfection (EFSA, 2011).

Again the application of mitigation strategies reflected in GMPs and GHPs in line with codes available from international organisations is recommended (CAC, 2003; FAO and WHO, 2008). Of particular importance at this stage may be, to:

- use water of adequate microbiological quality during further processing;
- ensure basic training on food hygiene practices to food handlers;
- ensure adequate design and hygiene management of food premises including pest control plans;

4.2.2. Marketing and retail

Guidelines from the WHO/FAO and the Codex Alimentarius Commission should be taken into account (CAC, 2003; FAO and WHO, 2008).

Of particular importance, beyond general GMPs and GHPs including hygiene practices of the staff handling the foodstuff, adequate management of the cold chain seems important. This is of particular interest for those products processed for ready-to-eat consumption (e.g. cut ready-to-eat vegetables, juices originating from vegetables that are not pasteurised).

4.2.3. Catering and home

GHP and GMP, including education and training are important to be followed under catering settings in order to control STEC in fresh vegetables.

Consumers are advised to follow GHPs when preparing food e.g. wash hands before and after preparing foods, wash all fruit and vegetables with potable running water, avoid cross-contamination, keep storage temperatures low for food. Peeling or cooking fruit and vegetables can also remove microbes. Some more hardy items such as root vegetable may be brush-washed to scrub surfaces for the physical removal of soil and microorganisms. This is often done in conjunction with a detergent followed by a rinse of potable water. Although these measures have been proven to be useful they cannot completely eliminate the risk.

Other routine hygiene practices such as washing hands after using the bathroom or after changing diapers are also important.

CONCLUSIONS

General Conclusions

- The German outbreak strain seems to share virulence characteristics of STEC and EAEC strains. STEC strains usually have an animal reservoir, while EAEC have a human reservoir.

- Infections in humans caused by similar strains (same serotype, same phylogroup, same MLST type, and with similar virulence gene array) have been reported in the past, and as such, the strain could not be regarded as "new". However the German outbreak strain is rare, and until now it has never been found to be responsible for the rate of infection and severity of disease seen during the current outbreak.

- Sequence analysis and comparative genomics will be able to show if the German outbreak strain is an EAEC that acquired EHEC virulence determinants, or if it is the other way around.

- Traditionally, STEC strains do not present a high level of resistance to antimicrobials. The antimicrobial resistance genotype of the outbreak strain, and the molecular typing of the *bla*$_{\text{CTX-M-15}}$ –containing plasmid, could provide some clues on the epidemiology of this pathogen.

Answer to the Terms of Reference

Answer to ToR1

Regarding the Exposure assessment,

- Many different types of foods have been identified as a potential source of STEC. These are usually raw or undercooked foodstuffs contaminated with faeces from ruminants, either during primary production (e.g. slaughtering, milking, fertilised vegetables) or further processing and handling.

- Data on STEC in food and animals are reported annually on a mandatory basis by EU Member States to the European Commission and EFSA. When interpreting this data it is important to note that they are not directly comparable due to differences in sampling strategies and applied analytical methods. The most widely used analytical method only aims at detecting STEC O157, whereas fewer investigations have been conducted with analytical methods aiming at detecting all or selected serotypes of STEC.

- In the scientific literature, outbreaks of STEC infection are becoming increasingly recognised as associated with vegetables, particularly contaminated sprouting seeds and green leafy salad vegetables.

- Outbreaks may have more than one exposure route involved. For example, primary human infection may originate from consumption of contaminated food or direct contact with an animal carrying STEC, while secondary infection may occur by the faecal-oral route, after contamination of food through handling by an infected person shedding the bacteria. As a result, especially during the late stages of an outbreak multiple exposure routes are likely.

- Ready-to-eat fresh vegetables and fruits demand has continuously increased over the last decades reflecting the consumer's preference for fresh and healthy foods with minimal preparation.

- Contamination of fresh produce with STEC is rare but has been linked to some severe outbreaks.

- In some outbreaks, the origin of contamination was suspected to be contaminated irrigation water and access of farm animals to the immediate environment of fresh-produce. In most outbreaks however, the origin of contamination was not elucidated.

- Contamination of vegetables with STEC can occur in different steps of the food chain: during primary production; during harvest and post-harvest including handling and processing, at marketing and retail and during catering and in the care of the consumer after sale during transport and in domestic settings.

- Bacterial contamination of vegetables occurs mostly on the surface of the tissues of the plants but it may also be internal. Three basic modes of bacterial interaction with growing plants could be considered: (a) attachment of bacteria to the surface of plants (epiphytes), (b) access of bacteria to the plants through natural openings (stomata) or damaged tissue, and (c) internalisation via root colonization

- Although there is no conclusive data, in theory internalisation of STEC would result in increased survival both in pre-harvest and post-harvest due to protection from exposure to UV and desiccation, as well as increased protection to surface decontamination treatments.

- Pre-harvest contamination can derive from infected farm animals. The possible routes of contamination are: irrigation water contaminated with animal waste as well as sewage, application of organic fertilizers of animal and/or human origin and direct contact of animals with fresh produce growing fields.

- There is considerable debate on the possibility of STEC being present internally in leafy vegetables, particularly if exposed during the pre-harvest phase. It should be noted that internalisation of *E. coli* in plants has been only shown in experimental laboratory conditions specially in the case of root inoculations with very young plants and using high inoculation doses.

- Processing of vegetables involves many points of contact with people, surfaces, water and the environment (soil, dust) and this represents potential opportunities for contamination with food-borne pathogens.

- Minimally processed vegetables and sliced fruit exhibit a characteristic high humidity. This fact, together with the high number of cut surfaces, can provide ideal conditions for microbial growth, including that of food-borne pathogens and spoilage micro-organisms.

- Cutting practices increased the chance of bacterial cross-contamination and can result in increased susceptibility of bacterial attachment.

- Some practices (e.g. vacuum cooling) used in the leafy greens industry can promote the internalisation of *E. coli* O157:H7 into lettuce. However, it has also been shown that *E. coli* O157:H7 can grow on iceberg lettuce held at refrigeration temperatures, even if the bacterium has only been applied externally, on the surface of the leaves.

- There is scarce information on the prevalence and quantity of STEC in vegetables both from surveillance and outbreak investigations. It is currently not possible to estimate the relative exposure to humans from pre-harvest or post-harvest contamination of vegetables by STEC.

At the same time, scarcity of data also hampers the estimation of the relative significance of surface or internal contamination of vegetables by STEC for human exposure.

Answer to ToR2

Regarding mitigation options,

- The use of Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs), and Hazard Analysis and Critical Control Point (HACCP) in the fresh fruit and vegetable industry provide the basic framework for safe products for the consumer. Good Agricultural Practices (GAPs) describe preventive measures implemented in farming operations to reduce product contamination and provide guidance for food-safety practices in the field. Implementing HACCP programs in processing and packaging facilities is a requisite for food safety.

- Since there is evidence of asymptomatic carriers of STEC in humans, screening of humans involved in food handling is relevant. The monitoring and/or exclusion of STEC carriers from food handling should be considered as a mitigation option.

- Pre-harvest mitigation options: the application of mitigation strategies reflected in GAPs in line with codes available from international organisations is recommended. In particular, to:

    - avoid access of farm animals (in particular ruminants) to the immediate environment of fresh produce;

    - use irrigation and agricultural water which are of adequate microbiological quality;

    - control the sourcing, handling and treatment of manure and slurry that are to be used for fertilising fields intended to grow produce for human consumption.

- Post-harvest mitigation options:

    - Current technologies or practices do not effectively eliminate any microbiological hazard acquired during post-harvest processing of fresh vegetables. Therefore the main focus should be on prevention of contamination both during pre-harvest and post-harvest.

    - The only effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating (e.g. cooking or pasteurization) or irradiation.

    - Adhesion of pathogens to surfaces and, when it occurs, internalisation of pathogens limits the usefulness of conventional processing and chemical sanitizing methods in preventing transmission from contaminated produce.

    - The application of mitigation strategies reflected in GMPs and GHPs in line with codes available from international organisations is recommended. In particular,

        - To use of water of adequate microbiological quality during further processing;

        - To ensure basic training on food hygiene practices to food handlers;

    - To ensure adequate design and hygiene management of food premises including pest control plans;
    - The correct management of cold chain seems of particular importance for those products processed for ready-to-eat consumption (e.g. cut vegetables, unpasteurised vegetable juices).
  - Catering and home:
    - GHPs when preparing food e.g. wash hands before and after preparing foods, wash all fruit and vegetables with potable running water, avoid cross-contamination, keep storage temperatures low for food. Peeling or cooking fruit and vegetables can also remove microbes. Although these measures have been proven to be useful they cannot completely eliminate the risk.

REFERENCES

Ackers ML, Mahon BE, Leahy E, Goode B, Damrow T, Hayes PS, Bibb WF, Rice DH, Barrett TJ, Hutwagner L, Griffin PM and Slutsker L, 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J Infect Dis, 177, 1588-1593.

Bae WK, Lee YK, Cho MS, Ma SK, Kim SW, Kim NH and Choi KC, 2006. A case of hemolytic uremic syndrome caused by *Escherichia coli* O104:H4. Yonsei Med J, 47, 437-439.

Barak JD and Ivey ML, 2011. Research funded by USDA-NIFA in the area of human pathogen-plant interactions. Phytopathology News.

Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P and Frankel G, 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology. 12, 2385-2397.

Beuchat LR and Ryu JH, 1997. Produce handling and processing practices. Emerg Infect Dis, 3, 459-465.

Boerlin P, 1999. Evolution of virulence factors in Shiga-toxin-producing *Escherichia coli*. Cell Mol Life Sci, 56, 735-741.

Bolton DJ, 2010. Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: virulence factors and pathogenicity in the farm to fork paradigm. Foodborne Pathog Dis, 8, 357-365.

Bonardi S, Brindani F, Pizzin G, Lucidi L, D'Incau M, Liebana E and Morabito S, 2003. Detection of *Salmonella* spp., *Yersinia enterocolitica* and verocytotoxin-producing *Escherichia coli* O157 in pigs at slaughter in Italy. Int J Food Microbiol, 85, 101-110.

Bonnet R, 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother, 48, 1-14.

Borie C, Montreal Z, Guerrero P, Sanchez ML and Martinez JCA, 1997. Prevalencia y caracterizacion de *Escherichia coli* enterohemorragica aisladas de bovinos y cerdos sanos fenados en Santiago, Chile. Archivos de Medicina Veterinaria WWiW: 205-212.

Brandl MT and Amundson R, 2008. Leaf age as a risk factor in contamination of lettuce with *Escherichia coli* O157:H7 and *Salmonella enterica*. Appl Environ Microbiol, 74, 2298-2306.

Breuer T, Benkel DH, Shapiro RL, Hall WN, Winnett MM, Linn MJ, Neimann J, Barrett TJ, Dietrich S, Downes FP, Toney DM, Pearson JL, Rolka H, Slutsker L and Griffin PM, 2001. A multistate outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. Emerg Infect Dis, 7, 977-982.

CAC (Codex Alimentarius Commission), 2003. Code of hygenic practice for fresh fruits and vegetables. CAC/RCP 53-2003. Adopted 2003. Revision 2010 (new Annex III for Fresh Leafy Vegetables). Available at: www.codexalimentarius.net/download/standards/10200/CXP_053e.pdf.

Canton R and Coque TM, 2006. The CTX-M beta-lactamase pandemic. Curr Opin Microbiol, 9, 466-475.

Canton R, Novais A, Valverde A, Machado E, Peixe L, Baquero F and Coque TM, 2008. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect, 14 Suppl 1, 144-153.

Caprioli A, Morabito S, Brugere H and Oswald E, 2005. Enterohaemorrhagic *Escherichia coli:* emerging issues on virulence and modes of transmission. Vet Res, 36, 289-311.

Carattoli A, 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob Agents Chemother, 53, 2227-2238.

Cassar CA, Ottaway M, Paiba GA, Futter R, Newbould S and Woodward MJ, 2004. Absence of enteroaggregative *Escherichia coli* in farmed animals in Great Britain. Vet Rec, 154, 237-239.

CDC (Centre for Disease Control), 2006. Ongoing Multistate Outbreak of *Escherichia coli* serotype O157:H7 Infections Associated with Consumption of Fresh Spinach --- United States, September 2006. September 29, 2006/55(38):1045-1046.

Clermont O, Bonacorsi S and Bingen E, 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol, 66, 4555-4558.

Cooley MB, Miller WG and Mandrell RE, 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl Environ Microbiol, 69, 4915-4926.

Critzer FJ, D'Souza DH, Saxton AM and Golden DA, 2010. Increased transcription of the phosphate-specific transport system of *Escherichia coli* O157:H7 after exposure to sodium benzoate. J Food Prot, 73, 819-824.

Dipineto L, Santaniello A, Fontanella M, Lagos K, Fioretti A and Menna LF, 2006. Presence of Shiga toxin-producing *Escherichia coli* O157:H7 in living layer hens. Lett Appl Microbiol, 43, 293-295.

Doane CA, Pangloli P, Richards HA, Mount JR, Golden DA and Draughon FA, 2007. Occurrence of *Escherichia coli* O157:H7 in diverse farm environments. J Food Prot, 70, 6-10.

Duffell E, Espié E, Nichols T, Adak GK, De Valk H, Anderson K and Stuart JM, 2003. Investigation of an outbreak of *E. coli* O157 infections associated with a trip to France of schoolchildren from Somerset, England. Euro Surveillance; 8(4):pii=406.

EFSA (European Food Safety Authority and European Centre for Disease Prevention and Control), 2005. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2004. The EFSA Journal, 310.

EFSA (European Food Safety Authority and European Centre for Disease Prevention and Control), 2006. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union in 2005. The EFSA Journal, 94.

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

EFSA (European Food Safety Authority), 2009. The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. The EFSA Journal, 271.

EFSA (Euroepan Food Safety Authority), 2011. Statement summarising the Conclusions and Recommendations from the Opinions on the safety of Irradiation of Food adopted by the BIOHAZ and CEF Panels. The EFSA Journal, 9(4), 2107. .

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

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2007. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Unioni in 2006. The EFSA Journal, 130.

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

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2010. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and food-borne outbreaks in the European Union in 2008. The EFSA Journal, 1496.

EFSA and ECDC (European Food Safety Authority, European Centre for Disease Prevention and Control), 2011. The European Union Summary Report on Trends and sources of Zoonoses and Zoonotic Agents and Food-borne Outbreaks in 2009. The EFSA Journal, 2090.

Erickson MC, Webb CC, Diaz-Perez JC, Phatak SC, Silvoy JJ, Davey L, Payton AS, Liao J, Ma L and Doyle MP, 2007a. Infrequent internalization of *Escherichia coli* O157:H7 into field-grown leafy greens. J Food Prot, 73, 500-506.

Erickson MC, Webb CC, Diaz-Perez JC, Phatak SC, Silvoy JJ, Davey L, Payton AS, Liao J, Ma L and Doyle MP, 2007b. Surface and internalized *Escherichia coli* O157:H7 on field-grown spinach and lettuce treated with spray-contaminated irrigation water. J Food Prot, 73, 1023-1029.

FAO and WHO (Food and Agriculture Organisation of the United Nations and World Health Organisation), 2008. Microbiological hazards in fresh leafy vegetables and herbs: Meeting Rerport. Microbiological Risk Assessment Series No. 14. Rome 151pp.

Ferguson DD, Scheftel J, Cronquist A, Smith K, Woo-Ming A, Anderson E, Knutsen J, De AK and Gershman K, 2005. Temporally distinct *Escherichia coli* 0157 outbreaks associated with alfalfa sprouts linked to a common seed source--Colorado and Minnesota, 2003. Epidemiol Infect, 133, 439-447.

Frank C, Faber MS, Askar M, Bernard H, Fruth A, Gilsdorf A, Höhle M, Karch H, Krause G, Prager R, Spode A, Stark K and Werber D, 2011. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. Euro Surveill. 2011;16(21):pii=19878.

Franz E, van Diepeningen AD, de Vos OJ and van Bruggen AH, 2005. Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar typhimurium in manure, manure-amended soil, and lettuce. Appl Environ Microbiol, 71, 6165-6174.

Franz E, Visser AA, Van Diepeningen AD, Klerks MM, Termorshuizen AJ and van Bruggen AH, 2007. Quantification of contamination of lettuce by GFP-expressing *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. Food Microbiol, 24, 106-112.

Friesema I, Sigmundsdottir G, van der Zwaluw K, Heuvelink A, Schimmer B, de Jager C, Rump B, Briem H, Hardardottir H, Atladottir A, Gudmundsdottir E and van Pelt W, 2008. An international outbreak of Shiga toxin-producing *Escherichia coli* O157 infection due to lettuce, September-October 2007. Euro Surveill, 13,

Garrood MJ, Wilson PD and Brocklehurst TF, 2004. Modeling the rate of attachment of *Listeria monocytogenes*, *Pantoea agglomerans*, and *Pseudomonas fluorescens* to, and the probability of their detachment from, potato tissue at 10 degrees C. Appl Environ Microbiol, 70, 3558-3565.

Gelting RJ, Baloch MA, Zarate-Bermudez MA and Selman C, 2011. Irrigation water issues potentially related to the 2006 multistate *E. coli* O157:H7 outbreak associated with spinach. Agric. Water Manage.,doi:10.1016/j.agwat.2011.04.004.

Grant J, Wendelboe AM, Wendel A, Jepson B, Torres P, Smelser C and Rolfs RT, 2008. Spinach-associated *Escherichia coli* O157:H7 outbreak, Utah and New Mexico, 2006. Emerg Infect Dis, 14, 1633-1636.

Gyles CL, 2007. Shiga toxin-producing *Escherichia coli*: an overview. J Anim Sci, 85, E45-62.

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 Res, 8, 11-22.

Heaton JC and Jones K, 2008. Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. J Appl Microbiol, 104, 613-626.

Heuvelink AE, Zwartkruis-Nahuis JT, van den Biggelaar FL, van Leeuwen WJ and de Boer E, 1999. Isolation and characterization of verocytotoxin-producing *Escherichia coli* O157 from slaughter pigs and poultry. Int J Food Microbiol, 52, 67-75.

Hilborn ED, Mermin JH, Mshar PA, Hadler JL, Voetsch A, Wojtkunski C, Swartz M, Mshar R, Lambert-Fair MA, Farrar JA, Glynn MK and Slutsker L, 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch Intern Med, 159, 1758-1764.

Huang DB, Jiang ZD and Dupont HL, 2003. Association of virulence factor-positive and -negative enteroaggregative *Escherichia coli* and occurrence of clinical illness in travelers from the United States to Mexico. Am J Trop Med Hyg, 69, 506-508.

Huang DB, Mohanty A, DuPont HL, Okhuysen PC and Chiang T, 2006. A review of an emerging enteric pathogen: enteroaggregative *Escherichia coli*. J Med Microbiol, 55, 1303-1311.

Hunter PA, Dawson S, French GL, Goossens H, Hawkey PM, Kuijper EJ, Nathwani D, Taylor DJ, Teale CJ, Warren RE, Wilcox MH, Woodford N, Wulf MW and Piddock LJ, 2010. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. J Antimicrob Chemother, 65 Suppl 1, i3-17.

Ibarra-Sanchez LS, Alvarado-Casillas S, Rodriguez-Garcia MO, Martinez-Gonzales NE and Castillo A, 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. J Food Prot, 67, 1353-1358.

Ingram DT, Patel J and Sharma M, 2011. Effect of Repeated Irrigation with Water Containing Varying Levels of Total Organic Carbon on the Persistence of *Escherichia coli* O157:H7 on Baby Spinach. J Food Prot, 74, 709-717.

Islam M, Doyle MP, Phatak SC, Millner P and Jiang X, 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. J Food Prot, 67, 1365-1370.

Itoh Y, Sugita-Konishi Y, Kasuga F, Iwaki M, Hara-Kudo Y, Saito N, Noguchi Y, Konuma H and Kumagai S, 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl Environ Microbiol, 64, 1532-1535.

Jablasone J, Warriner K and Griffiths M, 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. Int J Food Microbiol, 99, 7-18.

Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A and Hooper DC, 2006. qnrB, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother, 50, 1178-1182.

James J, 2006. Microbial Hazard Identification in Fresh Fruit and Vegetables, John Wiley & Sons, Inc., pp. 1-36.

Jay MT, Cooley M, Carychao D, Wiscomb GW, Sweitzer RA, Crawford-Miksza L, Farrar JA, Lau DK, O'Connell J, Millington A, Asmundson RV, Atwill ER and Mandrell RE, 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast. Emerg Infect Dis, 13, 1908-1911.

Jeter C and Matthysse AG, 2005. Characterization of the binding of diarrheagenic strains of *E. coli* to plant surfaces and the role of curli in the interaction of the bacteria with alfalfa sprouts. Mol Plant Microbe Interact, 18, 1235-1242.

Johannessen GS, Bengtsson GB, Heier BT, Bredholt S, Wasteson Y and Rorvik LM, 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. Appl Environ Microbiol, 71, 2221-2225.

Johannessen GS, Froseth RB, Solemdal L, Jarp J, Wasteson Y and L MR, 2004. Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce. J Appl Microbiol, 96, 787-794.

Johnsen G, Wasteson Y, Heir E, Berget OI and Herikstad H, 2001. *Escherichia coli* O157:H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. Int J Food Microbiol, 65, 193-200.

Johnston LM, Jaykus LA, Moll D, Anciso J, Mora B and Moe CL, 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. Int J Food Microbiol, 112, 83-95.

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. J Clin Microbiol, 41, 4930-4940.

Kaspar C, Doyle ME and Archer J, 2009. FRI Food Safety Review: non-O157:H7 Shiga Toxin-producing *E. coli* from meat and Non-Meat Sources. Food Research Institute, UW-Madison. Available at: http://fri.wisc.edu/docs/pdf/FRI_Brief_NonO157STEC_4_10.pdf.

Knutton S, 1995. Electron microscopical methods in adhesion. Methods Enzymol, 253, 145-158.

Koseki S and Isobe S, 2005. Prediction of pathogen growth on iceberg lettuce under real temperature history during distribution from farm to table. Int J Food Microbiol, 104, 239-248.

Li H, Tajkarimi M and Osburn BI, 2008. Impact of vacuum cooling on *Escherichia coli* O157:H7 infiltration into lettuce tissue. Appl Environ Microbiol, 74, 3138-3142.

Literak I, Dolejska M, Rybarikova J, Cizek A, Strejckova P, Vyskocilova M, Friedman M and Klimes J, 2009. Highly variable patterns of antimicrobial resistance in commensal *Escherichia coli* isolates from pigs, sympatric rodents, and flies. Microb Drug Resist, 15, 229-237.

Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L and Woodford N, 2007. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother, 59, 165-174.

Livermore DM and Hawkey PM, 2005. CTX-M: changing the face of ESBLs in the UK. J Antimicrob Chemother, 56, 451-454.

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*. Emerg Infect Dis, 14, 1287-1290.

Michino H, Araki K, Minami S, Takaya S, Sakai N, Miyazaki M, Ono A and Yanagawa H, 1999. Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. Am J Epidemiol, 150, 787-796.

Mohle-Boetani JC, Farrar JA, Werner SB, Minassian D, Bryant R, Abbott S, Slutsker L and Vugia DJ, 2001. *Escherichia coli* O157 and *Salmonella* infections associated with sprouts in California, 1996-1998. Ann Intern Med, 135, 239-247.

Moon JY, Park JH and Kim YB, 2005. Molecular epidemiological characteristics of virulence factors on enteroaggregative *E. coli*. FEMS Microbiol Lett, 253, 215-220.

Mootian G, Wu WH and Matthews KR, 2009. Transfer of *Escherichia coli* O157:H7 from soil, water, and manure contaminated with low numbers of the pathogen to lettuce plants. Journal of Food Protection, 72, 2308-2312.

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. J Clin Microbiol, 36, 840-842.

Mshana SE, Imirzalioglu C, Hossain H, Hain T, Domann E and Chakraborty T, 2009. Conjugative IncFI plasmids carrying CTX-M-15 among *Escherichia coli* ESBL producing isolates at a University hospital in Germany. BMC Infect Dis, 9, 97.

Nataro JP, Steiner T and Guerrant RL, 1998. Enteroaggregative *Escherichia coli*. Emerg Infect Dis, 4, 251-261.

Pebody RG, Furtado C, Rojas A, McCarthy N, Nylen G, Ruutu P, Leino T, Chalmers R, de Jong B, Donnelly M, Fisher I, Gilham C, Graverson L, Cheasty T, Willshaw G, Navarro M, Salmon R, Leinikki P, Wall P and Bartlett C, 1999. An international outbreak of Vero cytotoxin-producing *Escherichia coli* O157 infection amongst tourists; a challenge for the European infectious disease surveillance network. Epidemiol Infect, 123, 217-223.

Peirano G, Costello M and Pitout JD, 2010. Molecular characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* from the Chicago area: high prevalence of ST131 producing CTX-M-15 in community hospitals. Int J Antimicrob Agents, 36, 19-23.

Perna NT, Plunkett G, 3rd, 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.

Pitout JD, Gregson DB, Church DL, Elsayed S and Laupland KB, 2005a. Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing *Escherichia coli* strains in the Calgary health region. J Clin Microbiol, 43, 2844-2849.

Pitout JD and Laupland KB, 2008. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis, 8, 159-166.

Pitout JD, Nordmann P, Laupland KB and Poirel L, 2005b. Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother, 56, 52-59.

Prazak AM, Murano EA, Mercado I and Acuff GR, 2002. Prevalence of *Listeria monocytogenes* during production and postharvest processing of cabbage. J Food Prot, 65, 1728-1734.

Rodriguez-Bano J, Alcala JC, Cisneros JM, Grill F, Oliver A, Horcajada JP, Tortola T, Mirelis B, Navarro G, Cuenca M, Esteve M, Pena C, Llanos AC, Canton R and Pascual A, 2008. Community infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli*. Arch Intern Med, 168, 1897-1902.

Rodriguez-Bano J, Navarro MD, Romero L, Martinez-Martinez L, Muniain MA, Perea EJ, Perez-Cano R and Pascual A, 2004. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. J Clin Microbiol, 42, 1089-1094.

Rodriguez-Bano J, Navarro MD, Romero L, Muniain MA, de Cueto M, Rios MJ, Hernandez JR and Pascual A, 2006. Bacteremia due to extended-spectrum beta -lactamase-producing *Escherichia coli* in the CTX-M era: a new clinical challenge. Clin Infect Dis, 43, 1407-1414.

Ryall AL and Pentzer WT, 1982. Handling, transportation and storage of fruits and vegetables. Volume 2. Fruits and tree nuts, 2nd edition, pp426-473. Westport, CT, USA: AVI Publishing Company.

Schouten JM, van de Giessen AW, Frankena K, De Jong MC and Graat EA, 2005. *Escherichia coli* O157 prevalence in Dutch poultry, pig finishing and veal herds and risk factors in Dutch veal herds. Prev Vet Med, 70, 1-15.

SCVPH (Scientific Committee on Veterinary Measures relating to Public Health), 2003. Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Verotoxigenic *E. coli* (VTEC) in foodstuffs. Adopted on 21-22 January 2003. Available at:http://ec.europa.eu/food/fs/sc/scv/out58_en.pdf

Shaw RK, Berger CN, Feys B, Knutton S, Pallen MJ and Frankel G, 2008. Enterohemorrhagic *Escherichia coli* exploits EspA filaments for attachment to salad leaves. Appl Environ Microbiol, 74, 2908-2914.

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. Epidemiol Infect, 132, 915-919.

Sivapalasingam S, Friedman CR, Cohen L and Tauxe RV, 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot, 67, 2342-2353.

Söderström A, Lindberg A and Andersson Y, 2005. EHEC O157 outbreak in Sweden from locally produced lettuce, August-September 2005. Euro Surveill, 10, E050922 050921.

Solomon EB, Pang HJ and Matthews KR, 2003. Persistence of *Escherichia coli* O157:H7 on lettuce plants following spray irrigation with contaminated water. J Food Prot, 66, 2198-2202.

Solomon EB, Potenski CJ and Matthews KR, 2002a. Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. J Food Prot, 65, 673-676.

Solomon EB, Yaron S and Matthews KR, 2002b. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Appl Environ Microbiol, 68, 397-400.

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. J Appl Microbiol, 88, 335-341.

Takeuchi K and Frank JF, 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. J Food Prot, 63, 434-440.

Takeuchi K, Hassan AN and Frank JF, 2001. Penetration of *Escherichia coli* O157 : H7 into lettuce as influenced by modified atmosphere and temperature. Journal of Food Protection, 64, 1820-1823.

Tenaillon O, Skurnik D, Picard B and Denamur E, 2010. The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol, 8, 207-217.

Trias R, Baneras L, Badosa E and Montesinos E, 2008. Bioprotection of Golden Delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. Int J Food Microbiol, 123, 50-60.

Tyrrel SF, Knox JW and Weatherhead EK, 2006. Microbiological water quality requirements for salad irrigation in the United Kingdom. Journal of Food Protection, 69, 2029-2035.

Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, Reid-Smith RJ, Tellier PP, Tellis PA, Ziebell K and Manges AR, 2010. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis, 16, 88-95.

Wachtel MR, Whitehand LC and Mandrell RE, 2002. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. J Food Prot, 65, 18-25.

Warriner K, Huber A, Namvar A, Fan W and Dunfield K, 2009. Recent advances in the microbial safety of fresh fruits and vegetables. Adv Food Nutr Res, 57, 155-208.

Warriner K, Ibrahim F, Dickinson M, Wright C and Waites WM, 2003. Internalization of human pathogens within growing salad vegetables. Biotechnol Genet Eng Rev, 20, 117-134.

Webster D, Cowden J and Locking M, 2007. An outbreak of *Escherichia coli* O157 in Aberdeen, Scotland, September 2007. Euro Surveill, 12, E070927 070921.

Wendel AM, Johnson DH, Sharapov U, Grant J, Archer JR, Monson T, Koschmann C and Davis JP, 2009. Multistate outbreak of *Escherichia coli* O157:H7 infection associated with consumption of packaged spinach, August-September 2006: the Wisconsin investigation. Clin Infect Dis, 48, 1079-1086.

WHO 1998. Surface decontamination of fruits and vegetables eaten raw: a review. WHO/FSF/FOS/98.2. Food Safety Unit, World Health Organisation, Geneve. Available at: http://www.who.int/foodsafety/publications/fs_management/en/surface_decon.pdf.

Wick LM, Qi W, Lacher DW and Whittam TS, 2005. Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. J Bacteriol, 187, 1783-1791.

Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, Johnson AP, Pike R, Warner M, Cheasty T, Pearson A, Harry S, Leach JB, Loughrey A, Lowes JA, Warren RE and Livermore DM, 2004. Community and hospital spread of Escherichia coli producing CTX-M extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother, 54, 735-743.

Xicohtencatl-Cortes J, Sanchez Chacon E, Saldana Z, Freer E and Giron JA, 2009. Interaction of *Escherichia coli* O157:H7 with leafy green produce. J Food Prot, 72, 1531-1537.

Ziebell K, Konczy P, Yong I, Frost S, Mascarenhas M, Kropinski AM, Whittam TS, Read SC and Karmali MA, 2008. Applicability of phylogenetic methods for characterizing the public health significance of verocytotoxin-producing *Escherichia coli* strains. Appl Environ Microbiol, 74, 1671-1675.

APPENDICES

A. EU PRODUCTION AND TRADE DATA OF TOMATOES, LETTUCE AND CUCUMBERS

1. Production data (Tables 1 to 3)

According to production data downloaded from the Eurostat “food_in_pagr5” database[13]:

- Spain, the Netherlands and Poland were the main cucumber producers in the EU with harvest accounting for 682,900, 430,000 and 390,700 tonnes respectively in 2010 (Table 1);
- Spain was the main producer of lettuce in the EU in 2010 with 809,200 tonnes. Data of 2008 production indicate that Italy and France are also important producers with 467,700 and 316,900 tonnes respectively (Table 2);
- Italy followed by Spain are the main tomato producers in the EU; their harvest accounted for 6,382,700 and 4,749,200 tonnes respectively in 2009 (Table 3).

2. Trade Data

Trade data (imports and exports) were downloaded from the Eurostat Comext database[14].

2.1. Intra EU trade (Tables 4 to 9).

- Commodity “fresh or chilled cucumbers and gherkins”; Table 4 demonstrates exports from each MS to other MSs in 2010; Spain and the Netherlands are the main exporters with 433,017 and 376,628 tonnes respectively. Table 5 displays imports of each MS from other MSs in 2010; Germany and the Netherlands are the main importers with 495,956 and 151,374 tonnes respectively.
- Commodity “lettuce, fresh or chilled (excluding cabbage lettuce)”; Spain and the Netherlands are the main exporters in the EU with 184,077 and 59,364 tonnes respectively (Table 6), whereas Germany and the UK are the main importers with 166,752 and 77,255 tonnes respectively (Table 7).
- Commodity “tomatoes, fresh or chilled”; the Netherlands and Spain are the main exporters in the EU with 874,085 and 720,717 tonnes respectively (Table 8), whereas Germany and the UK are the main importers with 678,151 and 362,735 tonnes respectively (Table 9).

2.2. Imports from third countries (Tables 10 to 12).

- EU imports of the commodity “cucumbers and gherkins, fresh or chilled” from third countries accounted for 37,263 tonnes in 2010 (Table 10); Turkey was the main country of origin with 23,693 tonnes.

- EU imports of the commodity “lettuce, fresh or chilled (excluding cabbage lettuce)” from third countries accounted for 5,319 tonnes in 2010, with the main exporter being Tunisia with 1,552 tonnes (Table 11).

- EU imports of the commodity “tomatoes, fresh or chilled” from third countries (accounted for 496,997 tonnes in 2010 (Table 12). The main exporters into the EU in 2010 were Morocco and Turkey with 307,783 and 108,813 tonnes respectively.

---

[13] http://epp.eurostat.ec.europa.eu/portal/page/portal/food/data/database

[14] http://epp.eurostat.ec.europa.eu/portal/page/portal/external_trade/data/database

Table 1: Cucumber production in the EU by MS.

	Annual production of cucumbers (1000 tonnes)			
Member State	2010	2009	2008	2007
Spain	682.9 (p)	:	:	558.8
Netherlands	430.0	435.0	425.0	430.0
Poland	390.7 (p)	180.3 (e)	190.7	209.9
Greece	:	:	124.2	160.2
France	:	:	115.6	113.2
Germany	58.2	86.9	73.7	72.0
Romania	80.8	95.9	98.8	71.1
Italy	:	:	66.7	64.4
Hungary	26.4 (e)	36.3	55.4	51.7
United Kingdom	:	:	:	49.4
Bulgaria	63.7	61.7	53.2	48.5
Sweden	27.0	22.7	20.5	31.3
Finland	31.8	29.4	31.4	29.2
Austria	28.9	26.4	24.3	24.9
Belgium	:	19.8	19.2	20.3
Cyprus	15.3 (p)	15.7 (p)	13.8	15.4
Lithuania	25.1	8.1	6.3	6.8
Estonia	0.0	:	5.6	5.7
Latvia	7.2	0.6	0.8	4.5
Czech Republic	3.9	5.0	4.4	3.5
Slovakia	3.6	3.8	3.2	2.7
Slovenia	:	3.4	2.6	2.1
Malta	:	0.7	0.9	0.7
Luxembourg	0.0	0.0	0.0	0.0
Denmark	15.4 (p)	:	:	:
Ireland	:	:	:	:
Portugal	:	:	:	:

Notes: MSs are shorted in the first column according to the production figures of 2007; data extracted on 30/5/2011 from Eurostat "food_in_pagr5" database. (e): estimated value; (p): provisional value; (:) not available.

Table 2: Lettuce production in the EU by MS

	Annual production of lettuce (1000 tonnes)			
Member State	2010	2009	2008	2007
Spain	809.2 (p)	:	:	947.6
Italy	:	330.0	467.7	485.5
France	:	:	316.9	347.8
Germany	169.5	193.9	180.9	197.8
United Kingdom	:	:	:	117.0
Greece	:	:	90.4	94.8
Netherlands	83.0	86.0	90.5	85.5
Belgium	:	69.4	76.1	76.4
Austria	38.6	44.5	47.0	51.7
Sweden	24.1	28.5	28.5	26.6
Poland	27.8 (p)	14.8(e)	14.4	20.5
Hungary	17.2(e)	8.4	7.5	7.6
Slovenia	5.9(p)	8.7	8.5	7.0
Finland	4.5	6.4	5.8	5.0
Bulgaria	3.0	3.3	2.1	3.6
Malta	:	3.7	3.6	3.6
Cyprus	2.1 (p)	2.0(p)	1.7	1.5
Romania	1.3	1.3	1.1	1.1
Lithuania	1.4	0.3	0.3	0.2
Luxembourg	0.1	0.1	0.2	0.2
Slovakia	0.3	0.4	0.5	0.2
Latvia	0.0	0.1	0.1	0.1
Czech Republic	0.0	:	:	:
Denmark	11.5 (p)	:	:	:
Estonia	0.0	:	:	:
Ireland	:	:	:	:
Portugal	:	:	:	:

Notes: MSs are shorted in the first column according to the production figures of 2007; data extracted on 1/6/2011 from Eurostat “food_in_pagr5” database. (e): estimated value; (p): provisional value; (:) not available.

Table 3: Tomato production in the EU by MS

	Annual production of tomatoes (1000 tonnes)			
Member State	2010	2009	2008	2007
Italy	:	6382.7	5982.1	6528.0
Spain	4312.7 (p)	4749.2(p)	3847.8 (p)	4081.5
Greece	:	:	1338.6	1423.9
Portugal	:	1346.7	1147.6	1236.2
Netherlands	815.0	800.0	730.0	685.0
France	:	:	714.6	679.6
Romania	381.6	470.9	536.3	407.1
Poland	677.6 (p)	265.3 (e)	257.4	277.4
Hungary	131.5 (e)	192.8	206.0	227.6
Belgium	:	232.1	226.2	222.6
Bulgaria	112.4	104.2	134.1	133.2
United Kingdom	:	:	:	85.6
Germany	73.3	66.6	65.1	62.6
Austria	44.2	41.5	42.1	44.9
Finland	39.2	38.4	40.5	38.2
Cyprus	25.7(p)	26.5 (p)	23.4	29.4
Slovakia	10.5	25.9	30.6	29.2
Sweden	13.8	13.6	16.2	16.4
Malta	:	11.6	15.7	14.8
Czech Republic	7.9	14.5	9.9	9.2
Slovenia	4.0 (p)	4.3	4.7	4.4
Lithuania	13.0	1.5	1.3	1.5
Estonia	0.0	:	1.3	1.3
Latvia	5.3	0.0	0.0	0.3
Luxembourg	0.1	0.1	0.1	0.1
Denmark	15.0 (p)	:	:	:
Ireland	:	:	:	:

Notes: MSs are shorted in the first column according to the production figures of 2007; data extracted on 1/6/2011 from Eurostat "food_in_pagr5" database. (e): estimated value; (p): provisional value; (:) not available.

Table 4: 2010 exports of "fresh or chilled cucumbers and gherkins"as reported by the MSs of the first column to other MSs

Exporter	EU27	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	IE	IT	LT	LU	LV	MT	NL	PT	RO	SE	SI	SK	PL	HU
EU27	973943	23744	11696	2580	75	78374	440459	23497	4079	1246	10428	58278	125324	1591	5478	9996	6699	1586	4631	121	81696	3208	3945	26517	3391	11997	25157	8143
ES	433017	4796	3832	72		48153	142771	12955	2225		4459	34114	53551	4	1148	8156	3587	85	1981		64827	3174	656	17821	428	3124	16848	4252
NL	376628	3386	6432	9	4	9380	237803	9673	459	571	3051	14425	70505	1330	3622	383	1398	52	657	34		9	26	7169	7	504	5114	616
BE	34920	129		2		187	17141			3	0	7124	53			10		1271			8752					2	247	
DE	29216	4720	1237	38		5395		680	397	138	2654	1836	1			908	102	18	0		6800	1		1017	1	489	861	1924
GR	19857	1311	2	2339	66	1591	12390	148					12			193					114		1026			253	388	23
AT	11783		26	0		437	8662	3		0		519	0	0		88				2	1018		0	90	458	18	158	305
RO	11441	6918		113	0	2521	488			1						5					6					3	682	706
BG	10861	14				4411	4997			0		18		214			178						1011					18
HU	10183	149				2094	5292						0		0								748		599	877	424	
CZ	10165	1					3543			91				0		0					4		199	4		6064	172	88
PL	8953	3	3		0	3219	2919	2	338	4			77		40	1	1024		602			1	23	225		470		4
IT	5986	2208	12	7	0	320	626	4		5	3	110	15	31	51		1	0		85	2	9	244	2	1891	178	38	144
FR	5830	98	134			109	3800	29	1	112	0		725	0	105	224		149	0		87	13	9	5	7	4	218	0
LT	1454							0	150	29			17		20				1229		4						5	
LV	677				6		0		455	19				11			184										3	
SK	642	11				557	3					2		1	0						3		2		0			63
GB	514					1	0	1		6		3			492	5					4	1		0				
EE	402							0			15						225		162									
IE	353						7						342								3							
SE	247							2			245																	
DK	230						2			24		16	0								4			184				
PT	229						0			207		11						11										
SI	143	0								36						26					68					12		2
LU	134		19				13					102																
FI	54								54										0									
CY	26						0					0	26		0									0				
MT																												

Notes: Product by HS: 070700-cucumbers and gherkins, fresh or chilled; Quantities in tonnes; data extracted on 30/5/2011 from Eurostat Comext database. Example: Spain (ES) reported exports of 433,017 tonnes to the EU MSs and 4,796 tonnes to Austria (AT) in 2010. EU27: total exports to MSs.

Table 5: 2010 imports of “fresh or chilled cucumbers and gherkins”as reported by the MSs of the first column to other MSs

Importer	EU27	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	IE	IT	LT	LU	LV	MT	NL	PT	RO	SE	SI	SK	PL	HU
EU27	1133217	12179	31703	9563	51	11735	69253	267	523	468644	68	6527	2573	27378	411	7694	1358	58	1098	23	452206	203	4959	1154	56	2647	15116	5770
DE	495956	5867	14985	3904	26	4300		17		177140		3181	370	17297	8	612		0	1	23	262730		20				3986	1489
GB	151374		56	190			4235			71821		763			399	5	49				73847						10	
NL	81776	737	8838		0	61	9894	5		61912		222	68	5	4	20								3				7
CZ	75088	383	286	4710			16808			23226		161	7	2790		195					13377		2008	577		2483	5700	2379
FR	68074	951	6185				4127	0		40906			139			103		57			15589	9					9	
BE	50514						1286			4641		168	11	79		15		1			44313							
DK	33494		3				1627			20460		118		136		7					11027			67			48	
PL	31278	1248	26			664	3901	10		17667		587		98		229					5182		1432			8		225
SE	27024		3				1109	126		15228	11	0				0					10536						9	
AT	24496		61	0	6	0	8517			5196		182		2069		4343					3642		95		11	2	0	374
SK	15602	18	2			6244	3365		3	1526		2		328		43					579				1		3248	243
IT	13914	2083	19	2			2709			8091		259		164						0	293		249		44		0	
HU	13140	276				95	7496			2371		490	852	3		2					303		1105			146	3	
FI	10375						2641		12	4475						2					2751			495				
LT	10163		19	182			266		291	4128		0				2			362		3569		6	12			1326	
IE	5616		4				537			982		150	846			15	15				3057						9	
EE	4428						443			2305	57						233		714		382						293	
LV	4057								216	1584	0						1059				763						435	
RO	3732	0		323		71	54			863		0		1720		183					68					3	11	436
SI	3576	615					1			533		7		4		1810					3					5		600
PT	3528		4				0		0	3506		13				1					5							
BG	2665				2	7				68				2574							7		8					
LU	1479		1214				12			15		165				0					63	11						
ES	1272					294	145	108				58	280			15	2		21		82	183	36	0	0		30	18
GR	381	1		253	17		81									25					4							
CY	143												0	111							32							
MT	75															71					4							

Notes: Product by HS: 070700-cucumbers and gherkins, fresh or chilled; Quantities in tonnes; data extracted on 30/5/2011 from Eurostat Comext database. Example: Germany (DE) reported imports of 495,956 tonnes from the EU MSs and 5,867 tonnes from Austria (AT) in 2010. EU27: total imports from the MSs.

Table 6: 2010 exports of “fresh or chilled lettuce”as reported by the MSs of the first column to other MSs

Exporter	EU27	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SE	SI	SK
EU27	355316	16689	12488	160	372	5551	98065	14447	311	5400	6545	53601	33797	1755	4052	3897	33456	1726	1926	508	201	25142	11173	1720	1630	14337	4850	1472
ES	184077	4628	3727	94		2515	51484	2535	36		2430	44358	25976	391	2077	399	16299	249	40	10		13150	5299	1426	272	6493	9	180
NL	59364	735	5611		331	592	21573	3132	209	1954	2577	1178	3290	500	205	704	10319	1343	66	81	102		1346	2	104	3121	2	242
IT	38880	2395	2090	4	8	389	15459	1626	49	414	79	3306	2457	818	293	316		103	0	159	100	3138	742	106	482	567	3736	46
DE	34808	6762	430	29		1501		6543	0	1279	646	2088	102	44	62	4	4435	1	51	11		3699	3493	6	51	3452	64	55
FR	11799	46	630			19	6297	514	2	938	0		1454	1	0	104	804	6	314			457	16	158		39	1	
BE	10700	14		10		511	2296			94	61	2172	12	1			31	1	1454	2		4036	1	0		0		5
AT	4055			1		7	655			0		0	0	0	1389		771					2	140	0	3		1038	49
GB	3321				0	9	41			70		124				2369	38					649	0	22				0
HU	2589	2087					4			1													20		477			0
PT	1428						7	2		644		375	395									4				1		
DK	1273						128		12		8	1	69				384					6				665		
CZ	1266						75			5					2		277					0	14					892
SE	941							96			744												102					
LT	250									2			0			1	0			246		2						
SI	204	7					3										97					0			97			
GR	104			22	29		11										1								41			
BG	102																								102			
PL	65	15			5	6	20				1							14							2			4
IE	50						11						40															
SK	25	0				1	1								23		0						0					
LV	11								2									9										
CY	2												2	1														
FI	2								2											0								
RO	1			0											1													
LU	0		0				0					0																
EE	0										0																	
MT																												

Notes: Product by HS: 070519-lettuce, fresh or chilled (excluding cabbage lettuce); Quantities in tonnes; data extracted on 1/6/2011 from Eurostat Comext database. Example: Spain (ES) reported exports of 184,077 tonnes to the EU MSs, of which 4,628 tonnes to Austria (AT) in 2010. EU27: total exports to MSs.

Table 7: 2010 imports of "fresh or chilled lettuce"as reported by the MSs of the first column to other MSs

Importer	EU27	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SE	SI	SK
EU27	452574	2391	21942	0	0	1469	28118	595	28	240652	8	40980	2234	726	800	946	61434	28	6	69	0	43848	1312	2955	104	1801	74	56
DE	166752	516	5489					50		95816		14975	400	700	80	1	33465		1			15004	257	0				
GB	77255		92		0		2222	15		51839		12040		0		945	4975	1				4354	0	771				
NL	45066	0	7305			4	5201	9		29867		694	282	5	1	0	1453	1	0		0					244		
FR	34726	3	6627				1544			23721			114				1446		0			883		388				
AT	22681		4			0	8350			6324		345		2	17		6920					664	5			0	48	1
BE	22573						833			5705		5299					3246		4			7479		5			3	
DK	17802		4				4205			8021		1922	0				1232					2380				38		
IT	11613	163	47				166			5348		977	32	1				1				4618	16		2	230	12	
ES	10548		1294				353	6				3861	142				1692					1410		1789			1	
PL	7934		59			380	2595	1		2267		176		4	43		991					1399				20		
SE	5165		12				103	491		3001		19	163				741					635		1				
HU	4935	949				328	420			3148							77					7	5		0			1
CZ	4248	10	95				404			1793		1	12		28		309					794	748					54
SI	3914	654					28					10					3221					2						
LT	3311					0	0	24	19	615		1					283			53		2198	116			4		
IE	3036	0	0				446			489		369	1084				12					635						
FI	2686		20				714		2	122		0					155					410	0			1262		
PT	2230		98				5		0	1905		121	5				96					1						
SK	2052	93	2			670	313			201					4		186					438	146				0	
RO	1228	4				88	86					0		0	627		404					4	4				10	
LU	1002		792				12			28		169					0							1				
GR	923		0	0			109			264							327					220	5					
BG	296						8			167				1			1					7	10		102			
EE	249						1			7	8	3					22			16		192				0		
LV	205		1				0		6	5	0						118	25				47				2		
CY	78													13			2					64						
MT	66							0		1						0	61					5						

Notes: Product by HS: 070519-lettuce, fresh or chilled (excluding cabbage lettuce); Quantities in tonnes; data extracted on 1/6/2011 from Eurostat Comext database. Example: Germany (DE) reported imports of 166,752 tonnes from the EU MSs, of which 516 tonnes from Austria (AT) in 2010. EU27: total imports from the MSs

Table 8: 2010 Exports of "fresh or chilled tomatoes"as reported by the MSs of the first column to other MSs

Exporter	EU27	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SE	SI	SK
EU27	2291331	42930	59888	5186	955	85896	715671	36571	9181	115311	21852	230298	346843	17318	18989	28405	89958	20409	5340	10690	1251	164220	95044	31898	17322	76925	13265	29673
NL	874085	9924	25023	165	682	28312	373921	20363	5021	20968	13421	36134	163956	8981	3901	21200	38178	7454	424	6295	587		28968	92	2168	50362	2983	4558
ES	720717	5591	16136	507		24893	156903	8775	1868		5143	127064	136482	246	5813	4494	23708	9389	93	1564	28	101354	36550	31398	1348	15201	1455	4719
BE	178081	983		1	4	8727	60979	45	46	3985	53	50055	9139	3635	359		4482	10	3491	45		25949	3025			112	565	2392
FR	174946	1792	17177	21		11315	62635	2148	574	12123	542		11244	46	1110	315	14647	229	1129	532		15816	16188	406	105	4445	70	338
IT	122133	19007	1002	159	74	2509	40855	3816	18	325	2	8852	11539	2365	2361	48		81	34	50	633	9588	4790		6733	246	6430	617
PT	80698						2			76468		2082	1910	0			20		70				146					
PL	38275	876	1	19	4	6358	4730	33	633	704	444	1778	10756	1322	701	49	458	1681		412		931			2589	2249	5	1545
DE	27956	3903	258	25		811		1008	122	317	2207	2441	35	291	9		7357		100	34		3302	2983	2	207	2543		1
AT	21384		107	0		1110	12378	3		1		523	19	0	2204		177				4	1562	819		0	444	1735	299
CZ	13776	14					23			15					78		0					35	90		165			13357
SI	7832	340				14	1104	44		157			696		10		411					4665	75		206			110
HU	6133	251				124	1544			169			19			0	29	19				693	254		1268		22	1739
GR	4940		133	3705	182	4	214		0			56	10		10							11	411		205			
BG	4433					35	283					56		411	23			618		91			589		2326			
SK	4273	251				1654	39			22					2306	0							1		2		1	
GB	3427		0				22			1		821				2298						279	6					
LV	1709				9		0		732					20				916				1	32					
LT	1603							0		24			3			0				1576								
DK	1488						1			5		152					1					7				1321		
RO	1334			584		29	0			10					104		489						118					0
IE	1073						4			19			1037									13						
SE	365						4	336			9											16						
LU	364		50				31					283																
FI	172								169											1						2		
EE	136							0			32							13		90								
CY	1													1														
MT																												

Notes: Product by HS: 070200-tomatoes, fresh or chilled; Quantities in tonnes; data extracted on 1/6/2011 from Eurostat Comext database. Example: The Netherlands (NL) reported exports of 874,085 tonnes to the EU MSs, of which 9,924 tonnes to Austria (AT) in 2010. EU27: total exports to MSs.

Table 9: 2010 Imports of "fresh or chilled tomatoes"as reported by the MSs of the first column to other MSs

Importer	EU27_INTRA	AT	BE	BG	CY	CZ	DE	DK	EE	ES	FI	FR	GB	GR	HU	IE	IT	LT	LU	LV	MT	NL	PL	PT	RO	SE	SI	SK
EU27	2314564	10702	143608	2617	39	9476	74878	2073	387	692055	198	144370	12354	8563	2788	1003	109279	1479	606	1979	37	941694	32759	111074	463	386	3014	6682
DE	678151	6519	41572	76	7			8		134796		52406	18	164	1525	35	38730		93	1	30	399235	2229				709	
GB	362735		5583				9916			136471		9094				949	13089					174675	12519	439				
FR	224523	146	43626				3063	1		130552			840				8280		156			34854	1620	1385				
NL	156082	148	25997		25	116	5571	95		100408	14	12669	2970	11	129	18	6020			0			466	52	4	35	1337	
ES	142866		3140			207	6301	4				6111	783		20		278	181		39		15996	692	108962		12	141	
PL	90869	6	3135	19		105	6129	2	1	33645		10653	5	262	210		5100	8			1	31466		108	14	1		3
CZ	87232	628	8949	38			6797			21098		10470	95	5	131		2039					27094	6476		6		6	3401
IT	86849	404	3501				8574	1		20824		13108	0	1							1	39573	675	22	21		146	
SE	85437		52				2599	1955		15465	13	3351					283					61207	512					
BE	74617						2141	1		17071		17266	61	6			1087		357		1	36469	156					1
DK	47466		20				1057			16960		3002	0	16			4164					21885	52			310		
AT	45839		712				9194			4687		1034			105		17761					10916	631				522	277
LT	43783		3	575			28		263	7086		249	141		39		329			1051		31440	2544	24			12	
IE	29774	1	37				777			5303		227	7239				25	1				16153	10					
PT	28776		1				39		22	28278		389	14									33						
SK	26584	34	2252			8835	2821			5496		344	92		436		1001					3985	1276				14	
FI	22788		41				2542	7	30	5649		540					2					13460	489			29		
SI	12324	1516	537				0			625		70		59	58		5841					3496	107					15
HU	12036	1283				25	2246			2303		1207	97	94			411					1210	175		0			2985
LV	12001		46	16			28		71	2210	1	574					13	1288				7412	341					
EE	10652		40				9			1633	171	430					13			889		6818	638	11				
GR	9716		1033	45	2		4907			11		1					1510					2084	121		4			
RO	8770	17	19	1848		174	55			1149		40		315	135		2718				5	1191	980				126	
BG	8614		0		6	17	8			324		42		7432			123					209	39		415			
LU	5182		3312				79			10		1094					52					564		72				
CY	507													200			90					205	13					
MT	389	0														2	323					64						

Notes: Product by HS: 070200-tomatoes, fresh or chilled; Quantities in tonnes; data extracted on 1/6/2011 from Eurostat Comext database. Example: Germany (DE) reported imports of 678,151 tonnes from the EU MSs, of which 6,519 tonnes from Austria (AT) in 2010. EU27: total imports from the MSs

Table 10: 2010 EU imports of cucumbers and gherkins from third countries

Country of origin	Quantity (in tonnes)
EU27_EXTRA	37263
Turkey	23693
Former Yugoslav Republic Of Macedonia	4360
Jordan	3480
Morocco	2350
Bosnia And Herzegovina	761
Serbia	684
Albania	591
Syrian Arab Republic (Syria)	573
Egypt	263
Israel	157
Ukraine	111
Lebanon	70
Croatia	62
Iran, Islamic Republic Of	41
Switzerland	33
Dominica	14
Dominican Republic	11
Countries And Territories Not Specified Within The Framework Of Trade With Third Countries	5
India	2
Kenya	2
Thailand	2
Countries And Territories Not Specified In The Framework Of Intra-Community Trade	1
Saudi Arabia	1

Notes: Product by HS: 070700-cucumbers and gherkins, fresh or chilled; Quantities in tonnes; data extracted on 30/5/2011 from Eurostat Comext database. EU27_EXTRA: total EU imports from third countries

Table 11: 2010 EU imports of lettuce from third countries

Country of origin	**Quantity (in tonnes)**
EU27_EXTRA	5319
Tunisia	1552
United States	1254
Egypt	944
Morocco	904
Turkey	355
Israel	186
Norway	34
Former Yugoslav Republic Of Macedonia	25
Argentina	22
Switzerland	18
Countries And Territories Not Specified Within The Framework Of Trade With Third Countries	8
Lebanon	4
Croatia	4
Albania	4
Korea, Republic Of (South Korea)	3
China	2

Notes: Product by HS: 070519-lettuce, fresh or chilled (excluding cabbage lettuce); Quantities in tonnes; data extracted on 1/6/2011 from Eurostat Comext database. EU27_EXTRA: total EU imports from third countries

Table 12: 2010 EU imports of tomatoes from third countries

Country of origin	**Quantity (in tonnes)**
EU27_EXTRA	496997
Morocco	307783
Turkey	108813
Israel	23080
Former Yugoslav Republic Of Macedonia	19254
Tunisia	9850
Senegal	8706
Jordan	6925
Egypt	3680
Albania	3194
Serbia	2297
Syrian Arab Republic (Syria)	1284
Dominican Republic	964
Occupied Palestinian Territory (West Bank - Including East Jerusalem And Gaza Strip)	244
Norway	209
Moldova	163
Switzerland	92
Croatia	88
Colombia	66
Kosovo	50
South Africa	37
Russian Federation (Russia)	28
Countries And Territories Not Specified Within The Framework Of Trade With Third Countries	28
Brazil	23
Mayotte	20
Montenegro	20
Bosnia And Herzegovina	20
Costa Rica	20
Panama	16
China	14
Belarus	6
Ukraine	6
Peru	4
Algeria	4
India	3
Suriname	2
Bangladesh	1
Saudi Arabia	1
Thailand	1
Ecuador	1

Notes: Product by HS: 070200-tomatoes, fresh or chilled; Quantities in tonnes; data extracted on 1/6/2011 from Eurostat Comext database. EU27_EXTRA: total EU imports from third countries

B. EU CONSUMPTION DATA OF ALFALFA SPROUTS, TOMATOES, LETTUCE AND CUCUMBERS

Table 1: EU consumption data by age category and gender for alfalfa sprouts cucumbers, lettuce (two type groups) and tomatoes expressed in average grams per day. Data retrieved from the EFSA comprehensive European Food Consumption Database. Full details on variables and survey methodology available at EFSA, 2010. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal, 9(3):2097, 1-34. url: http://www.efsa.europa.eu/en/efsajournal/pub/2097.htm.

Age category	**Gender**	**Alfalfa sprouts, fresh (Medicago sativa)**	**Cucumbers (Cucumis sativus)**	**Iceberg-type lettuce**	**Lettuce, excluding Iceberg-type lettuce (Lactuca sativa)**	**Tomatoes (Lycopersicum esculentum)**	**Grand Total**
Infants	Female	0	20.5	-	25.0	13.8	18.3
	Male	0	19.1	-		12.2	14.5
Toddlers	Female	0.000144113	19.1	6.6	8.0	22.6	16.2
	Male	7.20565E-05	19.4	6.3	12.0	22.2	17.4
Other children	Female	0.004397489	25.9	12.3	11.8	29.8	22.4
	Male	0.001259107	27.9	10.2	12.6	29.6	22.4
Adolescents	Female	0.002083333	22.5	11.6	20.0	43.0	27.2
	Male	0.000160875	26.1	9.4	20.0	45.3	28.8
Adults	Female	0	23.5	16.7	20.1	48.5	28.5
	Male	0.108977979	24.3	15.9	20.3	50.9	29.3
Elderly	Female	0.054488989	28.8	19.2	101.0	47.3	53.6
	Male	0	26.8	25.6	21.0	51.8	32.0
Very elderly	Female	0	26.5	17.0	22.6	42.1	28.6
	Male	0	23.2	19.6	22.6	52.0	31.1
Grand Total		0.007441681	24.7	14.0	23.6	38.8	27.2

- = No data available