Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on preventative measures and applicable recommendations for avoiding possible food-borne infections caused by strains of verotoxigenic/Shiga toxin-producing/enterohemorrhagic *Escherichia coli* (VTEC/STEC/EHEC)

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Reference number: AESAN-2012-007

Report approved by the Scientific Committee on plenary session September 19th, 2012

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Abstract

Escherichia coli is the predominant species of normal aerobic and facultative anaerobic microbiota found in the digestive tract of many species of animals, and it is excreted from the body through faeces. It can be found outside the body as it can survive for a certain period in water and in food. Its isolate in these elements is indicative of faecal contamination. Although most strains of *E. coli* are commensals and even beneficial, some are pathogens and can cause serious enteric infections (diarrhoea, hemorrhagic colitis, haemolytic uraemic syndrome) or extraintestinal infections (urinary tract infections, bacteraemia or septicaemia, meningitis, peritonitis, mastitis, and respiratory and wound infections) in humans and animals.

The verotoxigenic *E. coli* (VTEC) group, also known as Shiga toxin-producing *E. coli* (STEC) and enterohemorrhagic *E. coli* (EHEC), especially the highly virulent strains of the serotype O157:H7, is a dangerous group of pathogens which causes very serious diseases in human beings: hemorrhagic colitis (HC) and the haemolytic uraemic syndrome (HUS). Ruminants, especially cattle, are the main reservoir of this type of micro-organism, while the primary means of transmission are minced meat, hamburgers and vegetables which are consumed raw or lightly cooked.

The necessary preventative measures, covering the whole food chain, should consequently be put in place. These include: good agricultural practices, biosafety programmes in farms with livestock, good hygiene and abattoir inspection practices, and good practices in vegetable processing for fresh consumption. Also, workers in the food industry must adopt procedures based on the principles of Hazard Analysis and Critical Control Points (HACCP), and consumers must be taught the good practices of preserving and cooking food. All of these measures should help minimise the incidence of this food poisoning in human populations. These measures should, furthermore, be combined with specific analysis protocols which enable quick and accurate detection of the strains involved, and

Translated from the original published in the journal: Revista del Comité Científico de la AESAN, 16, pp: 71-100

more specifically, the highly virulent strains belonging to the serotypes: O104:H4, O157:H7, O26:H11, O103:H2, O111:H8, O121:H19 and O145:H-.

Key words

Enterohemorrhagic *E. coli*, enteroaggregative *E. coli*, Shiga toxin, O104:H4, O157:H7.

Introduction

1. Basis of the request

Escherichia coli is an ubiquitous enterobacteria and it is the predominant species of normal aerobic and facultative anaerobic microbiota found in the digestive tract of most mammals and birds. A high percentage of *E. coli*, amply excreted in faeces, can survive outside the body, even for long periods. Therefore, it can be present outside the body and its isolation can be an indicator of faecal contamination. Although most *E. coli* strains are non-pathogenic members of the host's intestinal microbiota and play a harmless or even beneficial role therein, some strains are pathogens due to the acquisition of specific virulence factors which give them the capacity to produce a wide range of infections in human beings and animals. These infections can be enteric (diarrhoea, dysentery, hemorrhagic colitis, haemolytic uraemic syndrome and oedema disease) as well as extraintestinal (urinary tract infections, bacteriemia or septicaemia, meningitis, peritonitis, mastitis, and respiratory and wound infections).

The diarrheagenic *E. coli* have been divided into six groups or categories, based on their mechanisms of pathogenesis and virulence factors:

1. Enteropathogenic *Escherichia coli* (EPEC).
2. Enterotoxigenic *Escherichia coli* (ETEC).
3. Enteroinvasive *Escherichia coli* (EIEC).
4. Enterohemorrhagic, verotoxigenic or Shiga toxin-producing *Escherichia coli* (EHEC/VTEC/STEC).
5. Enteroaggregative *Escherichia coli* (EAEC).
6. Diffusely adhering *Escherichia coli* (DAEC).

The EHEC/VTEC/STEC group is a group of *E. coli* strains which can produce toxins similar to those produced by *Shigella dysenteriae* type 1. Two types of toxins have been described in this group: the Shiga 1 (Stx1) toxin or verotoxin 1 (VT1), which differs from the true Shiga toxin in one to seven amino acids and the Shiga 2 (Stx2) toxin or verotoxin 2 (VT2), which is 60% homologous to Stx1. Despite the differences existing between these and the true Shiga (Stx) toxin, all Stx1 and Stx2 toxins are considered as belonging to the Shiga toxin family, giving rise to the name Shiga toxin-producing *E. coli* (STEC). Active Stx toxins can be detected by carrying out a toxicity test on Vero cells, which is why this group is called verotoxigenic or verotoxin-producing *E. coli* (VTEC). The *E. coli* Shiga toxins (verotoxins) cause different symptoms in human beings, ranging from mild diarrhoea to hemorrhagic colitis (HC), and can cause increasingly serious diseases, such as haemolytic uremic syndrome (HUS) with microangiopathic haemolytic anaemia, thrombocytopenia and severe acute renal failure. This clinical picture has resulted in this *E. coli* group being named enterohemorrhagic *E. coli* (EHEC).

Ruminants have been identified as the main reservoir of verotoxigenic or Shiga toxin-producing *E. coli* strains (henceforth STEC). While cattle, however, is probably the main source of human infection (meat, dairy products from cattle, and vegetables and water contaminated by cattle faeces), STEC has also been isolated in sheep and goats as well as in wild ruminants (roe deer, deer and fallow deer). Ruminant faecal wastes are reconniced as the last source of a large percentage of human STEC-related infections. It can contaminate meat during slaughter in the abattoir, it can be swept away to rivers, lakes or sources of drinking water or it can be deposited on fruit and vegetables through the use of

organic manure or irrigation water contaminated with waste water. Some animals such as insects, birds, rodents and other wild animals can transport these bacteria from faeces to drinking water and food. In addition, STEC strains can be unknowingly ingested by people who interact with or work with animals.

Human beings can therefore be infected through direct contact with an infected person or a carrier animal, or indirectly through the environment, food, drinking water or surface water containing STEC-contaminated faecal material of human or animal origin. Therefore, preventative measures to avoid STEC-related infections should be implemented across the whole food chain. These include adopting good agricultural practices and biosafety programmes in farms with livestock, hygiene in abattoirs, and educating consumers and those who handle animals. This can be done by using self-monitoring measures and food safety management techniques aimed at controlling this dangerous agent. This should, furthermore, be combined with specific analysis techniques which enable quick and accurate detection of the strains involved.

Although Decision No. 2119/98/EC (EU, 1998) established the Network for the Epidemiological Surveillance and Control of Communicable Diseases in the Community, it was not until Decision No. 2009/312/EC (EU, 2009), regarding dedicated surveillance networks for communicable diseases, that enterohemorrhagic *E. coli* was mentioned as a food-borne disease. As a result of this regulation, details of cases of STEC-related infections in humans must be reported to the European Surveillance System (TESSy) on a quarterly basis, although it should be noted that most countries only monitor the serogroup O157. According to the European Food Safety Authority (EFSA), this monitoring should be extended to other serogroups, mainly O26, O103, O104, O111 and O145, which have been identified as the most frequently occurring in the periodic analyses carried out in Europe.

Commission Regulation (EC) No. 2073/2005 (EU, 2005) and its amendment (Commission Regulation (EC) No. 1441/2007 (EU, 2007)), regarding microbiological criteria for foodstuffs, established a food safety criteria, which remains current, for *E. coli* in live bivalve molluscs and in live echinoderms, tunicates and gastropods, during their lifetime. *E. coli* is a micro-organism which is also used as a hygiene criterion, as an indicator of faecal contamination and/or of the hygiene level of establishments where different types of foodstuffs are produced. Such foodstuffs include chopped ready-to-eat fruit and vegetables, cheese made with milk or whey subjected to heat treatment, butter and cream made from raw milk or milk subjected to heat treatment less intense than that of pasteurisation, minced meat, mechanically separated meat, meat preparations, etc.). In order to use *E. coli* this way, a plate count is carried out using a validated microbiological method (the Regulation establishes the ISO reference method which should be used for the tests). On the other hand, point 14 of Regulation (EC) No 2073/2005 indicates it is unlikely that the application of microbiological criteria for STEC O157 in the final product lead to a significant reduction of the associated risk for consumers. However, establishing microbiological guidelines aimed at reducing faecal contamination in the entire food chain could reduce public health risks, including those induced by the presence of STEC.

2. Terms of reference

The Scientific Committee is required to perform an assessment on the possible prevention mechanisms and recommendations applicable to avoid food-borne infections caused by *E. coli* and, in particular, STEC.

Assessment

1. Hazard identification

E. coli are small rod-shaped gram-negative bacilli, facultative anaerobes, motile by means of peritrichous flagella and they do not form spores. Their development is good in ordinary environments at temperatures of between 20 and 40 ºC and a pH of between 6 and 8. It is a natural inhabitant in the intestine of mammals and birds, being amply excreted on a daily basis in the faeces. A high percentage of these microorganisms then survive outside the body, at least initially. Therefore, their environmental presence is possible since they are capable of surviving some time in water and foodstuffs. Their isolation is therefore an indicator of recent faecal contamination.

Using the antigenic classification system proposed by Kauffman, around 180 somatic antigens (O1 to O185) have been recognised in *E. coli* along with 56 flagellar antigens (H1 to H56) (EFSA, 2011). Furthermore, although the possible O:H combinations are numerous, only a few are present among pathogenic strains (http://www.usc.es/ecoli/E.coli2.html). These antigens are determined by agglutination techniques using absorbed antisera to avoid cross reactions (Guinée et al., 1981) (Orskov and Orskov, 1984) (Ewing, 1986).

Pathogenic strains of *E. coli* have different types of virulence factors that collectively contribute to increase their pathogenicity. The virulence of *E. coli* is therefore considered a multi-factor phenomenon. The most salient of these virulence factors is the ability to produce toxins, the expression of adhesins that allows for adhesion to body surfaces, the ability to invade different cells or their resistance to serum and phagocytosis.

Although strains of *E. coli* causing infection in humans and animals can share certain virulence factors, they generally present different serotypes and contain specific adhesins, which are the cause of its host specificity. Therefore, strains of *E. coli* pathogens in human beings do not usually cause infection in animals and vice versa. However, it has been verified that animals can act as a carrier of *E. coli* pathogens for humans. Consequently, the verotoxigenic *E. coli* (STEC) that cause hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) in humans form part of the normal microbiota of ruminants, in most cases as commensals.

Based on pathogenesis mechanisms and virulence factors, the strains of *E. coli* causing diarrhoea are classified into the following six pathotypes:

1. Enteropathogenic *Escherichia coli* (EPEC).
2. Enterotoxigenic *Escherichia coli* (ETEC).
3. Enteroinvasive *Escherichia coli* (EIEC).
4. Enterohemorrhagic, verotoxigenic or Shiga toxin-producing *Escherichia coli* (EHEC/VTEC/STEC).
5. Enteroaggregative *Escherichia coli* (EAEC).
6. Diffusely adhering *Escherichia coli* (DAEC).

Of these, EHEC/VTEC/STEC are those most related to food-borne illness (Nataro and Kaper, 1998) (Lee, 2004) (FSAI, 2005) (EFSA, 2007a) (EFSA, 2007b).

The STEC strains that cause infections in humans belong to a large number of O:H serotypes (over 400 different serotypes have been documented) (http://www.usc.es/ecoli/SEROTIPOSHUM.htm). The

majority of HUS cases and outbreaks have been attributed to O157:H7. As a result, given this serotype's clinical importance, it is common to talk of two categories within STEC: STEC O157 and non-O157 STEC.

All together the European Union Member States (EU) reported 16,263 cases of human infection caused by STEC from 2005 to 2009. Some 3,573 cases were declared in 2009, signifying a 13% increase compared to 2008. The notification ratio for the EU in 2009 was 0.75 for every 100,000 inhabitants. In 2010, a total of 4,000 confirmed cases of VTEC in humans were reported by 25 Member States, signifying an increase of 12.0% compared to 2009 (3,573). The EU notification rate was 0.83 for every 100,000 inhabitants, which was also slightly higher than in 2009 (0.75 per 100,000 inhabitants) (EFSA/ECDC, 2012).

The serogroup O157 was the most frequently declared in 2008 and 2009, representing 52% of confirmed cases of established serotypes. When analysing these data it must be take into account that many laboratories centre their diagnostic techniques solely on the detection of STEC O157 (ECDC/EFSA, 2011). Like in previous years, in 2010 the most commonly identified VTEC serogroup was O157 (N= 1,501) with a decrease of 18.8% compared to 2009 (N= 1,848).

Ruminant faecal matter is aconsidered to be the original source of a large percentage of human STEC-related infections. The faecal wastes can contaminate meat during slaughter in the abattoir, it can be swept away to rivers, lakes or sources of drinking water, or it can be deposited on fruit and vegetables through the use of organic manure or irrigation water contaminated with waste water. Some animals such as insects, birds, rodents and other wild animals can transport these bacteria from faeces to drinking water and food. Human beings can therefore be infected through direct contact with an infected person or a carrier animal, or indirectly through the environment, food, drinking water or surface water containing STEC-contaminated faecal material of human or animal origin (Mora et al., 2011).

Foods most frequently associated with outbreaks and illness related to *E. coli* O157:H7 in humans include: fresh fruits and vegetables (lettuce, radish, alfalfa, etc); non-pasteurized apple and orange juice in which the microorganism can survive for various days despite having a pH of 3.4; partially cooked meat like under-cooked burgers; contaminated water; freshly drawn milk and milk based products like mayonnaise, yogurt and cheeses (Riley et al., 1983) (Lee, 2004) (WHO, 2005) (EFSA, 2007b) (WHO, 2008).

2. Hazard characterisation

The virulence characteristics of the STEC group vary depending on the strain but in general by definition they all produce either one or both of the verotoxins VT1/Stx1 and/or VT2/Stx2 that are codified in the genomic prophage. In addition, various variants have been documented in each one of the Stx branches based on their phenotype differences, their biological activity and the hybridisation properties. There are different naming systems for Stx variants and their coding genes, but in the *7th International Symposium on Shiga Toxin (Verocytotoxin)-producing Escherichia coli infection* in Buenos Aires in 2009, a naming consensus was reached. Two types of Stx were established (Stx1, Stx2), along with three Stx1 subtypes (Stx1a, Stx1c, Stx1d) and seven Stx2 subtypes (Stx2a, Stx2b, Stx2c, Stx2d,

Stx2e, Stx2f, Stx2g). The capacity of STECs to cause human illness is clearly linked to toxin type and subtype. Stx2 is the most powerful toxin and the subtypes Stx2a and, albeit to a lesser extent, Stx2d and Stx2c are the most commonly associated with HUS (Friedrich et al., 2002) (Feng et al., 2011).

STEC strains can present additional virulence factors. The most important is intimin, an external membrane protein responsible for the intimate adhesion of bacteria to the intestinal epithelium. Intimin is codified in the *eae* gene which forms part of the chromosome and pathogenicity island called *locus for enterocyte effacement* (LEE). The *eae* gene is present in the strains of some of the most virulent serotypes: O157:H7, O26:H11, O103:H2, O111:H8 and O145:H-. Another important virulence factor is the codified enterohaemolysin *(ehxA)* in the EHEC plasmid. The presence of the LEE and the EHEC plasmid are markers of the classic enterohemorrhagic strains of the main serotypes involved in 80% of the cases of HC and HUS in Europe and the United States (Garmendia et al., 2005) (Garrido et al., 2006) (EFSA, 2007b) (Mora et al., 2009). In fact, statistical analyses indicate that the concomitant presence of some of the Stx2a, Stx2c, Stx2d toxins, along with the LEE *(eae)* and the EHEC plasmid *(ehxA)* are predictors of serious clinical symptoms in patients. As well as LEE, effector genes *(nle)* have been documented that are not codified in the LEE, they are localised in the chromosomal island (CI) and are strongly associated with HUS and HC. Different studies indicate that an efficient intestinal colonisation system is a pre-requisite for the onset of serious illness in humans (Beutin and Martin, 2012).

Karmali et al. (2003) proposed the classification of STEC strains into five seropathotypes (seropathotypes A to E), according to incidence and association to HUS cases and outbreaks. Seropathotype A includes highly virulent strains of serotypes O157:H7 (non-sorbitol-fermenting) and O157:H- (H-non-mobile, sorbitol fermenters), which have been the cause of numerous outbreaks and are frequently associated with HUS; infectious dose for strains of this type of serotype is between 10 and 100 cells. Seropathotype B includes serotype strains that have caused occasional outbreaks and they are relatively common in cases of HUS and HC (O26:H11, O103:H2, O111:H8,H-, O121:H19, O145:H-). Seropathotype C brings together those serotype strains that have not been involved in outbreaks but have been isolated from patients with HUS and CH (O5:H-, O91:H21, O104:H21, O113:H21, O121:H-, O165:H25 and others). Seropathotype D includes serotype strains that have never been associated with HUS but have been isolated from patients with diarrhoea and HC (O7:H4, O69:H11, O103:H25, O113:H4, O117:H7, O119:H25, O132:H-,O146:H21, O171:H2, O172:H-,O174:H8 and others). Finally, seropathotype E includes many isolated serotypes in animals, foods and the environment in general that have not been involved in any clinical cases in humans. For these reasons it is important to determine the STEC strain serotype in order to identify its potential pathogenic risk (Blanco, 2012).

The STEC serotype O157:H7 was identified for the first time in 1975 in a patient in California suffering from bloody diarrhoea. It was associated with an outbreak of food-borne infection (minced meat) in the United States in 1982 (Kaspar et al., 2009). Cases were then detected in Japan (WHO, 2005) (CDC, 2006), England (EC, 2002a) and North America due to juice consumption (USFDA, 2001) and contaminated spinach (Grant et al., 2008). In accordance with published data, non-O157 STEC strains were for the first time documented as a possible cause of sporadic cases of HUS in France in 1975. Hospital records refer to the presence of serotype O103 STEC strains in patients. The first

detected outbreak of non-O157 caused by serotype O145:HNM took place in 1984 but the vehicle of infection could not be determined.

In 2009 (EFSA, 2011) the total number of confirmed STEC cases in the EU reported by TESSy stood at 3,573; although this figure suggests an increase of 13.1% compared to confirmed cases in 2008, the EFSA report indicates that there was no increase in the number of cases in the EU from 2005 to 2009. In those cases confirmed in 2009 in the EU, 51.7% of isolates were of O157 whereas in 2010, was 41.1%. The second most frequently detected serogroup was O26 (7% of isolates); and in total, 18 different serogroups were detected with 33.7% of non-typeable isolated strains. In the EU data brought together by ECDC/EFSA (2011), a notable increase has been detected in the number of cases involving HUS; in 2009 the number of cases was 242 (66% higher than the 146 cases in 2008). In 2010, a total of 4,000 confirmed cases of VTEC in humans were reported by 25 Member States, signifying an increase of 12.0% compared to 2009 (3,573), (EFSA/ECDC, 2012). In the EU data brought together by EFSA/ECDC (2011) it was verified an increment in the SUH cases, in 2009 the number of cases was 242, being a 66% higher than those in 2008, with 146 cases reported. In 2010 a total of 4,000 confirmed cases of VTEC in humans were declared in 25 member States. This figure represents an increase of 12% regarding the reported cases in 2009 (3,573 cases) (EFSA/ECDC, 2012). Considering the data compiled by EFSA/ECDC (2012) for the EU it was observed that the number of SUH cases reported in 2010 was 222, lightly lower than that reported in 2009 (242) but higher that that for 2008 (146 cases)

The number of sporadic cases of non-O157 STEC far exceeds the number of outbreaks. The same is the case for *E. coli* O157:H7. What is more, comparing the data from outbreaks caused by O157:H7 and non-O157, the non-O157 STEC appears to be much less frequently associated with meat, water and vegetables as transmission vehicles and more frequently associated with person-to-person contact or unknown vehicles. These differences are probably in part due to greater availability of analysis methods for O157:H7. In addition, *E. coli* O157:H7 behaves in a more pathogenic manner compared to other non-O157 strains, meaning that outbreaks are recognised and investigated in depth a lot more quickly (Kaspar et al., 2009).

The groups most vulnerable to STEC O157:H7 are children under the age of 15 years and people over 70 years (EFSA, 2007a, 2007b). The average pathogen incubation period is between one and eight days (Lee, 2004) (EFSA, 2007b). The minimum infective dose for STEC O157:H7 is less than 100 cells (Lee, 2004).

In Germany in May 2011 there was a serious outbreak of 3,842 cases of infection in humans in which 855 developed HUS and 53 died. Almost at the same time a much smaller outbreak occurred near Bordeaux in France (15 cases of bloody diarrhoea, 9 of which developed into HUS) (Frank et al., 2011). The characteristics of the strain involved in both outbreaks (STEC and EAEC O104:H4), a Shiga toxin-producing *E. coli*, belonging to serotype O104:H4 with common virulence factors with enteroaggregative *E. coli*, have changed the perception of STEC as human pathogens (Beutin and Martin, 2012). EAEC strains of serotype O104:H4, whether or not producers of Stx toxins, have only been isolated on occasions. Therefore, to date it is not considered an important serotype (ECDC/EFSA, 2011) (Beutin and Martin, 2012). One of the most surprising things about the outbreak caused by STEC O104:H4 in Germany and France was the age of those affected: mainly adults whereas the 0 to 9 years

age group was less affected. This is a contrast to the outbreaks and sporadic cases caused by classic STECs. The prevalence of women developing HUS (68%) after infection has not yet been possible to explain (Frank et al., 2011).

The fact that the number of women affected is much higher than normal in the case of other outbreaks caused by STEC, in which children are mainly infected, can be attributed in the most part to culinary habits. After all, women consume much more salads with bean sprouts than children. All this information confirms that the hypervirulent strain responsible for the outbreaks in Germany and France is an atypical strain in many aspects (Blanco, 2012), such as:

a) It is a hybrid strain with two pathotypes since it is both enteroaggregative and a producer of the Shiga toxin subtype Stx2a. The combination of these pathotype's virulence genes is not frequent and had only been previously observed in one strain of another serotype (O111:H2) responsible for a small outbreak in children in France developing into HUS.
b) It belongs to a serotype (O104:H4) and a sequence type (ST678) very rarely observed up to now in humans and never observed in isolated strains from animals or foodstuffs. We have references of this serotype in isolated STEC strains in Germany (2001), France (2004), Korea (2005), Republic of Georgia (2009), Italy (2009) and Finland (2010). We know that strains in Germany, the Republic of Georgia, Italy and Finland were also enteroaggregative and producers of Stx2a. The strain in Korea is not enteroaggregative and is a producer of Stx1 and Stx2. It is not yet known whether the strain in France is enteroaggregative. Despite not being producers of Shiga toxins, enteroaggregative strains of serotype O104:H4 have also been isolated in the Central African Republic (1995/1996), Denmark (2000) and recently in the Republic of Mali (2009) and Spain (1996) (data not published).
c) It is lacking the chromosomal pathogenicity island known as "*Locus Entorocyte Effacement*" (LEE) carrying the *eae (attaching and effacing)* gene present in the more virulent STEC strains of serotypes A and B.
d) It is also different from the majority of STEC strains in that it is multiresistant, being a producer of the CTX-M-15 extended-spectrum beta-lactamase also present in the intercontinental clone of *E. coli* O25b:H4-ST131.
e) As well as STEC and EAEC virulence genes, the strain has some virulence factors of extraintestinal pathogenic *E. coli* (ExPEC).

The extent of STEC O104:H4 virulence is not surprising if we bear in mind the great amount of virulence genes contained within the strain responsible for outbreaks in Germany and France. However, it is unknown why the O104:H4 strain is so virulent. It has been speculated that its enteroaggregative capacity allows for better colonisation of the intestinal epithelium and that it has mechanisms allowing for the production and liberation of toxin Stx2a in greater quantities. It has at least three adhesins (AAF/I, LPF-lpfAO26 and Iha), two siderophores (aerobactin and yersiniabactin) and three types of SPATE proteases (Pic, Sig A and SepA). The combination of these three types of proteases occurs in very few strains (Blanco, 2012).

It is believed that it is a new strain of probable human origin that has recently emerged through the enteroaggregative strain (EAEC) of serotype O104:H4 that has acquired a phage carrier of the

stx2a gene through a STEC strain. From another strain it would acquire the plasmid that codifies for the CTX-M-15 enzyme. This was deduced after comparing the complete genome of various isolates of the strain responsible for the outbreak in Germany with the genome of enteroaggregative strains of serotype O104:H4 and other serotypes as well as with the genome of strains of other pathotypes of diarrheagenic *E. coli* (ETEC, EPEC, EIEC) (Blanco, 2012).

EAEC strains cause prolonged diarrhoea mainly in children and travellers coming from the third world but are also one of the main causes of diarrhoea in Europe, including Spain (Blanco et al., 2006). Their reservoir is human beings only. They display a plasmid (pAA) codifying a fimbrial adhesin responsible for the enteroaggregative adherence pattern to HEp-II cells and the intestinal epithelium. The strain causing the outbreaks in Germany and France has the fimbria AAF/I, whereas the enteroaggregative strain and producer of Stx2a isolated in Germany in 2001 has the fimbria AAF/III and was sensitive to antibiotics (Bielaszewska et al., 2011).

Against this backdrop it is important to consider the situation in Spain with regards to these microorganisms. This was done through studies carried out in some regions where cattle farming is important to the economy. In Spain between 1992 and 2011, a total of 14,653 stool cultures were examined for the presence of O157:H7 and non-O157 STEC. STEC strains were detected in 415 (2.8%) of the examined stool cultures. In total, 65 (0.4%) cases of infection due to STEC O157:H7 were detected and 271 (1.8%) cases of infection due to non-O157 STEC (Table 1). The serotype O26:H11 was the most commonly observed in the cases of non-O157 (Table 2). None of the verotoxigenic strains isolated in Galicia from humans from 1992 and 2011 belonged to the serotype O104:H4 involved in the outbreaks in Germany and France. The STECs are the third most frequently identified enteropathogen in the stool cultures taken in Lugo Hospital after *Salmonella* and *Campylobacter*, causing a significant number of infections in this health jurisdiction. From these data from Lugo we can deduce that STEC O157:H7 produces more than 500 cases of infection in humans and that non-O157 cause more than 200 cases each year in Spain (Blanco et al., 2004a) (Mora et al., 2011).

Table 1. Prevalence of ECVT in stool cultures taken from patients at Lucus Augusti Hospital

No of stool cultures taken from adults and children with diarrhoea							
Year	**Total stool cultures analysed**	**STEC**					
		Isolated O157:H7		**Isolated Non-O157**		**Total Detected Cases**	
1992-1999	5,054	24	0.5%	87	1.7%	126	2.5%
2003-2005	3,970	12	0.3%	75	1.9%	144	3.6%
2006-2010	4,692	27	0.6%	85	1.8%	119	2.5%
2011	937	2	0.2%	24	2.6%	26	2.8%
Total	14,653	65	0.4%	271	1.8%	415	2.8%

Source: (Mora et al., 2011) and non-published updated data.

Table 2. Most frequent serotypes in STEC strains in humans in Spain

Serotype	Phylogenetic group	Type sequence	Seropathotype	Intimin
O5:H-	A	ST342	C	ß1
O26:H11	B1	ST21	B	ß1
O103:H2	B1	ST17	B	ϵ1
O111:H8	B1	ST16	B	θ
O113:H21	B1	ST56	C	-
O118:H16	B1	ST21	-	ß1
O145:H-	D	ST32	B	γ1
O146:H21	B1	ST442	D	-
O157:H7	D	ST11	A	γ1

Source: (Mora et al., 2011).

3. Exposure Assessment

Food Contamination Pathways

STEC strains can withstand minimum temperatures of 7 to 10 °C and maximum temperatures of 50 °C. Their optimum temperature for growth is 37 °C. Unlike the other *E. coli* strains, they are also the most acid resistant as they can survive in a pH of less that 4.4 and are capable of growing in foods with a water activity of 0.95 (Lee, 2004) (EFSA, 2007b) (WHO, 2008). Established growth conditions for *E. coli* O157:H7 are minimum temperatures of 2.5 to 6.5 °C, maximum temperatures of 44 to 45 °C, a pH of 4 to 9. It tolerates salt concentrations of up to 8% (FSAI, 2005) (WHO, 2008).

Bovine livestock is the most significant source of human infections (beef, dairy products, bovine faecal contamination, etc). STEC O157 and non-O157 STEC prevalence data vary from country to country both in terms of dairy livestock (0.4-74%) and livestock for meat consumption (2.1 to 70.1%). Table 3 shows STEC prevalence in slaughter livestock (EFSA/ECDC, 2011, 2012). Livestock is usually a carrier of various serotypes, some of which do not appear to be of high risk to humans as they do not present any significant virulence factors. STECs are not considered as pathogenic for ruminants except when infection occurs in young animals before weaning (linked to neonatal diarrhoea) (Gyles, 2007) (ECDC/EFSA, 2011) (Mora et al., 2011). A study conducted in Germany found a positive association between infections caused by different STEC serotypes and the density of livestock populations in a given area. Using data of more than 3,000 cases of STEC, analyses indicated that the risk of infection increased to 68% for every 100 additional animals per km^2 (Friesema et al., 2010).

Table 3. STEC prevalence data in bovine and beef meat and livestock in the EU (2007-2010)

		2007			2008			2009			2010		
Animal/ Food category	**Number of Member State***	**N**	**STEC**	**STEC O157**	**N**	**STEC**	**STEC O157**	**N**	**STEC**	**STEC O157**	**N**	**STEC**	**STEC O157**
Beef	12-14	14,115	0.3%	0.1%	14,598	0.3%	0.1%	9,285	2.3%	0.7%	8,566	0.5%	0.1%
Lamb	3-5	285	1.8%	0%	1,263	0.7%	0%	248	3.2%	0%	394	7.4%	0%
Beef (animals)	9-12	5,154	3.6%	2.9%	5,368	2.2%	0.5%	5,555	6.8%	2.7%	6,800	13.5%	0.2%
Lamb (animals)	4-8	533	0.9%	0.4%	671	3.1%	1.6%	324	20%	0.3%	773	30%	0%

*Total values correspond to data supplied by the corresponding number of European Union Member States. **Source:** (EFSA/ECDC, 2011) (EFSA/ECDC, 2012).

A study carried out in the city of Lugo discovered that in recent years there has been a significant decrease in STEC prevalence in minced beef on sale. From the 1,539 samples analysed between 1995 and 2012, STEC O157:H7 was detected in 9 cases (0.6%) and non-O157 in 150 cases (9.7%). The fact that no positive sample for the highly virulent serotype O157:H7 was detected between 2005 and 2009 is especially important from a public health point of view. None of the verotoxigenic strains isolated in Lugo belonged to the serotype O104:H4 involved in the outbreaks in Germany and France (Table 4).

Table 4. Prevalence of STEC O157:H7 strains and other serotypes in minced beef in Lugo

Year	Samples	STEC O157:H7		Non-O157 STEC		Total	
1995	58	3	5.0%	8	14%	10	17%
1996	91	0	0.0%	8	9%	8	9%
1997	173	1	0.6%	20	12%	21	12%
1998	133	1	0.8%	18	14%	18	14%
2001	80	1	1.3%	6	8%	7	9%
2002	20	0	0.0%	1	5%	1	5%
2003	230	2	0.9%	29	13%	30	13%
2005	250	0	0.0%	29	12%	29	12%
2006-2007	160	0	0.0%	14	9%	14	9%
2008	170	0	0.0%	11	6%	11	6%
2009	100	0	0.0%	4	4%	4	4%
2011	40	1	2.5%	0	0%	1	3%
2012	34	0	0.0%	2	6%	2	6%
Total	1,539	9	0.6%	150	9.7%	156	10.1%

Source: (Mora et al., 2007) (Mora et al., 2011).

Table 5 shows STEC prevalence data in meat packaged in a modified atmosphere in a new study conducted between 2009 and 2011. A serotype O104:H7 strain with *stx2* gene was detected in one of the sample. This type of strain would have proven positive in the commercial and real time PCR tests designed for detecting the STEC O104:H4 strain responsible for the outbreaks in Germany and France since they are based on detection of genes that codify the antigen O104 and the toxin Stx2. Thus, the alarm would have been raised despite the fact that the strain present in meat was serotype D and therefore of low virulence. This confirms the importance of serotyping and characterisation of gene virulence, also known as PFGE, which allows for the comparison of the causes of infection in humans with the view of establishing virulence levels.

Table 5. Detection of STEC in beef, pork and chicken in Lugo

*Beef (n=140/October 2011-April 2012)				
Beef-Fresh Minced Meat				
			STEC	
NMP	**Totals**	**%**	**O157:H7**	**no-O157**
<10	59	79.7%	1	0
10-99	10	13.5%	0	1
100-999	3	4.1%	0	0
>999	2	2.7%	0	1
Total	**74**	**-**	**1**	**2**
			(1.4%)	**(2.7%)**
Beef-Meat packaged in a protective atmosphere				
			STEC	
NMP	**Totals**	**%**	**O157:H7**	**no-O157**
<10	45	68.2%	0	4
10-99	16	24.2%	1	3
100-999	4	6.1%	1	4
>999	1	1.5%	0	1
Total	**66**	**-**	**2**	**12**
			(3.0%)	**(18.2%)**

*Pork (n=170/January 2011-May 2012)				
Pork-Fresh Minced Meat				
			STEC	
NMP	**Totals**	**%**	**O157**	**no-O157**
<10	92	68.7%	0	3
10-99	24	17.9%	0	2
100-999	14	10.4%	0	3
>999	4	3.0%	1	3
Total	**134**	**-**	**1**	**11**
			(0.7%)	**(8.2%)**
Pork-Meat packaged in a protective atmosphere				
			STEC	
NMP	**Totals**	**%**	**O157**	**no-O157**
<10	33	91.7%	0	1
10-99	3	8.3%	0	1
100-999	0	0.0%	0	0
>999	0	0.0%	0	0
Total	**36**	**-**	**0**	**2**
			(0.0%)	**(5.6%)**

*Chicken (n=200/September 2009-December 2010)				
Chicken-Fresh meat				
			STEC	
NMP	**Totals**	**%**	**O157**	**no O157**
<10	84	49.4%	0	1
10-99	66	38.8%	0	1
100-999	20	11.8%	0	0
>999	0	0.0%	0	0
Total	**170**	**-**	**0**	**2**
			(0.0%)	**(1.2%)**
Chicken-Meat packaged in a protective atmosphere				
			STEC	
NMP	**Totales**	**%**	**O157**	**no-O157**
<10	5	16.7%	0	0
10-99	18	60.0%	0	0
100-999	6	20.0%	0	0
>999	1	3.3%	0	1
Total	**30**	**-**	**0**	**0**
			(0.0%)	**(0.0%)**

***Source:** *E. coli* Reference Laboratory in Lugo.

There have been various studies carried out in Spain on the role of bovine livestock as a reservoir for STEC. It was consequently possible to verify in Galicia (1993-1995) that a third of calves and cows were carriers, with highly virulent STEC strains of serotype O157:H7 present in 12% of calves in 22% of sampled farms. In 1998, a study in Navarra detected the presence of STEC O157:H7 in various cattle slaughterhouses and feedlots in proportions varying between 10% and 19% in slaughterhouses and 0% and 23% in feedlots. The data obtained in Spain are in line with those found in other countries. They confirm that around 10% of bovine livestock are colonised by STEC O157:H7 strains (Blanco et al., 2004b).

After determining the serotypes and virulence genes of 514 strains of bovine STEC isolated in Spain, it was verified that they belonged to 66 serogroups and 113 serotypes. However, 52% of the strains belonged to just 10 serotypes (O4:H4, O20:H19, O22:H8, O26:H11, O77:H41, O105:H18, O113:H21, O157:H7, O171:H2 and ONT:H19). Many of the bovine strains belonged to the seropathotypes that had been previously found amongst STECs responsible for human infection. None of the isolated bovine strains of STEC in Spain belonged to serotype O104:H4. However, the two O104:H21 strains *vt1 vt2* of the ST672 sequence type were detected (Blanco et al., 2004b) (Mora et al., 2011).

Blanco et al. (2003) performed a study in collaboration with the Caceres Veterinary School in 1997. After studying 1,300 samples from 93 farms in Extremadura, it was found that 0.4 % of lambs were colonised by ECVT O157:H7 and 36% by non-O157 STEC. After serotyping 384 ovine strains, 35 serogroups and 64 serotypes were observed. However, 72% of strains belonged to one of the following 12 serotypes: O5:HNM, O6:H10, O91:HNM, O117:HNM, O128:HNM, O136:H20, O146:H8, O146:H21, O156:HNM, O166:H28 and ONT:H21. The STEC bovine serotypes were different from the STEC bovine serotypes but many of them were also found in verotoxigenic strains of human origin. None of the ECVT ovine strains belonged to serotype O104:H4. However, we detected 10 strains of O104:H7 *vt1* (the majority of sequence type ST1817).

In a study carried out in collaboration with both the Madrid and Murcia Veterinary Schools in 2003 (Cortés et al., 2005) (Orden et al., 2008), some 222 faecal samples of goats and kids taken from 12 farms in Murcia were analysed. We found that 0% of animals were colonised by STEC O157:H7 strains and 48% by non-O157. After serotyping 106 goat strains, 25 serotypes were observed, the most frequent being the following: O5:HNM, O76:H19, O126:H8, O146:H21, ONT:HNM and ONT:H21. None of the ECVT goat strains belonged to serotype O104:H4. Likewise, none of the 106 strains displayed the *eae* gene. However, 16% of the ECVT goat strains belonged to serotypes involved in HUS.

It should be highlighted that the *eae* gene was not detected in any STEC goat strains and was only present in 6% of ovine strains, compared to 29% in bovine strains and 56% of those responsible for human infection. Considering that the *eae* gene is normally associated with strains that have a higher virulence potential, it is clear that large ruminants are a more problematic reservoir. They thus require special attention when it comes to prevention measures.

Although slaughter ruminants, especially livestock cattle, are the main reservoir of STEC strains, wild animals (including game) play an important role as a reservoir of these pathogens. In a recent study from the LREC-USC, Mora et al. (2012) isolated non-O157 STEC strains in 53% of the roe deer sampled and STEC O157 in 0.56%, in accordance with that indicated by other authors for this and other types

of wild ruminants (Sánchez et al., 2009) (Sánchez et al., 2010). In the study by Mora et al. (2012) STEC strains of boars (8.4%) and foxes (1.9%) were also isolated and it was shown that isolated STEC strains in these wild animals (roe deer, boar, fox) of serotypes O5:H-, O26:H11, O76:H19, O145:H28, O146:H21 and O157:H7 presented similarity of more than 85% when compared to macrorestriction profiles of the STEC strains of the same characteristics in human patients. In this study it was therefore demonstrated that certain wild animals play an important epidemiological role in maintaining and transmitting STEC strains that are potentially pathogenic for humans. However, only 14 (10%) of the 135 strains of STEC displayed the *eae* gene: 7 from roe deer, 4 from boar and 3 from fox. Studies have also been conducted outlining game meat as a potential source of STEC transmission for humans (Miko et al., 2009) (Martin and Beutin, 2011).

A significant source of infection for livestock is their feed and water being contaminated with infected animal faeces. Previous studies indicate that STEC O26 can survive for long periods of time in manure: up to three months in manure and slurry pits, and one year in fields fertilised with manure. Survival times depend on temperature and soil type. The persistence of these pathogens in environments contaminated with manure has significant health implications due to their transmission not just in agricultural production but also in other aspects, such as in livestock fairs and farms schools where children are exposed to the presence of microorganisms (Gyles, 2007) (Kaspar et al., 2009) (ECDC/EFSA, 2011) (Mora et al., 2011).

The faecal material can contaminate meat during slaughter, while the animal is being skinned or due to insufficient evisceration. It can spread into flowing water, rivers and drinking water fountains or it can be deposited on fruits and vegetables when manure is used for fertilisation or when contaminated waste water is used for irrigation. Humans can be directly infected through contact with an infected person or carrier animal, or indirectly infected through surroundings, foods, contaminated drinking water, consumption of non-drinking water or the surface of water being contaminated with faecal material containing STEC of human or animal origin (Kaspar et al., 2009) (Mora et al., 2011).

In 2010, EFSA outlines that in a total of 8,566 beef samples analysed in a total of 12 Member States, STECs were isolated in 0.5% of the samples with serotype O157:H7 being isolated in 0.1% of the samples analysed. Isolation percentages varied between 0% and 1.6% in abattoir samples, between 0% and 3.9% in processing plants and between 0% and 5.1% (Spain) in sales establishment samples (EFSA/ECDC, 2012).

Raw fruit and vegetables form an important part of the human diet and the consumer is fully aware of their health benefits. However, in scientific literature we can find more and more studies describing STEC infection outbreaks as being associated with raw fruits and vegetables, with particular reference to bean sprouts (Michino et al., 1999) (Breuer et al., 2001) (Mohle-Boetani et al., 2001) (Ferguson et al., 2005) and raw vegetable salads (Ackers et al., 1998) (Hilborn et al., 1999) (Söderström et al., 2005) (CDC, 2006) (Friesema et al., 2008) (Wendel et al., 2009). Table 6 shows STEC/VTEC prevalence in vegetable samples (Mora et al., 2011). The Standing Committee on Veterinary Measures Relating to Public Health (SCVPH) issued a report on verotoxigenic *E. coli* which identified fresh vegetables, amongst other foods, as a public health concern (SCVPH, 2003), especially in the case of bean sprouts and unpasteurised fruit and vegetable drinks. These food

categories have also been considered to be a significant contamination route from foods according to an EFSA report on STEC (EFSA, 2007b).

The most common vehicle in outbreaks caused by STEC is undercooked beef. However, in recent years, outbreaks caused by vegetable products have become more and more frequent, with particular reference to vegetables, especially salads and different types of bean sprouts. Strangely, the biggest of outbreaks in the number of cases of diarrhoea and CH was caused by a STEC O157:H7 strain in 1996 in the city of Sakai (Osaka, Japan) and was linked to radish sprout consumption. EAEC strains have also caused various sprout-related outbreaks, including a serotype ONT:H10 outbreak in Japan in 1993 affecting more than 2000 people. Epidemiological studies support the hypothesis that the outbreaks in Germany and France are linked since they were caused by the same enteroaggregative strain of STEC O104:H4 and the same foodstuff: sprouts from fenugreek seeds imported from Egypt. Future studies should examine whether enteroaggregative strains have special mechanisms that allow for their adhesion to the surface of vegetables. It is evident that conditions for outbreak production (humidity and temperature) favour the growth of contaminant microorganisms. It is unknown how seeds have been contaminated but the use of contaminated human waste water for irrigation could be the cause (Mora et al., 2011) (Blanco et al., 2012).

Table 6. Prevalence of STEC/VTEC strains in fresh vegetables and in 4th range

Product	No	<10	%	10-99	%	>99	%	ECVT	%	O157:H7	O104:H4
Cucumber	32	32	100%	0	0%	0	0%	0	0%	0	0
Tomato	36	36	100%	0	0%	0	0%	0	0%	0	0
Lettuce	54	50	93%	4	7%	0	0%	1	1,9%	0	0
Soybean sprouts	6	6	100%	0	0%	0	0%	0	0%	0	0
Endive	2	2	100%	0	0%	0	0%	0	0%	0	0
Broccoli	7	7	100%	0	0%	0	0%	0	0%	0	0
Leek	2	2	100%	0	0%	0	0%	0	0%	0	0
Pepper	6	6	100%	0	0%	0	0%	0	0%	0	0
Carrot	1	1	100%	0	0%	0	0%	0	0%	0	0
Spring onion	1	1	100%	0	0%	0	0%	0	0%	0	0
Salad bag	53	52	98%	1	2%	0	0%	0	0%	0	0
Total samples	**200**	**195**	**98%**	**5**	**2.5%**	**0**	**0%**	**1**	**0.5%**	**0**	**0**

Source: (Mora et al., 2011).

Fruit and vegetable contamination can occur on a surface level or in internal tissues. Although STEC contamination between tissues has mainly been seen in vegetable leaves, *E. coli* O157 internalisation has also been documented in tomatoes (Ibarra-Sánchez et al., 2004). The presence of viable enterohemorrhagic *E. coli* O157:H7 has been found in internal radish and stoma sprouts coming from seeds that had been experimentally contaminated with the bacteria (Itoh et al., 1998).

The contamination of fruits and vegetables can occur at different stages of the food chain (Beuchat and Ryu, 1997) (FAO/WHO, 2008):

1. During primary production including harvest.
2. During post-harvest including handling, transport and processing.
3. When placed on the market in shops and supermarkets.
4. In prepared meal processing establishments, after sale during transportation and in the consumer's home due to poor handling.

Possible contamination routes are as follows: irrigation water contaminated with waste from livestock farms and sewers; the use of organic fertilisers of animal or human origin and; direct contact between animals (wild or domestic) and the fresh vegetable product when growing in the field. Irrigation by flooding can also be a potential contamination route if livestock farms are close to vegetable fields. During marketing and food preparation, contamination routes are linked to the use of contaminated water when washing vegetables, utensils that have not been disinfected and insufficient hygiene management (Söderström et al., 2005) (Tyrrel et al., 2006) (Gelting et al., 2011).

Control Strategies

Research on STEC food and water contamination prevention and strategies for eliminating or radically reducing the growth of any STEC that could be present in foodstuff has mainly focused on *E. coli* O157: H7. Nonetheless, studies have also been carried out on other non-O157 strains of STEC. The susceptibility of STEC OH157:H7 and non-O157 to various intervention techniques is probable similar to that of other *E. coli* strains, although there are some differences between strains in terms of their tolerance to acid and their susceptibility to other agents like temperature (Kaspar et al., 2009).

Various interventions are currently being developed that are to be carried out before the animal is slaughtered. Numerous post-slaughter interventions have been approved and put in place geared towards *E. coli* O157:H7 detection and decontamination of carcasses after meat production.

The farm itself and the type of feed that animals are given seem to be the origin of contaminated abattoir meat. Good agricultural practices (GAP) and good manufacturing practices (GMP) constitute the first measures to be considered in the control of STEC and other pathogenic microorganisms in foods of both animal and vegetable origin. The application of the HACCP is a useful way of preventing contamination caused by these microorganisms, thus reducing their prevalence on both the farm and in the crop field. However, in the application of HACCP for primary production it is quite difficult to objectify some points of critical control. On the other hand, the effect of food type on *E. coli* livestock excretion has been studied in an attempt to decrease the presence of this microorganism before animals reach the abattoir. Some studies indicate that livestock fed on a barley-based diet (Berg et al.,

2004) or on wet distillers grains (Wells et al., 2009) increase *E. coli* O157:H7 faecal excretion compared to livestock fed on a normal corn-based diet.

An important investigation is underway on the feeding of livestock with probiotics in order to exclude pathogens through the competitive exclusion mechanism. Promising results have been obtained with some organisms in terms of *E. coli* O157:H7 incidence in calves (Zhao et al., 1998).

Water troughs also seem to be another source of contamination. It has been proven that chlorination of water is the best treatment for controlling pathogens than could be present in water troughs. However, the presence of organic material in abundance in water could give rise to a decrease in residual chlorine content. This in turn would limit the efficiency of chlorine. The hygienisation of drinking water using lactic acid and calcium sulphate combined with benzoate, caprylic acid, butyric acid and chlorine dioxide has proven to achieve more than three logarithmic reductions in *E. coli* O157:H7, O26:H11, and O111:NM (Zhao et al., 2006).

At present, analysis is underway of the interventions that involve the use of agents like vaccines and bacteriophages prior to slaughter in the abattoir. However, it is too early to know whether these results actually certify their use in STEC control (Loneragan and Brashears, 2005) (LeJeune and Wetzel, 2007) (Johnson et al., 2008).

The carcasses of the slaughtered animals are difficult to decontaminate because of their shape and structure. Many treatments require physical surface contact. The use of antimicrobials and other treatments is not permitted to decontaminate carcasses and the use of lactic acid in beef carcasses is not even allowed on a European level. Nonetheless, the use of pressurised steam, hot water or lactic acid to wash the carcase surface has been proven to effectively reduce *E. coli* O157:H7 contamination. These intervention techniques to eliminate *E. coli* O157:H7 from the surface of carcasses has also proven to be efficient against O26:H11 and O111:H8 (Cutter and Rivera-Betancourt, 2000). However, Regulation (EC) No 853/2004 (EU, 2004) must be borne in mind as it lays down specific hygiene rules for the hygiene of foodstuff of animal origin aimed at food business operators. Its provisions ensure that operators do not use any other substance but water to eliminate surface contamination on food products of animal origin unless this substance has been authorised in accordance with this Regulation. EFSA (2011c) adopted a scientific opinion on the evaluation of the safety and efficacy of lactic acid for the removal of microbial surface contamination of beef carcasses, cuts and trimmings. In their opinion, EFSA concludes that treatment with lactic acid for the surface decontamination of beef carcasses, cuts and trimmings should not be a matter for concern from a food safety point of view. This is only the case as long as the substance used complies with EU specifications on food additives. In addition, EFSA concludes that treatments with lactic acid bring about a significant reduction of microbiological contamination compared to no treatment or treatment with drinking water. They also conclude that such treatments can help the development of microbial resistance.

Recently, Aymerich et al. (2008) have revised the effects of processing technology on the survival of *E. coli* and other bacteria present in meat and meat products. The procedures discussed included irradiation, high hydrostatic pressure, natural antimicrobial substances, active packaging and thermal treatments. These studies did not include comparison between *E. coli* O157:H7 and non-O157 STEC, but they did indeed identify the general efficiency conditions in the face of *E. coli*. It must be pointed out that the

decision of the European Council of the 18 December 2008 indicates the following with regards to the use of antimicrobial substances: it rejects the proposal from the Commission for a Council Regulation implementing Regulation (EC) No 853/2004 as regards the use of antimicrobial substances to remove surface contamination from poultry carcasses. Investigations have also researched the combination of various thermal and non thermal conservation technologies, under the concept of barrier technologies aimed at increasing efficiency. Rapid heating thermal technology, like the microwave or radiofrequency or steam pasteurisation tunnels offer new ways of pasteurising meat products and especially ready-to-eat product containing meat. Implementing some of these technologies after the final packaging of the product can avoid cross contamination during post-processing handling (Aymerich et al., 2008).

High hydrostatic pressure and ultraviolet light seem to be the most promising technologies because they pose little problems in terms of approval by health authorities, they do not require any special labelling as they contain no chemical additives and, if used properly, they do not cause any change in the product's texture, taste or smell (Hayakawa et al., 1994) (Alpas et al., 1999) (Chun et al., 2010). In some recent studies, STEC inactivation has been studied using ionising radiation (Beuchat et al., 1998) (EFSA, 2011b). The authors concluded that a dose of 1.8 kGy would provide five logarithmic reductions.

In a report, the WHO (2008) carefully reviewed the microbiological risks of fruits, vegetables, spices and medicinal herbs including guidance on mitigation options. The *Codex Alimentarius* Commission also published a Hygiene Code of Practice for fresh fruit and vegetables (CAC, 2003).

The use of Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) in crops and the industrialisation of fresh fruit and vegetables constitutes the basic framework in the production of safe food for the consumer (EFSA/ECDC, 2011). Good Agricultural Practices (GAP) describe the preventative measures applied during agricultural procedures as a way of reducing product contamination and providing guidance on procedures to reduce food hazards from the field. The implementation of Good Agricultural Practices sufficiently assures the retailer that the product is as harmless as reasonably possible.

In general, the implementation of the mentioned mitigation strategies included in the Good Agricultural Practices is recommended, in line with recommendations from international organisations (CAC, 2003) (WHO, 2008). As a result, it could be of particular interest to implement the following measures during cultivation:

- Avoid farm animals (especially ruminants) from entering the immediate surroundings of the crop fields.
- The water used for irrigation and agriculture in general should have an adequate microbiological quality.
- Control of procurement, handling and treatment of manure and slurry that will be used to fertilise fields destined for growing fruit and vegetables for human consumption.

After research into the food-borne outbreaks linked to leafy greens, spices and medicinal herbs, the conclusion was reached that bad hygiene practices during handling are a significant cause of contamination (WHO, 2008). Training and awareness of hygiene practice across the entire food chain is essential. In addition, given the existence of asymptomatic STEC carriers in humans, detection of those

carriers involved in food handling is of utmost importance (Stephan et al., 2000) (Silvestro et al., 2004). As a consequence, monitoring and exclusion of STEC carriers in the food handling process should be considered as a control or mitigation option.

According to the WHO (2008), current technologies and practices applied during post-harvest processing do not effectively eliminate those microorganisms that contaminate fruits and vegetables. Therefore, efforts should be mainly geared towards contamination prevention both pre- and post-harvest.

Despite the fact that various disinfectants can be used to reduce the microbial load in fruits and vegetables, their efficiency is variable and they do not guarantee the total elimination of pathogens (WHO, 1998). In general terms, the internal tissues of fruit and vegetables are considered sterile. Nonetheless, bacteria can still be present in small amounts as a result of irrigation water absorption, as a consequence of certain washing procedures and if the water used is contaminated with pathogens like STEC, which can also reach internal tissues (EC, 2002b). The adhesion of pathogens to the surface and internal tissues means that the use of conventional processes and disinfectant chemical methods is limited when preventing contamination transmission in fresh fruits and vegetables. The conclusion is that the only effective method of eliminating STEC from food is the introduction of bactericide treatments like thermal treatment (cooking or pasteurisation for example) or irradiation (EFSA, 2011b). On the other hand, it should be borne in mind that washing can help to spread contamination and internalise bacteria (WHO, 1998). Therefore, alternatives to chemical disinfection should be sought.

Cutting and chopping can eliminate the natural protective barriers of the whole plant. This therefore increases the risk of STEC presence as their survival and growth conditions are more favourable. Therefore, the implementation of HACCP during manufacturing and packaging is vital to decrease STEC risk in fruits and vegetables (James, 2006). However, it must be borne in mind that HACCP as a risk management tool can be difficult to implement as it is very hard to identify real Critical Control Points for fresh products unless methods are used that allow for a significant reduction of pathogens (irradiation for example) (EFSA, 2011b).

Once again, recommendations have been made to implement mitigation and control strategies included in GAP and GMP in line with recommendations from international organisations (CAC, 2003) (FAO/WHO, 2008). In order to control the presence of STEC during handling and processing, it is highly important to bear in mind the following recommendations:

- Use water with an adequate microbiological quality for washing.
- Ensure that the staff handling foods is properly trained in hygiene practices.
- Ensure the adequate design and management of hygiene procedures in premises used for food storage and processing. This includes pest control plans.

During the sale of fruit and vegetables in supermarkets and smaller shops, it is advisable to follow guidelines from the FAO/WHO and the *Codex Alimentarius* Commission (CAC, 2003) (FAO/WHO, 2008). Adequate management of the cold chain is vital and this should go above and beyond good manufacturing practices in general and good hygiene practices, including the hygiene practices of staff handling food products. This is of particular interest for processed ready-to-eat products (ready-to-eat cut vegetables and unpasteurised fruit or vegetable juices, for example).

The use of good hygiene and manufacturing practices for fresh fruits and vegetables, including training and educating the staff handling food, are important activities in catering centres as a way of controlling STEC strains. Consumers are recommended to follow good hygiene practices during food preparation. This includes washing hands before and after food preparation, washing all fruits and vegetables with drinking water, avoiding cross contamination and maintaining refrigeration temperature during storage. Peeling and cooking fruits and vegetables can also eliminate the risk of STEC presence. Some more resistant foods, such as root vegetables, should be washed with a brush to physical removed dirt and microorganisms. This can be done in combination with a detergent but the product must then be rinsed with drinking water. However, it must be mentioned that although these measures have proven useful, it is impossible to completely eliminate the risk of STEC presence in fresh fruits and vegetables.

Conclusions of the Scientific Committee

STEC food contamination routes

1. Many different types of foods have been identified as a potential source of STEC. These tend to be raw or poorly cooked foods or those that have been contaminated with ruminant faeces, whether during primary production (milking and fresh fertilised fruits, vegetables for example) or subsequent processing and handling (slaughter for example). Outbreaks of STEC infection are increasingly linked to fruits and vegetables, especially in the case of sprout vegetables and leafy greens for salads.
2. There could be more than one outbreak exposure path. For example, primary human infection could originate from the consumption of contaminated foods or direct contact with STEC carrier animals. Secondary infection could occur via fecal-oral transmission after foods have been contaminated due to them being handled by an infected person who excretes the bacteria in their faeces. As a result, there are probably multiple exposure paths, mainly during the last stages of an outbreak. These include asymptomatic carriers.
3. STEC contamination of meat can occur at different stages of the food chain: on the farm and in the slaughterhouse; during handling and processing; during placing on the market and retail sale; during food preparation for supply to third parties and; after sale during transportation and in the home due to poor handling on the part of the consumer.
4. STEC contamination of fruits and vegetables can occur at different stages of the food chain: during primary production, harvesting and post-harvesting, which includes handling and processing; during placing on the market, retail sale and catering and; after sale during transportation and in the home due to poor handling on the part of the consumer. Since current technologies and practices applied during post-harvest processing do not effectively eliminate those microorganisms that contaminate fruits and vegetables, efforts should focus mainly on contamination prevention both pre- and post-harvest.

Control measures to avoid STEC contamination and outbreaks

1. Good agricultural practices (GAP) and good manufacturing practices (GMP) are the main measures to be borne in mind in the control of STEC and other pathogenic microorganisms in foods of both animal and vegetable origin. Good Agricultural Practices (GAP) describe preventative measures to be applied during agricultural procedures aimed at reducing product contamination. They also provide guidance on those necessary agricultural practices geared towards increasing the harmlessness of foods from the field. The application of a HACCP system is a useful way of preventing STEC contamination, thus reducing their prevalence on both the farm and in the crop field. Nonetheless, during primary production when circumstances are so unpredictable, their application in many cases can be unfavourable. The incorporation of HACCP programmes in processing and packaging plants is an essential requisite in ensuring that foods are as harmless as possible.
2. Given the evidence of asymptomatic STEC carriers, detection of those STEC carriers involved in food handling is vital. Monitoring and/or exclusion of STEC carriers in food handling processes should be considered as a control option.
3. The type of feedstuff fed to farm animals seems to be the origin of contamination in animals for slaughter. It is therefore essential to consider the development of foods that decrease animals' *E. coli* excretion.
4. It is vital that the drinking water of animals has a good microbiological quality and that measures are taken for its hygienisation. These include chlorination and making sure that it contains no organic material. The water used for irrigation and agriculture in general should have an adequate microbiological quality. In processing plants and during subsequent packaging of fresh vegetables, water with an adequate microbiological quality should be used for washing.
5. Avoid farm animals (especially ruminants) from entering the immediate surroundings of the crop fields. Likewise, procurement, handling and treatment of manure and slurry that will be used to fertilise fields destined for growing fruit and vegetables for human consumption should be controlled.
6. The only effective method for eliminating STEC from food is the introduction of a bactericide treatment such as heating (cooking or pasteurisation for example) or irradiation. However, the combination of various thermal and non-thermal conservation technologies under the concept of barrier technologies along with rapid heating thermal technologies, like the microwave or radiofrequency or steam pasteurisation tunnels offer new ways of pasteurising meat products and especially ready-to-eat products containing meat.
7. The use of pressurised steam, hot water or lactic acid to wash the carcase surface has been proven to effectively reduce *E. coli* O157:H7 contamination. These intervention techniques to eliminate *E. coli* O157:H7 from the surface of carcasses has also proven to be efficient against O26:H11 and O111:H8. However, despite its benefits, the use of lactic acid to reduce contamination of the microbiological surface of beef carcasses, half-carcasses or quarter-carcasses is not permitted under current regulations. As a result, the industry should meet EU legislation requirements on food hygiene, according to that established in Regulations (EC) No 852/2004, No 853/2004 and

No 2073/2005. In any case, its use should always involve good hygiene practices and systems based on HACCP. The use of lactic acid should under no circumstances be considered as a hygienic substitution for slaughter practices and operation procedures, or even as an alternative to meeting the requisites of said Regulation.

8. Basic training should be provided for staff handling food on hygiene practices. Special training should be provided for slaughter men and other slaughterhouse and cutting room handlers. Amongst other aspects, special attention should be paid to control the use of knives during the evisceration. They should be disinfected with hot water at the right temperature and for the right amount of time. In the same way, in the evisceration process all manoeuvres should be controlled to avoid contamination with faecal matter from carcasses and entrails. Staff handling food should wash their hands well after any kind of contact with raw meat. Likewise, the adequate design and management of hygiene procedures should be guaranteed in premises used for food storage and processing. This includes pest control plans. Correct management of the cold chain is of particular importance for fresh and ready-to-eat products (chopped vegetables, unpasteurised juices, fruits, vegetables and meat products, for example).
9. The public should be informed of risks associated with improper food handling and preparation. Meat should be cooked for enough time to inactivate STEC. This is particularly the case for minced meat. It is important to avoid cross contamination of foods that are eaten raw (fruits and vegetables) with raw meat prior to cooking. Foods should be properly placed in the domestic refrigerator in such a way that liquid from foods to be eaten cooked (meat or fish) do not spill onto foods to be eaten raw or unheated (fruits and vegetables). Vegetables to be eaten raw in salads should be carefully washed at home.
10. Due to the presence of STEC in raw milk, only milk that has undergone thermal pasteurisation can be considered suitable for consumption as it can possibly contain STEC.
11. It is advisable to have data on the MPN of *E. coli* per gram in order to assess the level of faecal contamination in foods. However, it must be pointed out that low MNP of *E. coli* does not guarantee the absence of STEC. In this sense, real time and conventional PCR tests and PFGE classification through comparison with other strains and, in particular, the causes of infections with humans could prove very useful.

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